BIOCHEMICAL EFFECT OF THE ETHANOLIC ROOT EXTRACT OF *CROTON MEMBRANACEUS* ON THE TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

BY

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DECLARATION

I declare that this project was duly carried out by me at the Unit of Chemical Pathology, School of Biomedical and Allied Health Sciences Korle-Bu under the supervisions of Dr. George A. Asare, Dr. R.A. Ngala and Dr. W.K.B.A. Owiredu.

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DEDICATION

TO GOD BE THE GLORY

This research work is dedicated to my lovely husband and children.

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ABSTRACT

Benign prostatic hyperplasia (BPH) is one of the most common diseases of aging men, characterized by noncancerous enlargement of the prostate gland which is often associated with lower urinary tract symptoms (LUTS) and poses a threat to the quality of life (QoL). The prevalence of BPH is age-dependent, with the initial development usually after 40 years. The condition accounts for 80% of prostate disorders in Ghana with a peak age of 65 years. Treatment options are basically by orthodox or traditional means. Due to the fear of loss of libido in men as a result of the use of orthodox medicine, BPH patients have resorted to complementary and alternative medicine (CAM) for treatment. An ethanolic extract of *Croton membranaceus (CMERE)* has been used over four decades for the management of BPH based on anecdotal evidence. However, no scientific study exists to back this practice. The study therefore aimed at investigating the effect of *CMERE* on BPH patients. Thirty (30) clinically diagnosed BPH patients were recruited by a convenience sampling technique. Baseline information on LUTS and sexual function was obtained using the International Prostate Symptom Score (IPSS) and the International Index of Erectile Function (IIEF) questionnaires, respectively. Blood samples were also obtained from subjects for biochemical assays [PSA, renal function tests (RFTs), liver function tests (LFTs), lipid profile and Apo proteins A1 and B] using spectrophotometric and ELISA techniques. Treatment outcome on glutathione s-transferase T1 and M1 (GSTT1 and GSTM1) genotypes was determined by molecular techniques. Abdomenopelvic ultrasonography was also performed to determine the prostate volume. These parameters were again measured 3 months after administration with 60 mg per day *CMERE* in capsule form. The average age was 66 ±11 years. At the end of the study, no patient had severe prostate symptoms compared to baseline data of 37%. Furthermore, improvement in IPSS and QoL was significant (p = 0.0005 and 0.0001, respectively).

*CM*ERE did not adversely affect systemic organs generally. No significant differences were observed in RFTs, and the lipid profile except for Apo A1 which increased indicating an increase in high density lipoprotein cholesterol (HDL) (p=0.0248), LFT parameters were normal except for increases in total and indirect bilirubins (p = 0.0008, p = 0.0007, respectively). However, these were within the normal reference intervals and could be as a result of bilirubin functioning as an antioxidant. PSA decreased significantly by 40.87% (p =

0.0015), accompanied by a significant decrease of 39.71% in prostate volume (p = 0.0008) after treatment. Thirty-three percent (33%) and 67% of the subjects were GSTT1 negative and GSTT1 positive, respectively, whiles 20% of the subjects were GSTM1 negative and 80% GSTM1 positive, respectively. There was a significant reduction of PSA levels ($p<0.05$) in both GSTT1(+)/GSTM1(+) subjects but not GSTT1(-)/GSTM1(+) and GSTT1(+)/GSTM1(-) subjects. In conclusion, *C. membranaceus* use is safe and a highly desirable alternative for men with BPH. IPSS, QoL, PSA and prostate volume improved significantly. GSTT1(+)/GSTM1(+) polymorphism subjects also showed a significant and better response to treatment. This is the first evidence backing the anecdotal claims for the use of *C. membranaceus* in the management of BPH.

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5-ARIs	5-alpha reductase inhibitors
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BCG	Bromocresol green
BMI	Body mass index
BPH	Benign prostatic hyperplasia
BuBc	Bilirubin unconjugated and conjugated
BUN	Blood urea nitrogen
CAM	Complementary and alternative medicine
CHOL	Cholesterol
Cm	*Croton membranaceus*
CMERE	*Croton membranaceus* ethanolic root extract
Cr	Creatinine
Cu^{2+}	Cupric ions
CV	Cardiovascular
DHT	Dihydrotestosterone

DRE	Digital rectal examination
ED	Erectile dysfunction
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
ERSPC	European randomized study of screening for prostate cancer
fPSA	Free prostate specific antigen
g	grams
GGT	Gamma glutamyltransferase
GSH	Glutathione
HCL	Hydrochloric acid
HDL	High density lipoprotein
IDL	Intermediate density lipoproteins
IIEF	International index of erectile function
IPSS	International prostate symptom score
K^+	Potassium
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LFT	Liver function test
RFT	Renal function test
LUTS	Lower urinary tract symptoms
$MgCl_2$	Magnesium chloride
mmol/l	Millimole per litre
Na^+	Sodium
NIH	National Institute of Health
^OC	Degree Celsius

P-5-P	Pyridoxal-5-phosphate
PC	Prostate cell
PCa	Prostate cancer
PEG	Polyethylene glycol
PSA	Prostate specific antigen
QoL	Quality of life
S-D	Sprague-Dawley
TBIL	Total bilirubin
TMB	Tetramethyl bendidine
TP	Total protein
tPSA	Total prostate specific antigen
TRIG	Triglyceride
TX	Triton X
U/L	Units per litre
UK	United Kingdom
VLDL	Very low density lipoproteins
µl	microlitre

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Benign prostatic hyperplasia (BPH), one of the most common diseases of aging men, is characterized by noncancerous enlargement of the prostate gland and is often associated with lower urinary tract symptoms (LUTS) (Ajit and Poduri, 2012). It is progressive and a highly prevalent disease which clinically manifests as LUTS, posing a financial burden on the patients (Saigal and Joyce, 2005). The prevalence of BPH is age-dependent, with the initial development usually after 40 years of age (Nan *et al.*, 2012). Approximately, 60% of men aged over 50 years have histological evidence of BPH and, after the age of 70 years, the proportion increases to 80% (Ajit and Poduri, 2012). However, in Ghana the condition accounts for 80% of prostate disorders and it mostly affects men aged over 60 year with a peak age of incidence at 65 years (Yeboah, 2009).

It has been reported that approximately 4.5 million visits were made to a hospital in China for a primary diagnosis of BPH in the year 2000 with a treatment cost of $1.1 billion for outpatient pharmaceuticals (Nan *et al.*, 2012). One-third of the Swedish male population aged over 50 years have been reported to have LUTS, which is associated with BPH (Stranne *et al.*, 2009). About 150 new cases report monthly to the Korle-Bu teaching hospital with LUTS associated with BPH (Yeboah, 2009).

Although BPH is rarely fatal, it affects the quality of life (QoL) and when left untreated, serious life-threatening complications may result.

Prostatic growth and development are governed by genetic (Sanda *et al.,* 1994), hormonal (Marker *et al.,* 2003) and dietary factors (Bravi *et al.,* 2006). However, literature indicates that apart from steroids and peptides, lipids also play a crucial role in the pathogenesis of BPH (Cai *et al.,* 2001; Kaplan-Lefko *et al.,* 2008; Escobar *et al.,* 2009; Vikram *et al.,* 2010; Rick *et al.,* 2011). Although the effects of peptides and lipids on the growth of the gland may be milder compared to that of steroids, chronic change in their levels due to dietary habit or genetic predispositions can significantly contribute to the initiation and/or progression of the disease over a period of time (Escobar *et al.,* 2009).

Although the underlining cause of BPH is not well understood, as men age, the amount of active testosterone in the blood decreases, leaving a higher proportion of estrogen. It has been suggested that BPH may occur because the higher amount of estrogen within the gland increases the activity of substances that promote cell growth (Ross *et al.,* 1986). Another theory focuses on dihydrotestosterone (DHT), a substance derived from testosterone in the prostate, which may help control its growth. Epidemiologic studies also suggest an environmental etiology and nutrition (Pohar *et al.,* 2003; Gonzalez and Salas-Salvado 2006), but evidence for specific factors are not consistent (Giovannucci *et al.,* 2007). Some potentially modifiable factors such as lycopene from tomatoes have been identified (Chan *et al.,* 2005), but still debatable (Magbanua *et al.,* 2011). As lycopene is the most efficient quencher of oxygen radicals among the common carotenoids (Di Mascio *et al.,* 1989), investigators have undertaken trials of supplementation of □-carotene (Hennekens *et al.,* 1996; Arab *et al.,* 2001), T-tocopherol
(Virtamo *et al.,* 2003) and vitamin E (Chan *et al.,* 1999; ATBC, 1994). It was therefore observed that the anticancer effect of supplemental tocophenol and □-carotene during post intervention follow-up of subjects disappeared (ATBC, 1994).

The use of medicinal plants for the treatment and management of diseases by all manner of people is on the increase worldwide (Calixto, 2000). It plays a vital role for the development of new drugs and has considerable importance in international trade. Currently, an estimated 75-80% of the world population, especially from the developing countries, depend on plant-derived medicine as the first line of treatment of diseases (Monteagudo, *et al.*, 2006). Furthermore, medicinal plants continue to attract the attention of drug investigators and the pharmaceutical industry. Thus, over the past decade, herbal medicines have been accepted as alternative medicines and have also made an impact on international trade (Madhuri and Govind 2009).

The US herbal medicine market has experienced tremendous growth reaching about $3.2 billion in 1996 and $5 billion in 1999 (Calixto, 2000). It has been estimated that the European market alone reached about $7 billion in 1997 with the German market corresponding to about $3.5 billion (50%) of the European market (Calixto, 2000). Phytomedicine sales in Asia and Japan reached about US$2.3 billion and $2.1 billion respectively (Grunwald, 1995). In the United States, the National Centre for Complementary and Alternative Medicine at the National Institutes of Health spent approximately US$33 million on herbal medicine in 2005 whereas the National Canadian Institute also committed nearly US$89 million for research into traditional medicine (Wachtel-Galor, 2011).

Croton membranaceus (*Cm*) Mull. Arg., a plant of the genus Euphorbiaceae, locally called *Bokum,* is an emerging plant of interest from the medicinal point of view. Its aqueous root extract is currently employed, in varying dosage forms, for the treatment and management of prostatic cancers and other related tumours (Aboagye *et al.*, 2000; Mshana *et al.*, 2000). Also, marked cytotoxic activity of *crotomembranafuran*, betasitosterol-3-D-glucoside and DL-thrietol against human prostate (PC-3) cells has been reported (Bayor *et al.*, 2008).

The promising antitumour potential of *Cm* has led to concerns over safety and efficacy despite its long history of use and the perception that such "natural" products offer a safer alternative to conventional medicines (Adewunmi and Ojewole, 2004). Toxicological data on medicinal plant extracts are currently required as part of the evaluation process of these plants in many countries to ensure safety before marketing (Morgan *et al*., 1994; Gericke, 1995). Additionally, toxicity studies on medicinal plants provide insight into specific toxic effects on target organs; the mode of toxic action and also dosing range guidance for other toxicity studies (OECD, 1998; OECD 2001). However, the virtual non-existence of extensive toxicological and other therapeutic data on *Cm*, despite claims of success in its use for prostatic tumours and other related cancers, limits its widespread acceptance as a potential antitumour, anti-atherogenic and anti-ischemic agent. Indeed, numerous drugs used in cancer chemotherapy exhibit cell toxicity with the potential to induce genotoxic, carcinogenic and teratogenic effects on non-tumor cells (Chung *et al.,* 1998). The toxicological effect of *Cm* on target organs such as the testes and its consequences on male sex hormones remain unknown.

Some toxicological studies have been documented on *Cm* to confirm its safety (Asare *et al*., 2011). Furthermore its anti-atherogenic potential has been explored (Afriyie *et al*., 2013) and its hypoglycaemic and anti-hypertensive effects are beginning to emerge (Asare *et al*., 2015a). Histological evaluation of a recent study has shown that *Cm* treatment causes considerable shrinking to the prostate (Afriyie *et al.,* 2013) with no adverse effect on the testes, which is suggestive of its efficacy in treating BPH.

1.2 PROBLEM STATEMENT

Biochemical studies on medicinal plants provide insight into specific effects on target organs; the mode of biochemical action and also dosing range guidance for other studies

(OECD, 1998; OECD 2001). Most medicinal plants on the market have not been extensively studied but treatment of conditions such as BPH, has a global shift from orthodox to plant medicine due to the fear of loss of libido after the use of orthodox medicine for treatment.

Although *Cm* is said to be generally non-toxic, the virtual non-existence of extensive biochemical and other therapeutic data on *Cm*, despite claims of success in its use for prostatic tumours and other related cancers, limits its widespread acceptance as a potential anti-tumour agent. The biochemical effect of *Cm* on target organs such as the prostate and its consequences on male sexual function as well as LUTS remain unknown in humans. There is therefore the need to investigate the effect of *CMERE* on the prostate and other general biochemical markers of systemic organs, in order to verify anecdotal claims for the use of *Cm* in the management of BPH.

1.3 JUSTIFICATION

The use of medicinal plants for the treatment and management of diseases is generally on the increase. It plays an important role for the development of new and effective drugs. Currently, an estimated 75-80% of the world population, especially from the developing countries, depend on plant-derived medicine as the first line of treatment of diseases (Monteagudo, *et al.*, 2006).

Cm, a plant of the genus Euphorbiaceae, is an emerging plant of interest from the medicinal point of view. Its aqueous root extract is currently employed, in varying dosage forms, for the treatment and management of prostatic cancers and other related tumours (Aboagye *et al*., 2000; Mshana *et al*., 2000). Histological evaluation of a recent study has shown that *Cm* causes considerable shrinking to the prostate in rats (Afriyie *et al.*, 2013)

with no adverse effect on the testes, which is suggestive of its efficacy in treating BPH. However, throughout literature, no evidence has been provided on its effect in humans.

1.4 HYPOTHESIS

H_0: *Cm* has no therapeutic effect on the prostate in the management of BPH.

1.5 AIM

The aim of the study was to investigate the effect of *Cm* on BPH patients opting for Complementary and Alternative Medicine (CAM) in the management of BPH.

1.6 OBJECTIVES

1. To determine the effect of *Cm* on the prostate (free / total PSA levels, and prostate volume).
2. To determine the effect of *Cm* on some systemic organs and biochemical markers (kidney function, liver function and lipid profile).
3. To determine if *Cm* has any adverse effect on sexual function and quality of life.
4. To determine GSTT1 and GSTM1 distribution and their effect on PSA after treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE PROSTATE GLAND

2. 1.1 Anatomy

The prostate gland (Figure. 2.1), which also surrounds the first two centimetres of the urethra, is located beneath the bladder and in front of the rectum in men. It is surrounded

by a capsule of fibrous tissue called the prostate capsule. The urethra is the tube in the penis which conducts urine and semen to the tip of the penis. The prostate is part of the genitourinary system which consists of the kidney, ureters, bladder, urethra, the two testes, the scrotum and the prostate gland. The adult prostate is shaped like a pyramid and of the size of a golf ball (Yeboah, 2009). The prostate weighs approximately 20 g in a healthy man (Rhee, 2008).

The gland is small at birth and enlarges at puberty and after forty years; its normal growth is hormonally controlled with other growth factors produced by the testes and the prostate itself. The enlargement of the gland depends on the age and race. Growth is fast in African Americans and whites in America but less in Orientals. Familial, genetic and environmental factors also influence the growth of the prostate gland as well as dihydrotestosterone (DHT) and hormonal imbalance (Yeboah, 2009).

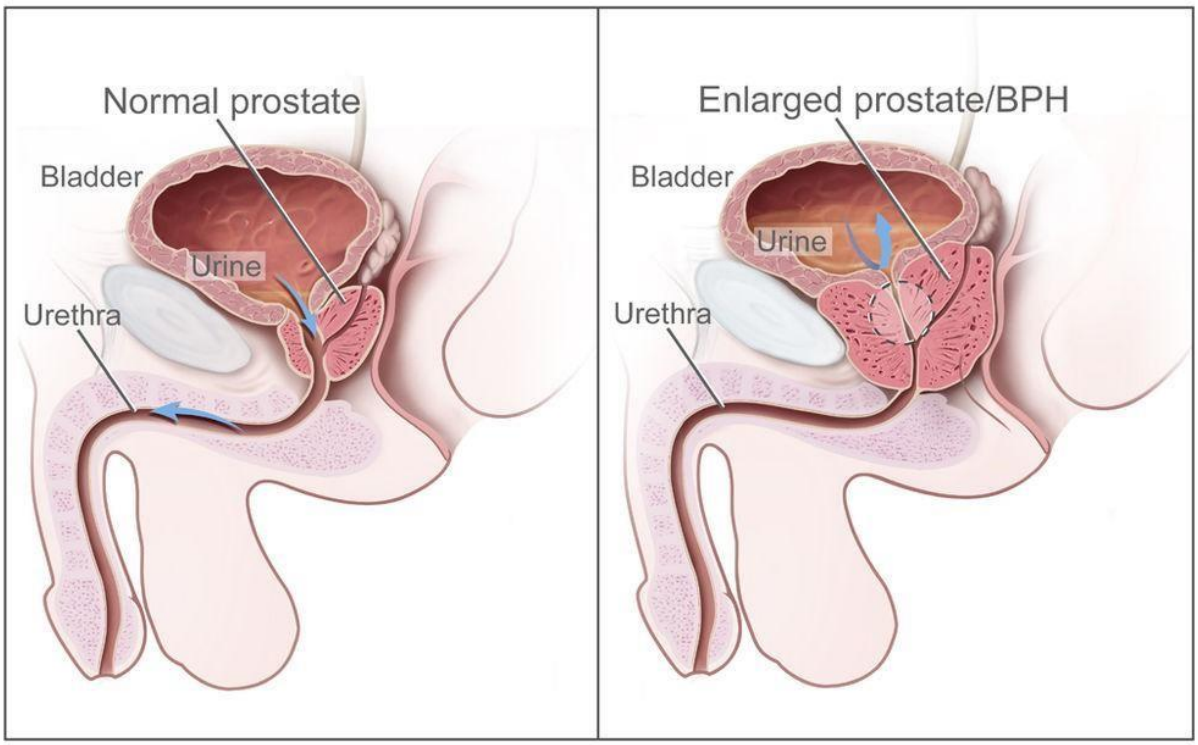


Figure. 2.1: Anatomy of the male reproductive and urinary systems, showing the normal prostate and an enlarged prostate (testicles, bladder, and the direction of urine flow (arrowed). Source: Andriole *et al.*, 2012

2.1.2 Functions

The prostate gland is composed of both glandular tissue that produces prostate fluid and muscle tissue that helps in male ejaculation. Prostate fluid also helps to keep sperm, which is found in semen, healthy and lively, thereby increasing the chances of fertilization (Encarta, 2009). However, the glands main function is to produce prostatic fluid which forms the major part of the semen ejaculate. Furthermore it secretes prostate specific antigen (PSA) and prostatic acid phosphatase which liquefies semen and acts as a medium through which sperms travel for fertilization. The secretions may also have an antibacterial effect (Bhavin *et al.*, 2013). The prostate also contracts at the time of ejaculation to prevent retrograde (or backward) flow of semen into the bladder (Wedro, 2009). The prostate gland is affected by several disorders such as prostate cancer, prostatitis and BPH (Yeboah, 2009).

2.2 COMMON DISEASES OF THE PROSTATE

2.2.1 Prostate cancer

Cancer of the prostate is now the commonest cause of cancer death in males with urinary problems in Ghana. It accounts for about 18% of prostate disorders and the prevalence is about 5% in men over the ages of 50 years and about 120 to 150 new cases are seen at the Korle-Bu Teaching Hospital every year (Yeboah, 2009). Most patients are over 60 years with the peak age of incidence at 65 years. There is no known cause but hormones especially oestrogen imbalance, growth factors, aging, race, diet (red meat) and high animal fat, early sex , virus and prostatitis have been incriminated as confounding factors

of prostate cancer (Yeboah, 2009), although a number of risk factors such as oxidative stress and genetic predisposition have been identified (Alba *et al*., 2009).

2.2.2 Prostatitis

Inflammation of the prostate gland caused by infection accounts for 2- 2.5% of prostate disorders (Yeboah, 2009). These are acute prostatitis, prostatic abscess and chronic prostatitis. The common causative organism is *Escherichia coli*, a gut organism. The infection usually gets into the prostate gland through the blood stream or occasionally urethral catheter wearing (Yeboah, 2009).

2.2.3 Acute prostatitis

Acute prostatitis is swelling and irritation (inflammation) of the prostate gland that develops suddenly causing difficulty in urination or slow urine flow. It is usually caused by a bacterial infection such as *Enterococci, E. coli* and *Staphylococcus aureus* (Encarta, 2009).

2.2.4 Prostatic abscess

An abscess is a localized collection of pus in the prostate that is surrounded by swelling (inflammation). Fluid collected may contain living and dead white blood cells, dead tissue and bacteria (pus) at the damaged area (Encarta, 2009).

2.2.5 Chronic prostatitis

This is recurrent urinary tract infection and/or chronic infection of the prostate. It may be as a result of bacteria or non-bacterial infection (Rhee, 2008).

2.2.6 Benign prostatic hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is the most common male benign proliferative disease, and approximately eight million patients are estimated to visit physicians and are diagnosed of primary or secondary BPH annually world-wide. (Kouji *et al.*, 2013). It is a non-cancerous condition (Encarta, 2009) of which blacks are mostly affected. It is high in Africans, African Americans and Caucasians but low in Asians and Japanese men. The condition occurs as a result of the enlargement of the prostate gland (Kouji *et al.*, 2013; Clinicalkeys, 2014). The prostate enlarges in two different ways. In one type of enlargement, cells multiply around the urethra. The second type of enlargement occurs in the middle-lobe of the prostate where cells grow into the urethra and the bladder outlet area (Stanley, 2013). As the prostate enlarges, it compresses the urethra by the contractions of the smooth muscles within it and causes a gradual obstruction to the bladder outflow, leading to progressive severity in lower urinary tract symptoms (LUTS) such as frequency, urgency, nocturia, incomplete voiding, weak urinary stream, acute urine retention and haematuria (Chin-Hsiao, 2013).

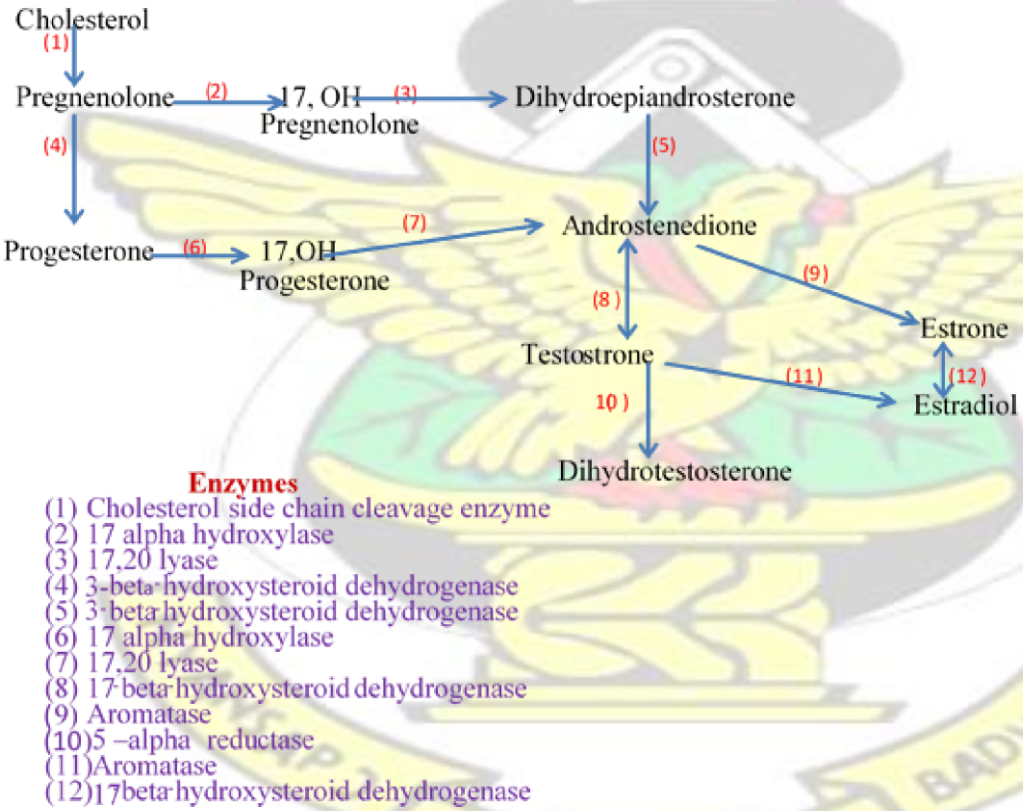
2.2.6.1 Prevalence

It is difficult to determine the exact incidence and prevalence of BPH because research groups often use different criteria to define the condition. Its prevalence increases with age and may affect 75% of men in their sixties (Chin-Hsiao, 2013).

According to the National Institutes of Health (NIH), BPH affects more than 50% of men over age 60 and as many as 90% of men over the age of 70 (Stanley, 2013). The incidence of gross enlargement of the prostate gland has been reported as 40% in 70year -old men, and microscopic foci of the prostate gland are present in up to 80% of these men (Kouji *et al.*, 2013). A study conducted to investigate the high prevalence of BPH in the UK

community defined prevalence rate of BPH as enlargement of the prostate gland of equivalent weight greater than 20 g in the presence of symptoms of urinary dysfunction and/or a urinary peak flow rate less than 15 ml per second without evidence of malignancy**.** The study showed a rise from 138 per 1000 (13.8%) in men aged 40-49 years to 430 per 1000 (43%) men aged 60-69 years (Garraway *et al.*, 1991). However, in Ghana the condition accounts for 80% of prostate disorders and it mostly affects men aged over 60 years with a peak age of incidence at 65 years (Yeboah, 2009).

BPH is rarely fatal, but affects the quality of life (QoL), and if left untreated, serious life-threatening complications may arise. Prostatic growth and development are multifactorial



and governed by genetic (Sanda *et al.*, 1994), hormonal (Marker *et al.*, 2003) and dietary factors (Bravi *et al.*, 2006).

2.2.6.2 Causes

Testosterone is produced by the Leydig cells of the testes and is converted by 5αreductase to dihydrotestosterone (DHT) [Figure 2.2]. Testosterone and DHT promote prostatic

epithelial and stromal cell proliferation, apoptosis inhibition, and prostatic angiogenesis. Balance between cellular proliferation and apoptosis exists in patients with normal intraprostatic levels of androgen and estrogen, but DHT imbalance occurs with advancing age, favouring prostatic epithelial and stromal cell proliferation (Paolone, 2010). Production and accumulation of DHT increase with age resulting in the induction of the condition. Furthermore, high estrogen level as a result of aging stimulate cell growth (Bhavin *et al.*, 2013).

Figure 2.2: DHT biosynthesis pathway showing the enzymes that catalyse the reaction steps. Source: Collins, 2013.

Obstructive LUTS are produced through two mechanisms. The enlarged prostate can obstruct the prostatic urethra and impede continuous urinary flow and also the prostatic stromal smooth muscle cells can become hyperactive and constrict the prostatic urethra. Irritative urinary symptoms result from bladder instability. The obstructing prostate increases the intravesicular pressure, and the bladder's smooth muscles adapt by increasing in size. When the bladder muscles become hypertrophic, the bladder becomes hypersensitive causing irritative (storage) urinary symptoms (Thorner, 2009).

2.2.6.3 Risk factors

2.2.6.3.1 Genetic factors

Genetic factors have been implicated in the risk of developing BPH in monozygotic twins with an affected sibling which suggests that family history may be a risk factor (ClinicalKeys, 2014). Other genetic factors may include: Vitamin D_3-vitamin D receptor which regulates both epithelial and cell growth proliferation, Cytochrome P45017 which mediates sex steroid hormone synthesis and 5α-reductase enzyme which converts testosterone to DHT which promotes prostate cell proliferation (Thorner *et al.*, 2009).

2.2.6.3.2 Effect of lipids

High level of total cholesterol, LDL-cholesterol, triglyceride and decreased level of HDL-cholesterol increases the risk of BPH whiles cholesterol-reducing drugs may lower the risk (Moyad and Lowe, 2008). Increased LDL in serum is associated with higher risk of BPH (Parsons *et al.,* 2008). Physical exercise, decreases the serum lipid level and this is associated with decreasing the risk for BPH (Parsons and Kashefi, 2008). Hyperlipidemia has an associated with obesity and higher body mass index (BMI), and these parameters has a positive correlation with the BPH (Hammarsten *et al.,* 1998; Hammarsten and Hogstedt, 1999; Dahle *et al.,* 2002; Parsons *et al.,* 2006; Parsons *et al.,* 2009). Patients with increased BMI have been said to have large prostate volume with high International Prostate Symptom Score (Kim *et al.,* 2011). Obesity and sedentary lifestyle increases the risk for BPH and this has been indicated in several studies (Dahle *et al.,* 2002; Parsons *et al.,* 2006; Parsons *et al.,* 2009; Parsons, 2011).

The increase in adipose aromatization of testosterone to estrogen and the altered levels of testosterone and sex hormone–binding globulin in patients with increased adipose stores promote prostatic epithelial and stromal cell proliferation (Wein *et al.,* 2012).

2.2.6.3.3 Dietary factors

Increased intake of polyunsaturated fats has an increased BPH risk. A high meat intake diet is said to increase the risk of BPH by 25% (ClinicalKeys, 2014). A diet rich in fatty acids increases Acetyl-coA, which increases androgen synthesis. Glucose regulation is also hypothesized to influence prostate growth that is, elevated fasting plasma glucose is said to increase the risk of BPH whiles elevated insulin levels are said to be associated with increased prostate volume (Wein *et al.,* 2012).

2.2.6.4 Preventive Measures

Although advanced age has always been thought of as the primary cause of BPH, moderate to vigorous exercise reduces the risk of BPH. The level of physical activity is inversely proportional to the risk of BPH and LUTS. According to a follow-up study by Health Professionals, men who walked two to three hours weekly had 25% lower risk for BPH than those who did not (Wein *et al.,* 2012).

2.2.6.5 Diagnosis

BPH is diagnosed based on the clinical history and digital rectal examination (DRE) findings. Urinalysis is a laboratory test universally recommended for BPH. Serum prostate-specific antigen (PSA) levels can be used as a marker for prostatic diseases including BPH as well as abdominal ultrasonography.

2.2.6.5.1 Prostate Specific Antigen

Prostate Specific Antigen (PSA), a protease secreted by the prostate epithelial cells was first described in 1979. This became useful as a biomarker and a potential tool for screening and early detection of prostate cancer (PCa). However, PSA has been found to be elevated in 30% to 50% of patients with an enlarged prostate (Meelan *et al.,* 2011).

PCa being the cancer that is most frequently diagnosed in men, plays an important role in cancer-related deaths worldwide. The European Randomised Study of Screening for Prostate Cancer (ERSPC) showed that PSA-based screening can lower PCa deaths by up to 30%. However, notwithstanding the value of PSA in terms of lowering PCa deaths, its use in screening is limited due to its lack of specificity in low PSA values (Meelan *et al.,* 2011). A study carried out to assess the incidence and disease-specific deaths for PCa in

subjects with a baseline PSA < 3.0 ng/ml, which had been used by the ERSPC as an indication for biopsy since 1997 showed that a total of 915 (5.8%) PCa cases were diagnosed in 15,758 at an average age of 62.3years within 1993 and 2008. However, the risk of PCa diagnosis was said to have increased from 1.7 per 1000 life year in men with PSA levels < 1.0 ng/ml to 14.5 per 1000 life year in men with PSA levels of 2.0–2.9 ng/ml (Meelan *et al.*, 2011).

Other studies carried out to investigate the relationship between age, PSA and prostate volume indicated that prostate volume and serum PSA concentration were significantly correlated and increases with age advancement (Vesely *et al.*, 2003). Prostate volume, serum PSA and International Prostate Symptom Score (IPSS) also correlated significantly with age (Vesely *et al.*, 2003).

2.2.6.5.2 Free: total PSA ratio

The percentage measurement of the free form of PSA (free: total PSA ratio) is helpful in the assessment of the risk of prostate cancer in subjects with borderline or moderately high total PSA (4.1-10.0 ng/mL). It is also used to aid in the selection of subjects who need a follow-up prostate biopsy (Ito *et al.*, 2003). High total PSA levels with low percentages of free PSA are associated with high risks of prostate cancer (Ito *et al.*, 2003).

Free prostate specific antigen (fPSA) is high in BPH, but clinically an increased value alone is not diagnostic as a specific test for differential diagnosis of BPH. The ratio of free PSA/total PSA is a better marker and is used together with other clinical observations. Depending on the ratio, the probability is interpreted as follows:

Table 2.1: The probability of prostate cancer in relation to free and total PSA ratio.

Free PSA/total PSA	Probability of prostate cancer
0-10%	55%
10-15%	28%
15-20%	25%
>20%	10%

(AccuBind, 2013).

Studies done therefore reported that men with PSA levels of 4.1 to 10 ng/mL who are not suspected of having prostate cancer by whatever means should undergo percentage fPSA measurement and be carefully monitored at short intervals over the long-term if they have lower percentage fPSA levels (Ito *et al.*, 2003).

2.2.6.6 Treatment Options

Treatment options include watchful waiting, drug treatment, and surgical interventions (Bhavin *et al*., 2013). BPH patients often have LUTS, and need to be treated surgically or with medication. Although transurethral resection of the prostate is the most common surgical treatment for BPH worldwide, the procedure can lead to complications (e.g. bleeding, urethral stricture, incontinence) and may have limitations for people of advanced age (Kouji *et al.*, 2013).

Alpha-blockers are frequently prescribed for treatment of BPH and have a quick onset of action within 3 to 5 days. These drugs alone fail to shrink BPH volume and are often insufficient to eliminate symptoms (Kouji *et al.*, 2013). However, 5-alpha reductase inhibitors (5-ARIs), which suppress testosterone conversion into dihydrotestosterone (DHT), have greater efficacy in reducing BPH volume. Also, the combination of 5-ARIs with alpha -blockers leads to the best symptomatic response to date (Kouji *et al.*, 2013).

However, these medications cause adverse effect like impotence, muscle growth impairment, decreased libido, hypotension, fatigue, and premture ejaculation (Bhavin *et al*., 2013)

The option for an alternative treatment by patients may be as a result of the possible complication brought about by some treatment options, time and the cost involved.

2.3 Phytotherapy

Phytotherapy is a complementary and alternative medicine (CAM) and it is defined as the use of plants or plant extracts for medicinal purposes (Kim, 2012). Interest in phytotherapy is growing worldwide. Asian and western countries use it in the prevention and management of disease and improvement of general health (Kim, 2012). Plant based therapy is widely given in men with symptomatic BPH in Western Europe, where physicians prescribe herbal products in the same manner as they prescribe drugs. For example, in Italy plant based products are prescribed five times more often than alphablocking agents or finasteride (a 5-alpha reductase) and in Germany more than 90% of all medications prescribed for symptomatic BPH involve phytotherapy (Leonard *et al.*, 2000). Several studies about the efficacy of phytotherapy in urologic diseases such as BPH, erectile dysfunction (ED) and male infertility have emerged (Kim, 2012).

2.3.1 Medicinal Plants and BPH

Natural agents are commonly believed to be devoid of side effects and are thought to be efficacious as synthetic products. However, the use of medicinal plants are on the increase due to the availability and ease with which consumers can obtain them. A review study conducted on medicinal plants have revealed that some monoherbal and polyherbal products reduce BPH symtoms (Azimi *et al.*, 2012). In a review of animal studies, a monoherbal plant such as *Lepidium latifolium* has been observed to decrease prostate size

(Martinez *et al.,* 2004) as well as *Lepidium meyenii* (Gonzales *et al.,* 2007) after treating steroid induced BPH rats with the extract. However, *Echinacea purpurea* was observed to prevent the development of BPH when BPH was induced by the

administration of estradiol and testosterone in rats models (Skaudickas *et al.,* 2009).

Polyherbal plants such as *Curcuma longa* together with *Zingiber officinale* inhibited the growth of prostate with little effect on testis growth in normal immature rats as well as decreased prostatic index, serum testosterone and estradiol levels in mice. *Secale cereal* together with *Serenoa repens* decreased prostate size in androgen-induced prostate enlargement rats (Azimi *et al.,* 2012).

A review on human prostate studies using polyherbal plants including *Chimaphila umbellata, Populus tremula, Pulsatilla pratensis, Equisetum arvense Triticum aestivum* indicated a decrease in IPSS, QoL and PSA level after three months treatment when results were compared to pre-treatment parameters.

Pygeum africanum a monoherbal plant has also been observed to decrease IPSS and increase QoL in humans twelve months after treatment whereas *Secale cereal* improved symptomatic BPH and prevented the clinical progression of BPH after four years treatment comparing the results to a control group and pre-treatment parameters (Xu *et al.,* 2008). *Trifolium pretense* decreased PSA level, prostate volume, and IPSS twelve months after treatment whiles *Cucurbita pepo* decreased IPSS and improved QoL within the same period of time (Azimi *et al.*, 2012).

2.3.2 *Pygeum africanum* (African plum)

The bark of the African plum tree (*Pygeum africanum*) was used traditionally to improve genito-urinary tract symptoms. The purified bark extracts which has been used for the past 30 years in Europe has been postulated to have phytosterols, especially b-sitosterols, pentacyclic triterpenoids and esters of long-chain fatty alcohols as active components

which may suppress LUTS by reducing bladder hyperreactivity, decreasing inflammation, and protecting against abnormal prostate growth (Andro and Riffaud, 1995). Studies conducted on *Pygeum* extract with doses ranging from 75 mg to 200 mg per day for 16 weeks in duration noted an improvement in nocturia compared with placebo. Meanwhile, a dose range of between 100 mg and 400 mg per day in symptomatic BPH men, indicated a mean reduction in the IPSS between baseline and 6 months treatment. Extracts from *P. africanum* may therefore be a useful treatment option for BPH (Wilt *et al.*, 2000).

2.3.3 *Serenoa repens* (Permixon)

Serenoa repens (Permixon) is one of the available treatment options for men with BPH. The drug is the n-hexane lipidosterolic extract of the dwarf American palm and a complex mixture of various compounds. A pharmacodynamic demonstration of the effect the lipidosterolic extract of *Serenoa repens*, showed that the extract inhibits both type 1 and type 2 isoenzymes of 5 alpha-reductase and interferes with binding of dihydrotestosterone to cytosolic androgen receptors in prostate cells (Plosker and Brogden 1996). In controlled clinical trials in men with BPH, oral administration of *Serenoa repens* 160 mg twice daily for 1 to 3 months showed an improvement in dysuria, reduced the frequency of nocturia by 33 to 74% , decreased urinary frequency during the day by 11 to 43% and increased peak urinary flow rate by 26 to 50%. In a larger study, *Serenoa repens* showed a 37% efficacy as compared with a 39% efficacy of finasteride when the subjects were administered with the same dosage for six months. However, *Serenoa repens* have been indicated to improve symptom in men with BPH (Plosker and Brogden 1996).

Serenoa repens in combination with *Urtica dioica* have also been observed to decrease IPSS and increase urinary flow when BPH subjects were treated for six month and the results compared to finasteride (Sokeland, 2000; Azimi *et al.*, 2012). However, in its combination with *Cucurbita pepo,* urinary flow was improved three months after treatment whiles IPSS and mean residual urine volume dropped after six months.

2.3.4 Mechanism of Phytotherapy Action

It has been proposed that the liposterolic extract of plants has antiandrogenic effects, inhibits the type 1 and type 2 isoenzymes of 5-alpha-reductase, inhibits prolactin and growth factor-induced cell proliferation, and has antiestrogenic and anti-inflammatory effects (Lowe and Fagelman 1999). This has been found to be true for *Serenoa repens* (Saw Palmetto Berry Extract) (Carraro *et al.,* 1996).

2.4 CROTON MEMBRANACEUS

2.4.1 The Plant

Croton membranaceus, a plant of the Family Euphorbiaceae, is an emerging plant of interest from the medicinal point of view. The herb comprises of about 1,200 species, widespread in tropical regions of the world (Abbiw *et al*., 2002). It is mostly found in the Krobo-Gyakiti area near the Volta River and the Krobos literally refer to it as the "Bokum". In Nigeria, the plant is found near Wuru in the region of the confluence of the Niger and Benue rivers (Abbiw *et al*., 2002; Appiah *et al.,* 2013).

The plant grows to a height between 1 and 2 m above the ground in West Africa with slender and stellate-pubescent branches. The leaves are ovate and acutely acuminate.

The leaves grow to a size of between 2 cm and 8 cm long and 1 cm to about 5 cm wide (Burkill, 1986; Appiah *et al.,* 2013) and have entire margins covered with stellate hairs on

both surfaces. This plant bears only a few and very small monoecious flowers on 5-6 cm long racemes of which the male flowers are borne on the upper part while the female flowers are found at the lower part of the raceme. Their petals may by rudimentary or completely absent. The fruit is an ellipsoid capsule. The plant bears a characteristic pleasant odour in all parts of the plant including the roots. This fragrance may be useful for the purpose of identifying the plant in the event of doubt (Appiah *et al.*, 2013).

Fig. 2.3: A *Croton membranaceus* Müll. Arg image showing the stem, leaves, flower and fruits of the medicinal plant under observation. Source: Schmelzer, 2007.

2.4.2 Chemical Compounds

The root extract is said to contain an alkaloid, a coumarin, diterpenoids and phytosterols (Aboagye *et al.*, 2000; Maja *et al.*, 2005; Bayor *et al.*, 2008; Appiah *et al.*, 2013). Six compounds isolated from the active ethyl acetate fraction of *Cm* extract, include a new furano-clerodane diterpenoid, *crotomembranafuran*, in addition to the known glutarimide alkaloid (julocrotine, sitosterol, sitosterol-3- d-glucoside) labdane diterpenoid, gomojoside H and dl-threitol. The root bark contains scopoletin and julocrotine, as glutarimide alkaloids. It also contains calcium oxalate crystals (Asare *et al.*, 2011; Bayor *et al.*, 2008). Furthermore, N[N-(2-methylbutanoyl) glutaminoyl]-2phenylethylamine has

recently been isolated (Sarkodie *et al.,* 2014). The crude ethanol extract of *C. membranaceus* and julocrotine, are speculated to possess 5-□-reductase inhibitory activity (Appiah *et al.,* 2013).

2.4.3 Uses

C. membranaceus is one of the herbal plants that are locally used in Ghana by herbal medicine practitioners for the treatment of BPH. Its aqueous root extract is currently employed, in varying dosage forms, for the treatment and management of PCa and other related tumours as well as the treatment of measles (Bayor *et al.,* 2009). Furthermore, a study done to investigate the acute toxicity of *C. membranaceus* in Sprague–Dawley rats revealed that its ingestion does not produce general acute toxicity (Asare *et al.,* 2011).

In a study conducted to investigate the antimicrobial activity of *Cm* the methanolic root extract and compounds isolated *crotomembranafuran*, gomojoside H and julocrotine (Aboagye *et al.,* 2000) were shown to have an antimicrobial activity against the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, the fungi *Aspergillus niger* and *Candida albicans* (Bayor *et al.,* 2009). The methanolic extract showed a significant antibacterial and antifungal activity against the test organisms used with minimum inhibitory concentrations (Bayor *et al.,* 2009).

5-□-reductase inhibitory activity is the mechanism for the application of finasteride for the treatment of BPH. BPH treatment with the ethanolic root extract alongside finesteride, demonstrating similar efficacy as finesteride has been reported in an animal model of BPH (Afriyie *et al.,* 2014). Furthermore, the antiproliferative activity of *C. memebranaceus* on BPH-1 cells has been demonstrated (Afriyie *et al.,* 2014). Whether

C. membranaceus can treat prostate cancer is uncertain. However, the cytotoxic activity of the methanolic extract of *CM* roots has been shown against DLD-1 and MCF-7 cancer cell lines. Furthermore, *Cm*'s cyto and genotoxicity have been demonstrated using the rat bone marrow micronucleus assay (Asare *et al*., 2015a). Additionally, using human prostate cancer PC-3 cells, furano-clerodanec exhibited cytotoxicity at an IC_{50} of > 5 µg/mL (Bayor *et al.*, 2007).

2.4.4 Target organ of *CMERE*

A study aimed at investigating organs that the ethanolic root extracts of *Cm* target, was performed on experimental male Sprague-Dawley rats by feeding the animals with varying concentrations of (30-300) mg *CMERE* alongside a control group. It was observed that, among the major organs histologically examined; only the prostate was abnormal, showing thickening and infoldings of the epithelial cells and shrinkage in a dose dependent manner. It was therefore concluded that *Cm* targets the prostate with significant PSA reduction (Afriyie *et al.*, 2013).

2.5 GSTM1 AND GSTT1 GENES

Glutathione S-transferases (GSTs, EC 2.5.1.18) are phase II xenobiotic metabolizing enzymes, protecting the body from oxidative damage by conjugating glutathione with various electrophilic compounds generated after activation by phase I enzymes (Ntais *et al.,* 2005). Mammalian cytosolic GSTs form a superfamily of genes consisting of four distinct families, namely alpha, mu, pi and theta (Takahiko *et al*., 1996).

GSTM1 and GSTT1belong to the theta class gene family which are said to be isoenzymes of GST. GSTM1 has been reported to be absent in 35-60% of individuals whiles GSTT1

is said to be absent in 10-65% of human populations (Takahiko *et al*., 1996). The deletion of these genes lead to complete lack of their enzyme activity. The absence of these enzymes (null genotype) may be due to homozygous deletions which has been implicated in poor elimination of carcinogenic substances including constituents of tobacco the potential sources of reactive oxygen species (ROS) in the body. Individuals with these deletions are susceptible to oxidative injury (Rebbeck, 1997). GSTM1 is involved in the metabolism of a possible carcinogen, styrene oxide which is an intermediate of styrene synthesis and it also catalyses the metabolism of carcinogenic benzo-α-pyrene-7,8-diol oxide, found in tobacco smoke. On the other hand, the GSTT1 enzyme is reported to be involved in the metabolism of dichloromethane and ethylene oxide a potential carcinogenic substance (Kumar *et al*., 2011).

Studies have shown that, there is a positive association between GSTM1 or GSTT1 polymorphism with high risk of BPH and prostate cancer (Srivastava *et al.,* 2005; Agalliu *et al*., 2006; Kumar *et al*., 2011). A historical case-control study indicates that 58% of cases were both GSTT1 and GSTM1 positive compared to 61% of controls. 23% of cases were GSTT1(-)/GSTM1(+) compared to 26% controls. However, 10% of cases were observed to have a null genotype compared to 6% of controls (Asare *et al.,* 2015b unpublished data).

CHAPTER THREE

MATERIALS AND METHODS

3.1 CHEMICALS, REAGENTS AND EQUIPMENT

The sources and /or manufactures of chemicals, reagents and equipment used for the study are listed in Appendix I.

3.2 STUDY DESIGN

Three main research tools were employed. The first was the International Prostate Symptom Score (IPSS) questionnaire and the International Index of Erectile Function (IIEF) questionnaire. The second research tool was blood sampling for biochemical and molecular tests and the last, abdominopelvic ultrasonography. These tools were employed before and after 90 days of the herbal treatment.

3.2.1 IPSS Questionnaire

The IPSS is structured on the answers to seven (7) questions concerning urinary symptoms and one (1) question concerning quality of life (QoL). Each question concerning symptoms in passing urine allowed the patient to choose 1 out of 6 answers which represents increasing severity of a particular symptom. The answers were assigned points from 0 to 5, with 5 signifying worsening symptoms. The total score therefore ranged from 0 to 35 (asymptomatic to very symptomatic). The symptoms were graded as follows: incomplete emptying, frequency, intermittency, urgency, weak stream, straining and nocturia. The eighth question dealt with the patient's perceived

QoL and was scored 0-6, which were graded as: delighted, pleased, mostly satisfied, mixed-about equally satisfied and dissatisfied, mostly dissatisfied, unhappy and terrible in the increasing order of severity, indicating poor QoL (Appendix II).

3.2.2 IIEF Questionnaire

IIEF was based on the effects of erectile challenges on one's reproductive health life over the past 4 weeks. The 15-point questionnaire categorized the answers into five domains as follows: erectile function, orgasmic function, sexual desire, intercourse satisfaction and

overall satisfaction. Clinical interpretation ranged from the lowest score (no dysfunction) to the highest (severe dysfunction) and vice versa depending on the type of domain (Appendix II).

3.3 SUBJECTS

Patients presenting with BPH attending the Urology clinic of the Ghana Police Hospital, an approved centre by the Ministry of Health to administer medicinal plants were used. Patients opting for the use of *Cm* packaged as URO 500 (60 mg per day) who were willing to be part of the study were recruited. A convenient sampling technique was used to recruit the subjects.

3.3.1 Inclusion criteria

Newly diagnosed cases of BPH. Cases of individuals who had not started any orthodox or phytotherapy and cases of individuals who willingly opted for CAM (phytotherapy), un-coerced, and without induced consent were selected. Patients between fourty (40) and ninety (90) years old were selected. The subjects were sampled before and after treatment with *Cm* for three months.

3.3.2 Exclusion criteria

Patients without BPH, individuals with BPH who have already started treatment and cases of individuals with BPH who opted for orthodox treatment were excluded.

3.4 ETHICAL ISSUES

The study was approved by the Protocol and Ethics Review Committee of the School of Biomedical and Allied Health Sciences (SBAHS), UG, Korle-bu with ethics number

SAHS-ET/SAHS/PSM/ML/09/AA/26A/2012-2013 (Appendix II). The consent of patients (Appendix II) were sought before their samples were taken for the various assay.

3.5 BLOOD SAMPLE COLLECTION AND STORAGE

Five millilitres (5 ml) of venous blood samples were collected from the antecubital vein of each individual subject after an overnight fast. Two millilitres (2ml) of the blood was transferred into an EDTA tube and three millilitres (3ml) into a gel separator tube for DNA extraction and biochemical assays respectively. The blood samples were then safely transported to the laboratory, separated for buffy coat and serum and subsequently stored at -80^{O}C.

3.6 LABORATORY PROCEDURES

The following routine biochemical assays were performed on the blood samples; renal function test (RFT), liver function test (LFT), lipid profile [total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL), apolipoprotein A and B] using the Vitros 5,1 FS Chemistry System autoanalyzer (Rochchester, New York). LDL was calculated. The total and free PSA levels were however performed using an ELISA kits.

3.6.1 Biochemical Analysis

Liver function test, lipid profile, blood urea electrolytes and creatinine were analyzed using the VITROS system auto analyzer (version 5,1FS Rochchester New Yorks). The VITROS system analyzer uses a multilayered analytical element coated on a polyester support (spreading layer, reagent layer and support layer). The Apo (A and B) proteins were analyzed using an A25 Biosystems autoanalyzer.

3.6.1.1 Measurement of total cholesterol (T. CHOL)

3.6.1.1.1 Principle

The VITROS Cholesterol (CHOL) slide method was performed using the VITROS CHOL slides and Calibrator kits on a VITROS Chemistry System. The method used was based on an enzymatic method proposed by Allain *et al.* (1974).

Six microliter (6 µl) sample was deposited on the slide and eventually distributed by the spreading layer to the underlying layers. The Triton X-100 (TX 100) surfactant in the spreading layer aids in dissociating the cholesterol and cholesterol esters from lipoprotein complexes present in the sample. Hydrolysis of the cholesterol esters to cholesterol was catalyzed by cholesterol ester hydrolase. Free cholesterol was then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidized a leuco dye in the presence of peroxidase to form a colored dye. The incubation time for the entire reaction was 5 minutes at 37ºC. The intensity of the dye formed was proportional to the cholesterol concentration present in the sample and was measured by reflectance spectrophotometry at a wavelength of 540nm.

The reaction sequence was as follows:

1. $\text{lipoprotein} \xrightarrow{TX-100} \text{cholesterol} + \text{cholesterol ester} + \text{proteins}$
2. $\text{cholesterol ester} + H_2O \xrightarrow{\text{cholesterol ester hydrolase}} \text{cholesterol} + \text{fatty acids}$
3. $\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$
4. $H_2O_2 + \text{leuco dye} \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$

3.6.1.2. Measurement of triglyceride (TRIG)

3.6.1.2.1. Principle

The VITROS Triglyceride (TRIG) slide method was performed using the VITROS TRIG slides and Calibrator kit on VITROS Chemistry System. The method used in this study was based on the enzymatic method (Spayd *et al.*, 1978). Six microliter (6 µl) of patient sample was deposited on the slide and evenly distributed by the spreading layer to the underlying layers. The Triton X-100 surfactant in the spreading layer aids in dissociating the triglycerides from lipoprotein complexes present in the sample. The triglyceride molecules were then hydrolyzed by lipase to yield glycerol and fatty acids. Glycerol diffused to the reagent layer, where it was phosphorylated by glycerol kinase in the presence of adenosine triphosphate (ATP). In the presence of L-α-glycerol-phosphate oxidase, L-α-glycerophosphate was then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The final reaction involved the oxidation of a leuco dye by hydrogen peroxide, catalyzed by peroxidase, to produce a dye. The incubation time for the entire reaction was 5 minutes at 37^{o}C.The intensity of the dye formed was proportional to the triglyceride concentration present in the sample and was measured at a wavelength of 540 nm by reflectance spectrophotometry.

The reaction sequence is as follows:

1. $\text{lipoprotein} \xrightarrow{\text{surfactant}} \text{triglycerides} + \text{proteins}$
2. $\text{triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids}$
3. $\text{glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} L-\alpha-\text{glycerophosphate} + \text{ADP}$

   $L-\alpha-\text{glycerophosphate} + O_2 \xrightarrow{\text{oxidase}} \text{dihydroxyacetone phosphate}$

4. +

$2H_2O_2$

5. $H_2O_2 +$ leuco dye $\xrightarrow{\text{peroxidase}}$ dye $+ 2H_2O$

3.6.1.3 Measurement of high density lipoprotein (HDL)

3.6.1.3.1 Principle

The VITROS HDL cholesterol slide method was performed using the VITROS HDL cholesterol slides and Calibrator kit on VITROS Chemistry System. The method was based on a non-HDL cholesterol precipitation method followed by an enzymatic detection (Allain *et al.,* 1974).

Ten microliter (10 µl) of sample was deposited on the slide and was eventually distributed by the spreading layer to the underlying layers. HDL cholesterol was separated by the precipitation of non-high density lipoproteins (non-HDL) using phosphotungstic acid (PTA) and magnesium chloride ($MgCl_2$) in the spreading layer. The Emulgen B-66 surfactant in the spreading layer aids in the selective dissociation of the cholesterol and cholesterol esters from the HDL cholesterol complexes present in the sample. Hydrolyses of the HDL-derived cholesterol ester to cholesterol was catalyzed by a selective cholesterol ester hydrolase. Free cholesterol was then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidized a leuco dye in the presence of peroxidase to generate a coloured dye. The incubation time for the entire reaction was 5 minutes at 37ºC. The intensity of dye formed was proportional to the HDL cholesterol concentration present in the sample and was measured at a wavelength of 670 nm by reflectance spectrophotometry.

The reaction sequence was as follows:

1. $\text{HDL} - \text{C} + \text{non} - \text{HDL} - \text{C} \xrightarrow{\text{PTA / MgCl2nt}} \text{high density lipoproteins} + \text{non} - \text{HDL}$

2. $\text{High density lipoproteins} \xrightarrow{\text{Emulgen B}-66} \text{cholesterol} + \text{HDL Chol. esters} + \text{proteins}$

3. $\text{HDL}-\text{C esters} + \text{H}_2\text{O} \xrightarrow{\text{chol.ester hydrolase}} \text{cholesterol} + \text{fatty acids}$

4. $\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest} - 4 - \text{en} - 3 - \text{one} + \text{H}_2\text{O}_2$

5. $\text{H}_2\text{O}_2 + \text{leuco dye} \xrightarrow{\text{peroxidase}} \text{dye} + 2\text{H}_2\text{O}$

3.6.1.4 Measurement of serum electrolytes

3.6.1.4.1 Sodium (Na^+)

3.6.1.4.1.1 Principle

The VITROS Na^+ slide method was performed using the VITROS Na^+ slides and the VITROS calibrator kit on VITROS chemistry systems. This method uses direct potentiometry for measurement of sodium ions. The slide consists of two ion-selective electrodes each containing methyl monensin (an ionophore for sodium), a reference layer, and a silver and silver chloride layer coated on a polyester support. Ten microliters (10µl) of sample and 10µl of VITROS reference fluid placed on separate halves of the slide resulted in migration of both fluids towards the centre of the paper bridge. A stable liquid junction was formed that connects the reference electrode to the sample electrode. Each electrode produced an electrochemical potential in response to the activity of sodium. The sample was incubated for 2 minutes at 37°C. The potential difference between the two electrodes was proportional to the sodium concentration in the sample.

3.6.1.4.2 Potassium (K^+)

3.6.1.4.2.1 Principle

The VITROS K^+ slide method was performed using the VITROS K^+ slides and the VITROS calibrator kit on VITROS chemistry systems. This method uses direct potentiometry for measurement of ionic potassium. The slide consists of two ionselective electrodes each containing valinomycin (an ionophore for potassium), a reference layer, and a silver and silver chloride layer coated on a polyester support. Ten microliters (10 µl) of sample and 10 µl of VITROS reference fluid placed on separate halves of the slide resulted in migration of both fluids towards the centre of the paper bridge. A stable liquid junction was formed connecting the reference electrode to the sample electrode. Each electrode produced an electrical potential in response to the activity of potassium. The sample was incubated for 2 minutes at 37ºC. The potential difference between the two electrodes was proportional to the sodium concentration in the sample.

3.6.1.4.3 Measurement of blood urea nitrogen (BUN)

3.6.1.4.3.1. Principle

The VITROS BUN/UREA slide method was performed using the VITROS BUN/UREA slides and Calibrator kit on VITROS Chemistry System. Six microliter (6 µl) sample was deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Water and non-proteinaceous components then travel to the underlying reagent layer, where the urease reaction generates ammonia. The semipermeable membrane allows only ammonia to pass through to the colour forming layer, where it reacts with the indicator to form a dye. The incubation time for the entire reaction was 5 minutes at 37ºC. The reflection density of the dye was measured at a wavelength of 670 nm and was proportional to the concentration of urea in the sample.

The reaction sequence was as follows:

1. $H_2NCONH_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$
2. $NH_3 + \text{ammonia indicator} \rightarrow \text{dye}$

3.6.1.5 Measurement of creatinine

The VITROS creatinine (Cr) slide method was performed using the VITROS Cr slides and Calibrator kit on VITROS Chemistry System. Six microliters (6 µl) sample was deposited on the slide and evenly distributed by the spreading layer to the underlying layers. Creatinine diffused to the reagent layer, where it was hydrolyzed to creatine in the rate-determining step. The creatine was converted to sarcosine and urea by creatine amidinohyrolase. The sarcosine, in the presence of sarcosine oxidase, was oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involved the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product. During the initial reaction phase, endogenous creatine in the sample was oxidized. The resulting change in reflection density was measured at 2 time points. The difference in reflection density was proportional to the concentration of creatinine present in the sample. This was incubated at 37^{o}C for 5 minutes and measured at a wavelength of 670 nm.

The reaction sequence was as follows:

1. $\text{Creatinine} + H_2O \xrightarrow{\text{creatinine/ amidohydrolase}} \text{creatine}$
2. $\text{Creatine} + H_2O \xrightarrow{\text{creatitine/ amidinohydrolase}} \text{sarcosine} + \text{urea}$
3. $\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{sarcosine oxidase}} \text{glycine} + \text{formaldehyde} + H_2O_2$
4. $H_2O_2 + \text{leuco dye} \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$

3.6.1.6 Measurement of serum Aspartate Aminotransferase (AST)

3.6.1.6.1 Principle

The VITROS AST slide method was performed using the VITROS AST slides and the VITROS calibrator on VITROS chemistry systems. Seven microliters (7 µl) of sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. In the assay, the amino group of L-aspartate was transferred to αketoglutarate in the presence of pyridoxal-5-phosphate (P-5-P) to produce glutamate and oxaloacetate. The oxaloacetate formed in the deamination of the L-aspartate was converted to pyruvate and carbon dioxide by oxaloacetate decarboxylase. Pyruvate was oxidized to acetylphosphate and hydrogen peroxide by pyruvate oxidase. Finally, the peroxidase-catalyzed oxidation of a leuco dye produced a coloured dye. The rate of oxidation of the leuco dye was monitored by reflectance spectrophotometry. The rate of change in reflectance density was proportional to enzyme activity in the sample which was incubated at 37ºC for 5 minutes and measured at 670 nm.

The reaction sequence was as follows:

1. $\text{Aspartate} + \alpha - \text{ketoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{glutamate} + \text{Pyridoxal} - 5 - \text{phosphate}$
2. $\text{Oxaloacetate} \xrightarrow{\text{oxaloacetate decarboxylase}} \text{pyruvate} + CO_2$
3. $\text{Pyruvate} + \text{phosphate} + O_2 \xrightarrow{\text{pyruvate oxidase}} \text{acetylphosphate} + H_2O_2$
4. $H_2O_2 + \text{leuco dye} \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$

3.6.1.7 Measurement of Alanine Aminotransferase (ALT)

3.6.1.7.1 Principles

The VITROS ALT slide method was performed using the VITROS ALT slide and the VITROS calibrator on VITROS chemistry systems. Eleven microliters (11 µl) of sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzed the transfer of the amino group of L-alanine to α-ketoglutarate to produce pyruvate and glutarate. Lactate dehydrogenase (LDH) then catalyzed the conversion of pyruvate and NADH to lactate and NAD+. The rate of oxidation of NADH was monitored by reflectance spectrophotometry. The rate of change in reflectance density was proportional to enzyme activity. This was incubated for 5 minutes at 37^{o}C and measured at a wavelength of 340 nm.

The reaction sequence was as follows:

1. $\text{Alanine} + \alpha - \text{ketoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{glutamate} + \text{Pyridoxal} - 5 - \text{phosphate}$
2. $\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^+$

3.6.1.8 Measurement of Alkaline Phosphatase (ALP)

3.6.1.8.1 Principle

The VITROS ALP slide method was performed using the VITROS ALP slides and the VITROS calibrator on VITROS chemistry systems. Eleven microliters (11 µl) of sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the p-nitrophenyl phosphate substrate. The ALP in the sample catalyzes the hydrolysis of the p-nitrophenyl phosphate to

pnitrophenol at alkaline pH. The p-nitrophenol diffuses into the underlying layer and it was monitored by reflectance spectrophotometry. The rate of change in reflection density was converted to enzyme activity which was proportional to ALP concentration in the sample. The sample was incubated at 37°C for 5 minutes and measured at a wavelength of 400 nm.

The reaction was as follows:

$$\text{p} - \text{nitrophenyl phosphate} \xrightarrow{\text{ALKP}} \text{p} - \text{nitrophenol} + \text{glutamate} + \text{H}_3\text{PO}_4 + \text{Mg}^{2+}{}_+$$

AMP

3.6.1.9 Measurement of serum Gamma Glutamyltransferase (GGT)

3.6.1.9.1 Principle

The VITROS GGT slide method was performed using the VITROS GGT slides and the VITROS calibrator on VITROS chemistry systems. Eleven microliters (11 µl) of sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. GGT catalyzes the transfer of the □-glutamyl portion of L-□-glutamylp-nitroanilide to glycylglycine, simultaneously producing p-nitroaniline. The rate of change in reflection density was measured which was proportional to the GGT concentration in the sample. The sample was incubated at 37°C for 5 minutes and measured at a wavelength of 400 nm.

The reaction was as follows:

$$\text{L} - {}^{\gamma} - \text{glutamyl} - \text{p} - \text{nitroanilide} + \text{glycylglycine} \rightarrow \text{GGT} + \text{p} - \text{nitroaniline} + {}^{\gamma} - \text{glutamyl} + \text{glycylglycine}$$

3.6.1.10 Measurement of serum Total Bilirubin (TBIL)

3.6.1.10.1 Principle

The VITROS TBIL slide method was performed using the VITROS TBIL slides and the Calibrator kit on VITROS Chemistry System. The analysis was based on a modification of the classic diazo reaction. Ten microliters (10 µl) of sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers which provides a reflective background for measuring the diazo bilirubin product. Dyphylline is used in this method to dissociate unconjugated bilirubin from albumin. Unconjugated bilirubin, conjugated bilirubin and albumin-linked bilirubin (delta) subsequently reacts with the diazonium salt 4-(N-carboxymethylsulfonyl) benzenediazonium hexafluorophosphate to produce azobilirubin chromophores. The reaction was incubated at 37^{o}C for 5 minutes. The concentration for TBIL was determined by measuring the azobilirubin chromophorees at two wavelengths (540 nm and 460 nm). The reflectance measurement at 460 nm corrects for spectral interferences.

The reaction was as follows:

$$\text{Total bilirubin} \xrightarrow{\text{dyphylline}} \text{azobilirubinchromophores}$$

(Bu, Bc and delta) [4-(N-carboxymethylsulfonyl)-benzenediazonium hexafluorophosphate]

3.6.1.11 MeasuremSent of Bilirubin, unconjugated and conjugated (BuBc)

3.6.1.11.1 Principle

The VITROS BuBc slide method was performed using the VITROS BuBc slides and the Calibrator kit on VITROS Chemistry System. Ten microliters (10µl) of sample was deposited on the slide and was evenly distributed by the spreading layer aided by caffeine and sodium benzoate to the underlying layers. Bu dissociates from albumin and migrates with Bc through a masking layer to the reagent layer. Proteins (including delta bilirubin

and haemoglobin) and lipids are retained in the spreading layer. The masking layer blocked interfering compounds trapped in the spreading layer thereby preventing them from being measured. Bu and Bc bind to a cationic mordant to form a complex with Bu having a higher molar absorptivity than Bc. The reaction was incubated at 37^{o}C for 5 minutes.The concentrations of Bu and Bc were measured at 400 nm and 460 nm respectively.

The reaction sequence was as follows:

1. bilirubin complexes $\rightarrow$ bilirubin (Bu + Bc)
2. bilirubin + mordant $\rightarrow$ bilirubin − mordant complex

3.6.1.12 Measurement of serum albumin (ALB)

3.6.1.12.1 Principle

The VITROS Albumin (ALB) slide method was performed using the VITROS ALB slides and Calibrator kit on VITROS Chemistry System. Six micro liter (6 µl) sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. When the fluid penetrates the reagent layer, the bromocresol green (BCG) dye diffuses to the spreading layer and binds to albumin from the sample. This binding results in a shift in wavelength of the reflectance maximum of the free dye. Incubation time for the entire reaction was 3 minutes at a temperature of 37^{o}C. The amount of albumin bound dye was proportional to the concentration of albumin in the sample. The colour complex that formed was measured by reflectance spectrophotometry at a wavelength of 630 nm.

The reaction was as follows:

$$\text{albumin} + \text{bromocresol green (BCG)} \rightarrow \text{BCG} - \text{albumin complex}$$

3.6.1.13 Measurement of total protein (TP)

3.6.1.13.1 Principle

The VITROS Total Protein (TP) slide method was performed using the VITROS TP slides and Calibrator kit on VITROS Chemistry System. The method was based on the biuret reaction, which produces a violet complex when protein reacts with cupric ion (Cu^{2+}) in an alkaline medium (Kingsley, 1942). Ten microliter (10 µl) sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. When the fluid penetrates the reagent layer, the reagent diffuses up to the spreading layer and reacts with protein. The reaction between protein and copper tartrate takes place largely in the spreading layer where the protein was confined because of its high molecular weight. The entire reaction was incubated for 5 minutes at 37ºC.The amount of coloured complex formed was proportional to the amount of total protein in the sample and was measured at a wavelength of 540nm by reflectance spectrophotometry.

The reaction was as follows:

$$\text{Protein} + \text{copper tartrate} \xrightarrow{\text{LiOH}} \text{coloured complex}$$

3.6.2. PSA test

Accu-Bind Free PSA (fPSA) and total PSA (tPSA) ELISA kits were purchased from Monobind Inc. (Califonia, USA). The tests were performed according to the manufacturer's instructions. Serum samples were added alongside standards to a streptavidin- coated well and exogenously added a biotinylated highly specific

monoclonal PSA antibody. After mixing, a reaction resulted between the serum antigen and the antibodies without competition to form a soluble sandwich complex immobilized on the surface of the well. After incubation, decantation and washing, tetramethylbenzidine (TMB)/hydrogen peroxide (substrate) was added to react with the complex after which the reaction was stopped with 1N HCl solution. The final chromogen was then read at 450 nm using the laboratory system multiskan MS microplate reader. The enzyme activity was directly proportional to the serum antigen concentration. By utilizing reference standards, a curve was generated from which the antigen concentration of the samples were determined.

3.6.3 Apo A1

3.6.3.1 Principle

The turbidimetric method was used for the determination of Apo A1. Apo A1 in the presence of polyethylene glycol (PEG) buffer, anti-human Apo A1 antibody forms an immunocomplex. Fifteen microliters (15 µl) of sample was added to 1500 µl of the buffer and incubated for 3 minutes at 37^{o}C after which 500 µl of anti-human apolipoprotein A1 antibody (goat) was added, incubated for 5 minutes and measured photometrically at a wavelength of 340 nm at 37^{o}C against a reagent blank. The intensity was proportional to the Apo A1 concentration in the sample.

The reaction was as follows:

$$\text{Anti} - \text{human ApoA1 antibody} + \text{ApoA1} \quad \leftrightarrow \text{Immunocomplex (agglutination)}$$

3.6.4 Apo B

3.6.4.1 Principle

The turbidimetric method was used for the determination of Apo B. Apo B in the presence of polyethylene glycol (PEG) buffer, anti-human Apo B antibody forms an immunocomplex. Fifteen microliters (15 µl) of sample was added to 1500 µl of the buffer and incubated for 3 minutes at 37^{o}C after which 500 µl of anti-human apolipoprotein B antibody (goat) was added, incubated for 5 minutes and measured photometrically at a wavelength of 340 nm at 37^{0}C against a reagent blank. The intensity was proportional to the Apo B concentration in the sample.

The reaction was as follows:

$$\text{Anti} - \text{human ApoB antibody} + \text{ApoB} \quad \leftrightarrow \text{Immunocomplex (agglutination)}$$

3.6.5 MOLECULAR ANALYSIS

3.6.5.1 PCR Amplification

DNA was extracted from blood (buffy coat) using DNeasy Blood & Tissue Kit (Qiagen Inc,. USA). A single assay using multiplex polymerase chain reaction (PCR) was performed for gene amplification. In brief, 2.0 µl of the DNA sample was amplified in 15 µl multiplex reaction mixture containing 10 pM of each of the following GSTM1 primers (MF-5’ GAA CTC CCT GAA AAG CTA AAG C 3’ and MR - 5’ GTT GGG CTC AAA TAT ACG GTG G 3’) and GSTT1 primers (TF - 5’ TTC CTT ACT GGT CCT CAC ATC TC 3’ and TR- 5’ TCA CCG GAT CAT GGC CAG CA 3’). As an internal control, CYP1A1 gene fragment was also co-amplified (CF- 5’ GAA CTG CCA CTT CAG CTG TCT 3’ and CR- 5’ CAG CTG CAT TTG GAA GTG CTC 3’) at 0.6

µM. The master mix consisted of 1.5 mM $MgCl_2$, 200 µM dNTPs, 3 µl 5X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0) and 0.1 µl *Taq* DNA polymerase. The PCR conditions consisted of an initial denaturation at 94ºC for 5min followed by 35 cycles of 2 min at 94ºC, 1 minutes at 59ºC for annealing followed by extension for 1 min at 72ºC. A final extension for 10 minutes at 72ºC terminates the process. The final PCR product from co-amplification of GSTM1 (215 bp), GSTT1 (480 bp) and CYP1A1 (312 bp) a gene that encodes cytochrome P450 enzymes in drug metabolism and cholesterol synthesis. The product was visualized after electrophoresis (Fisher Biotech electrophoresis system FB-SB-B16 USA) in an ethidium bromide-stained 2% agarose gel (Kumar, *et al.,* 2011). Data was captured using UV Bio Dock It imaging system
(Analytik Jena US/Canada).

3.6.6 PROSTATE SCAN

Patients were asked to drink approximately 1.5 L of water and allowed to wait for 1-2 hours before the scan was performed. The purpose of this preparation was to get the urinary bladder filled with urine so as to act as an acoustic window through which the prostate could better be visualized. Each patient were then positioned on the ultrasound couch. With the pelvic area exposed, a liquid gel was applied to the probe surface to improve the contact between the patient's skin and the probe surface. The prostate volume and bladder volume at full capacity were obtained.

3.6.7 STATISTICAL DATA ANALYSIS

All the data for the biochemical analysis were entered into Microsoft Excel version 2013 and transported to GraphPad Prism version 6.01 for statistical analysis. Means and standard deviations were determined under the XY data analyses option and the Column

data analyses option was used to establish the significance of the biochemical analyst before and after treatment (student t-test for paired data). The correlation test (Pearson) within column analyses option was used to establish a relationship between age, IPSS, IIEF, QoL and PSA levels. A $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC DATA

Sixty (60) patients were recruited but 30 came back for the second assessment. In all, 30 subjects clinically diagnosed BPH patients with an average age of 66 ± 11 years attending the urology clinic, Police Hospital were considered for the study.

4.2 IPSS

IPSS relates to the degree of BPH or prostate cancer symptoms experienced by the patient. Thirty-seven percent (37%) of the patients had severe prostate symptoms, 40% moderate and 23% mild symptoms before the patients were treated with *Cm* root extract. Fifty-seven (57%) and 43% of the patients had moderate and mild symptoms respectively after treatment. However, there was no patient with severe prostate symptom after treatment.

In general, 33% of the patients recruited for this study were happy about their state of health before treatment while 67% of them were unhappy. After treatment with the *Cm,* 83% were happy about their present state of health whiles 17% still remained unhappy.

4.2.1 PROSTATE SYMPTOMS SCORE

The prostate symptom score, sexual function and QoL are shown in table 4.1. Generally, the change in prostate symptom score (IPSS) and QoL were statistically significant with

p-values 0.0005 and 0.0001, respectively, whiles the individual domains of sexual function were not significant. Increased score indicates severe symptoms.

Table 4.1*:* Prostate symptom score, sexual function and quality of life of BPH patients on *Croton membranaceus* treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value
Age (yrs)	66.0 ± 11.0		
Total IPSS	15.1 ± 8.7	10.3 ± 4.7	0.0005*
Erectile Function	10.0 ± 6.0	9.7 ± 4.1	0.7513
Orgasmic Function	2.6 ± 2.5	2.9 ± 2.4	0.6203
Sexual Desire	5.9 ± 2.5	5.0 ± 2.1	0.0528
Intercourse Satisfaction	3.8 ± 3.2	4.6 ± 2.5	0.1536
Overall Satisfaction	5.2 ± 3.6	4.8 ± 2.9	0.5983
Quality of Life	3.3 ± 1.6	1.7 ± 0.9	0.0001*

*Significance at 95% Confidence interval

4.2.2 Age and IPSS correlation

A positive relationship was observed between age and IPSS both before and after treatment. Though the p-values were not significant (p=0.981 and 0.189) respectively, the scatter plots were more precise after treatment and this is shown in figure 4.1.

Increased score indicates severe symptoms.

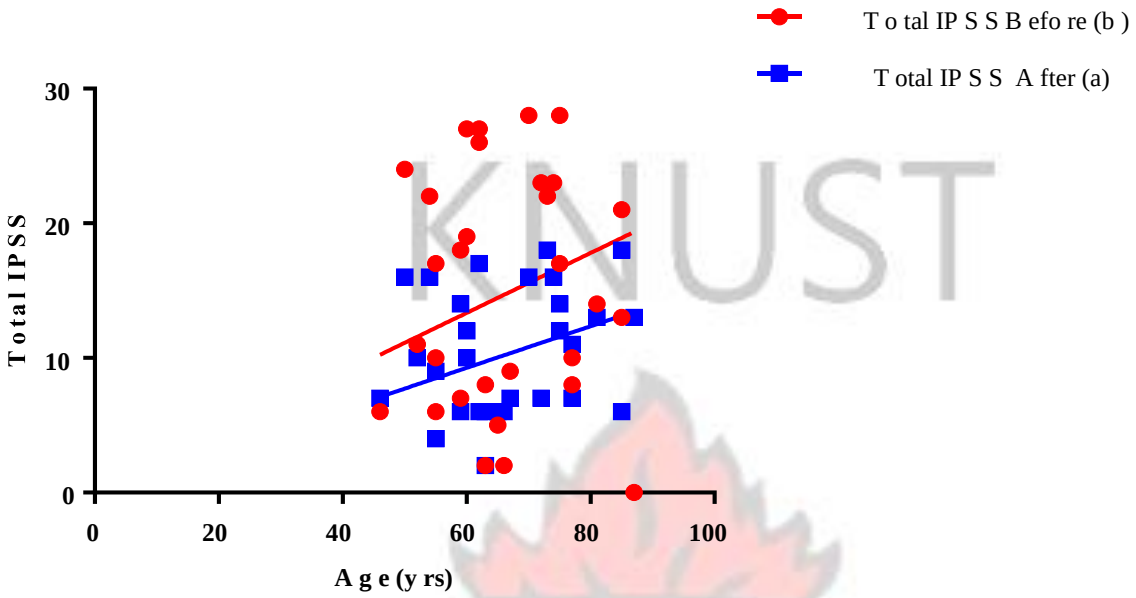
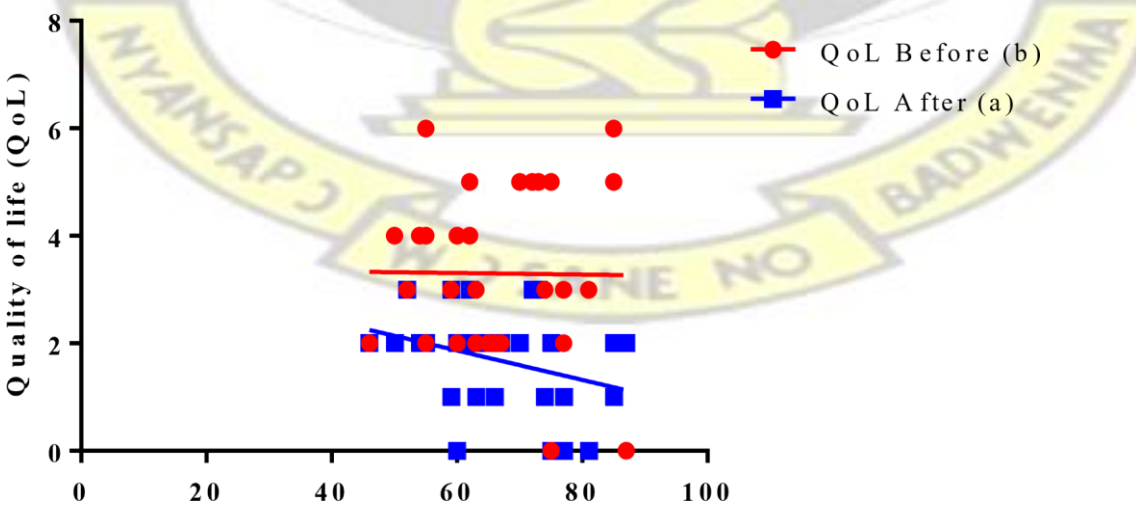


Figure 4.1 : A relation ship betw een age an d internatio nal pro state sy m p to m s sco re (IP S S) b efo re an d after treatm en t w ith C M E R E , r(b)= 0 .0 0 5 , p -v alu e (b)= 0 .9 8 1 an d r(a)= 0 .2 4 7 , p -v alu e (a)= 0 .1 8 9

4.2.3 QoL and age relationship

Figure 4.2 shows a negative relationship between age and quality of life both before and after treatment but p-values were not significant (0.956 and 0.075) respectively.



A g e (y rs)

F ig u r e 4 .2: A relationship betw een ag e and th e qu ality o f life befo re an d after treatm en t w ith C M E R E , r(b)= -0.0 1 0, p -v alu e (b)= 0 .95 6 an d r(a)= -0 .3 3 0 , p -v alu e (a)= 0 .0 7 5

4.2.4 QoL and IPSS relationship

A positive relationship was observed between quality of life and IPSS before treatment with a significant p-value (p= 0.0002). This relationship virtually disappeared after treatment with a non-significant p-value (p= 0.747). This is shown in figure 4.3 and figure 4.4 respectively.

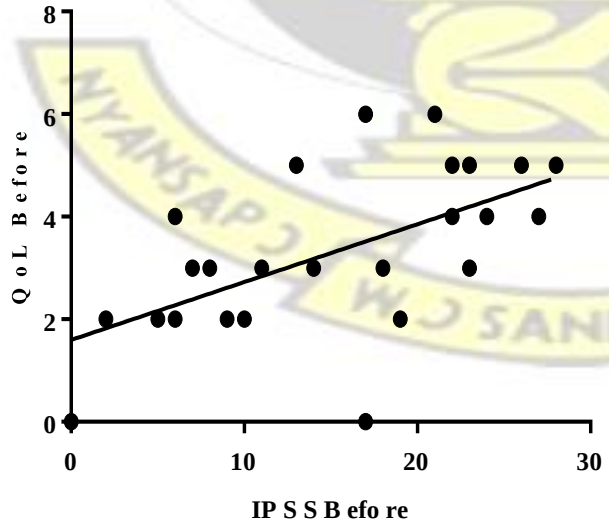


Figure 4.3: A correlation between quality of life (QoL) and IPSS before treatment with CMERE r= 0.634 and p-value 0.0002

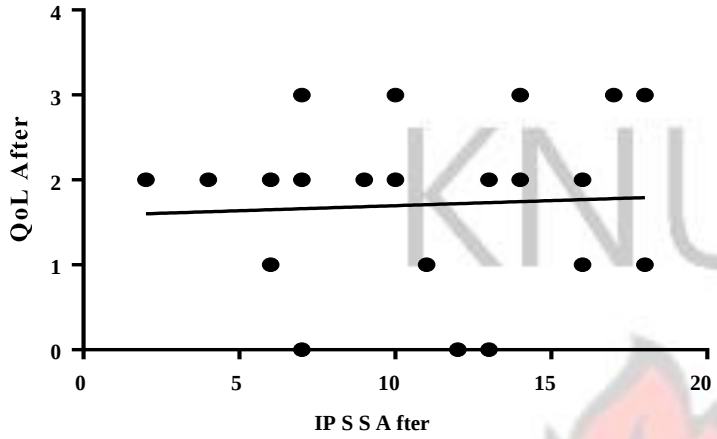


Figure 4.4: A correlation between QoL and IPSS after treatment with CMERE, r= 0.061 and p-value= 0.747

4.2.5 PSA and QoL relationship

A negative correlation was observed between total PSA and quality of life showing a significant change before treatment (p= 0.035). Though the relationship remained negative after treatment, the p-value turned to be insignificant (p= 0.219). This is shown in figure 4.5 and figure 4.6.

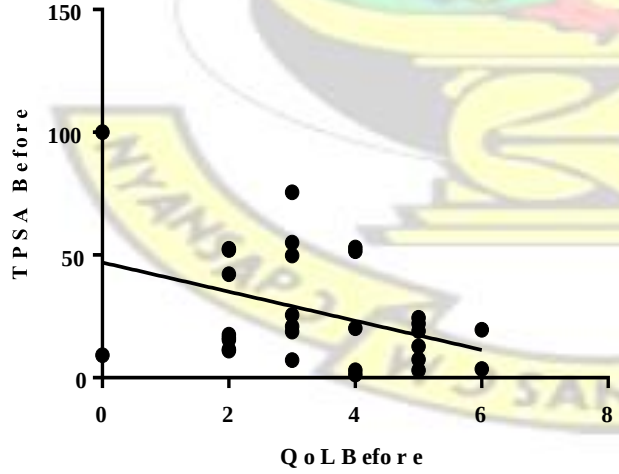


Figure 4.5: A correlation between TPSA and QoL before treatment with CMERE, r= -0.386 and p-value= 0.035

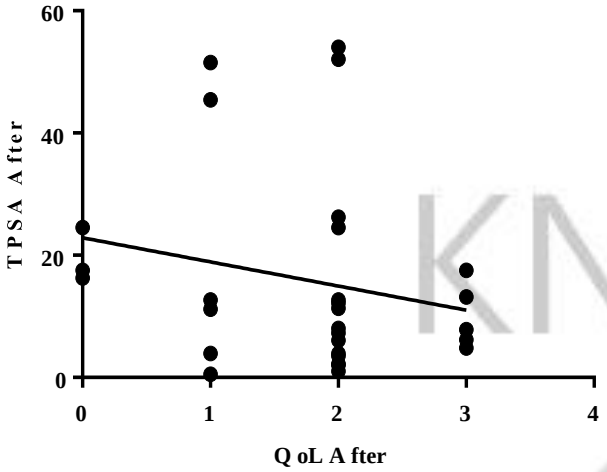


Figure 4.6: A correlation between TPSA and QoL after treatment with CMERE, r= -0.231 and p-value= 0.219

4.2.6 PSA and IPSS relationship

A negative relationship was observed between total PSA and IPSS both before and after treatment and the p-value were not significant (p= 0.640 and 0.239) respectively. This is shown in figure 4.7 and figure 4.8.

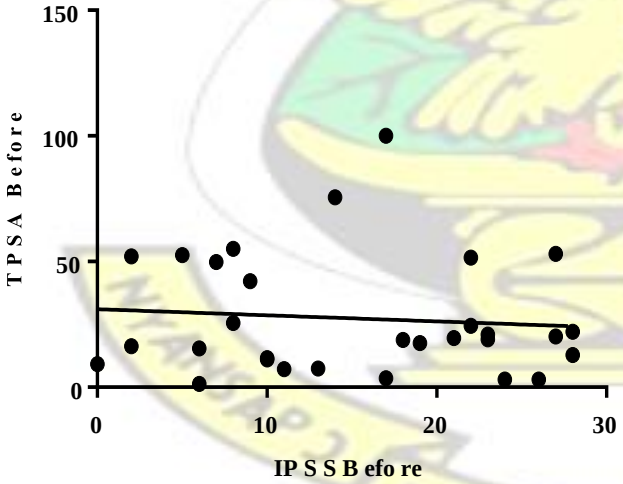
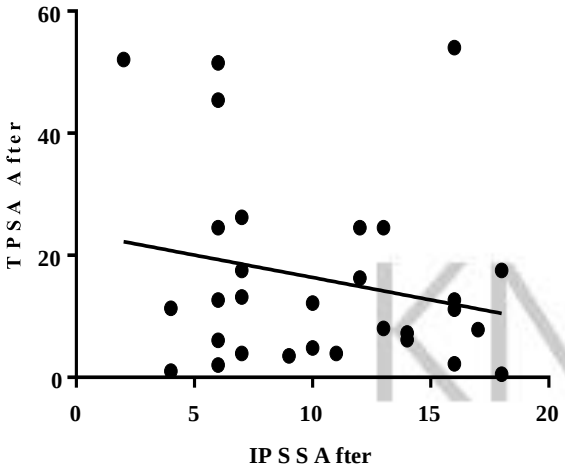


Figure 4.7: A correlation between TPSA and IPSS before treatment with CMERE, r= -0.089 and p-value= 0.640



F ig u r e 4 .8: A correlation betw een T P SA and IP S S after treatm ent w ith C M E R E , r= -221 and p-value= 0.23 9

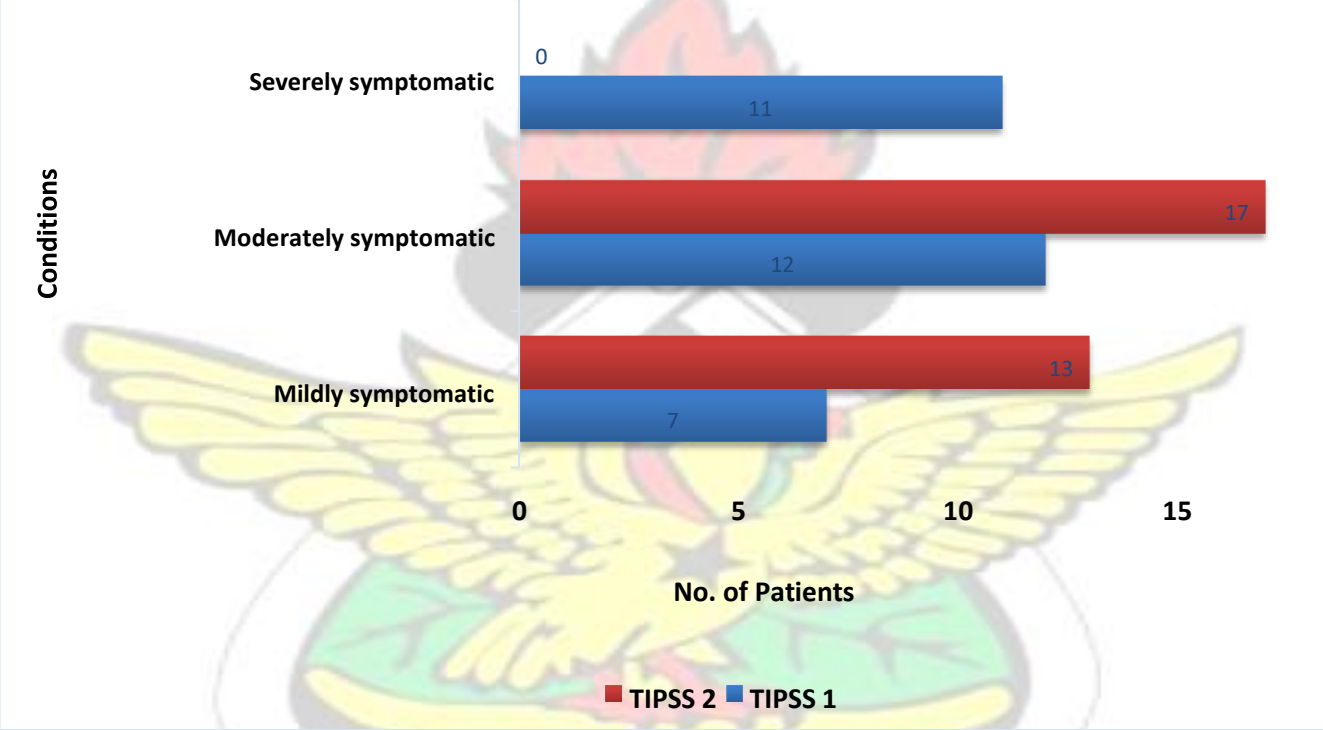
4.2.7 Urine excretion condition

Figure 4.9 shows the number of patients and their urination condition before and after treatment. It was observed that, 11 patients had severe symptoms, 12 had moderate and 7 had mild symptom before treatment. After treatment, there was no patient with severe symptoms and the moderate and mild symptom had increased to 17 and 13 respectively. Increased score indicates severe symptoms.

Figure 4.9: The prostate symptom score before and after *Cm* treatment

4.2.8 Sexual state

Figure 4.10 shows a general overview of the number of patients and their sexual function. The number of patients with severe dysfunctions in erectile, orgasmic, intercourse satisfaction and overall satisfaction decreased after treatment except for



sexual desire.

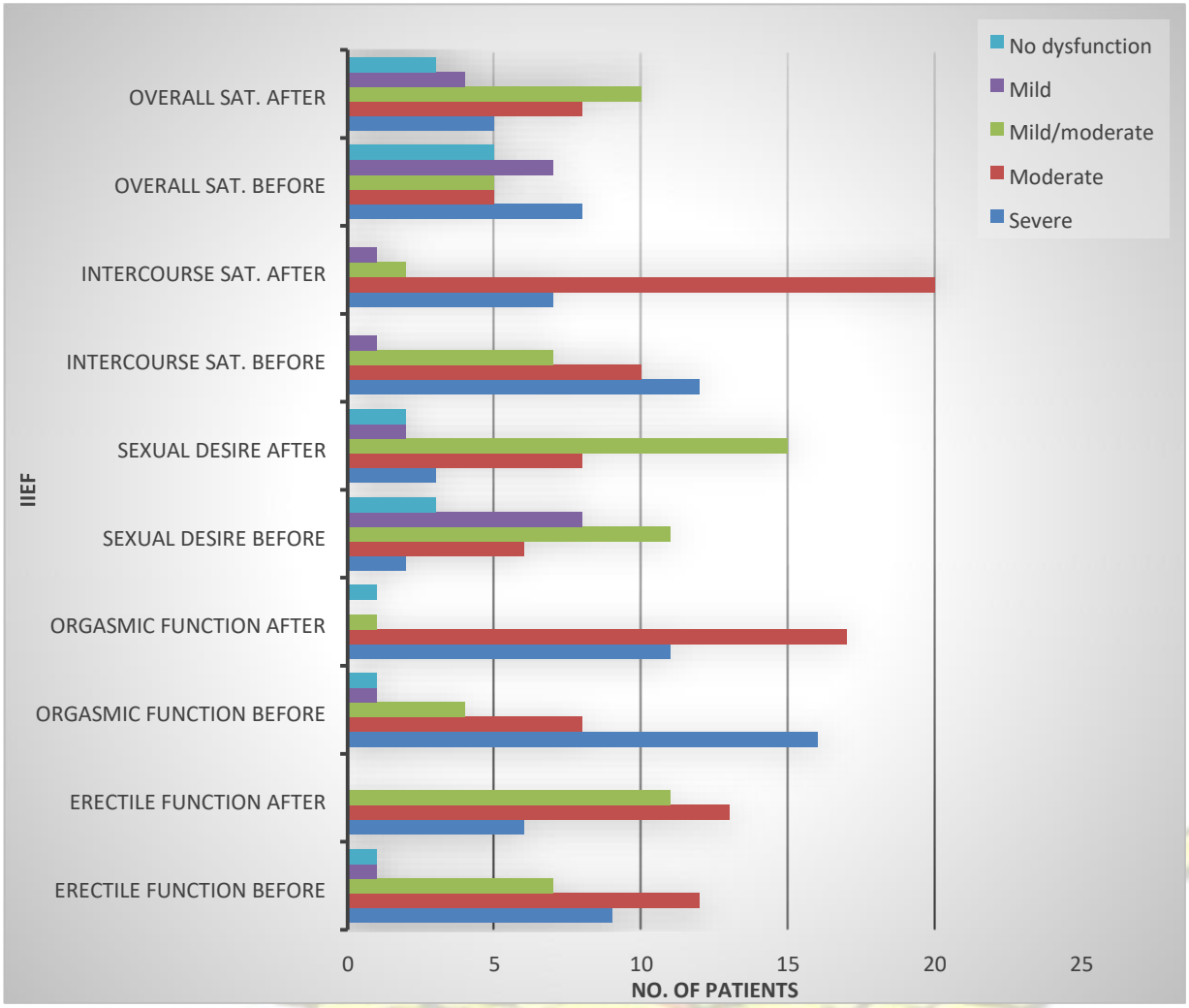


Figure 4.10: The general overview of patient's condition before and after treatment with CMERE.

4.3 IIEF

IIEF relates to the effect of erectile challenges on one's sexual life. Figure. 4.15 shows an overview of the number of patients and their sexual function. Generally, patients with severe dysfunctions in erectile, orgasmic, intercourse satisfaction and overall satisfaction dropped after treatment except for sexual desire. Those with moderate dysfuctions increased across all the five domains after treatment whiles mild to moderate dysfuction increased for erectile function, sexual desire, overall satisfaction and decreased in the case of orgasmic function and intercourse satisfaction. There were no patients with mild dysfuction for erectile and orgasmic functions after treatment but whiles the number of

patients with mild dysfuction decreased for sexual desire and overall satisfaction, that of intercourse satisfaction remained unchanged. However, there were no patients with no dysfuction for erectile function and intercourse satisfaction after treatment. Whiles patients with no dysfuction for orgasmic function remained unchanged, that of sexual desire and overall satisfaction decreased after treatment.

4.3.1 Sexual function

The severity or otherwise of the patient's sexual function is shown in table 4.2. Individual with severe dysfunctions in erectile, orgasmic, intercourse satisfaction and overall satisfaction dropped except sexual desire that increased slightly.

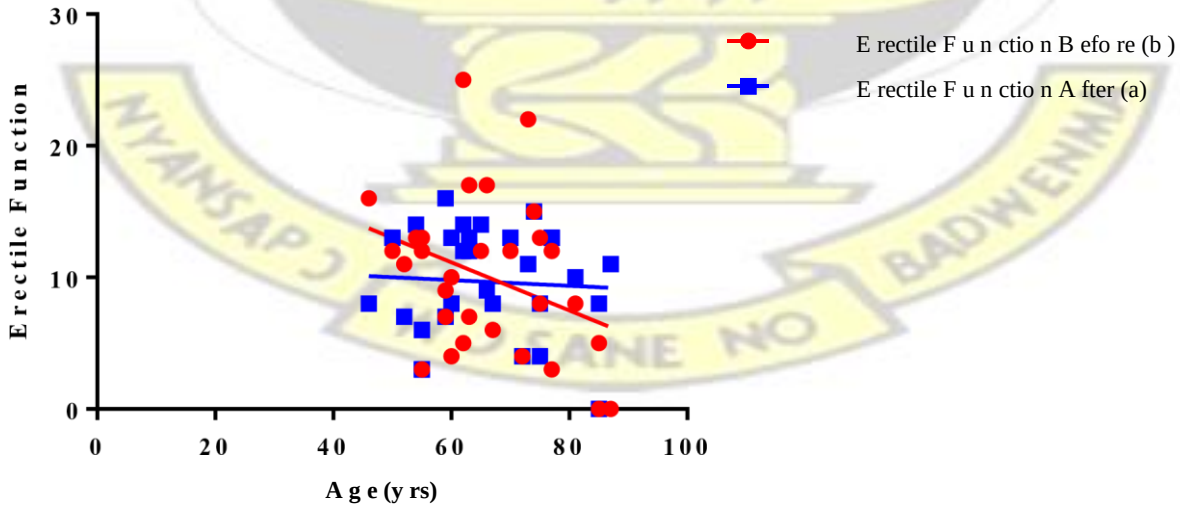
Table 4.2: Sexual function using the international index of erectile function (IIEF) before and after treatment.

Condition	Erectile function		Orgasmic function		Sexual Desire		Intercourse satisfaction		Overall satisfaction	
	Before (%)	After (%)	Before (%)	After (%)	Before (%)	After (%)	Before (%)	After (%)	Before (%)	After (%)
Severe dysfunction	30	20	54	37	6	10	40	23	27	17
Moderate dysfunction	40	43	27	57	20	27	33	67	17	27

	24	37	13	3	37	50	24	7	17	33
Mildmoderate Dysfunction										
Mild dysfunction	3	0	3	0	27	6	3	3	23	13
No dysfunction	3	0	3	3	10	7	0	0	16	10

4.3.2 Erectile function and age relationship

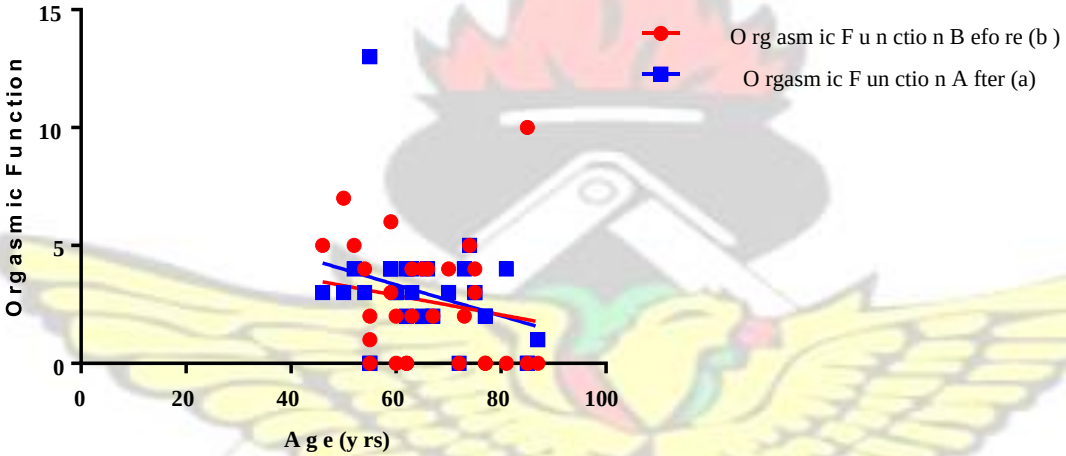
A negative relationship was observed between age and erectile function both before and after treatment. Though the p-values were not significant (p=0.067 and 0.761 respectively) the scatter plots were more precise after treatment and this is shown in figure 4.11



F ig u r e 4 .1 1: A relatio n sh ip b etw een age an d erectile fu n ctio n b efo re and after treatm en t w ith C M E R E , r(b)= -0 .33 9, p-v alue(b)= 0 .0 67 an d r(a)= -0 .0 5 8 , p -v alu e (a)= 0 .7 6 1

4.3.3 Orgasmic function and age relationship

Figure 4.12 also shows a negative relationship between age and orgasmic function both before and after treatment with insignificant p-values (p=0.344 and 0.103) respectively.



F ig u r e 4 .1 2: A relationship betw een ag e and orgasm ic functio n befo re an d after treatm en t w ith C M E R E , r(b)= -0.1 7 9, p -v alu e (b)= 0 .34 4 an d r(a)= -0 .3 0 4 , p -v alu e (a)= 0 .1 0 3

4.3.4 Sexual desire and age relationship

A negative relationship was observed between age and sexual desire before treatment but changed to positive after treatment and this is shown in figure 4.13. Although the pvalues were not significant (p= 0.408 and 0.968), the scatter plots were more precise after treatment.

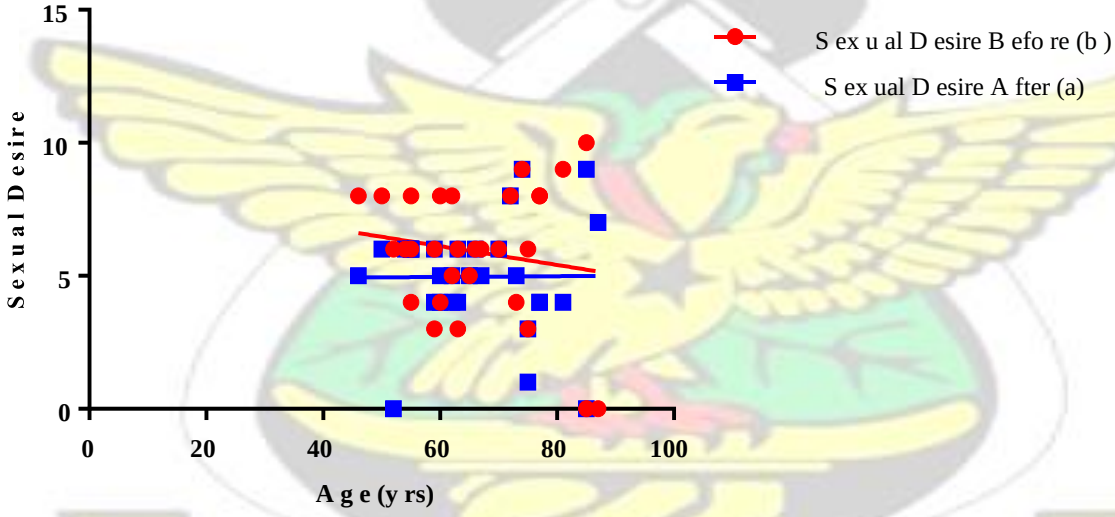


Figure 4.13: A relationship between age and sexual desire before and after treatment with CMERE, r(b)= -0.157, p-value (b)= 0.408 and r(a)= 0.008, p-value (a)= 0.968

4.3.5 Intercourse satisfaction and age relationship

Figure 4.14 shows a negative relationship between age and intercourse satisfaction both before and after treatment but the p-values were not significant (p= 0.050 and 0.278) respectively.

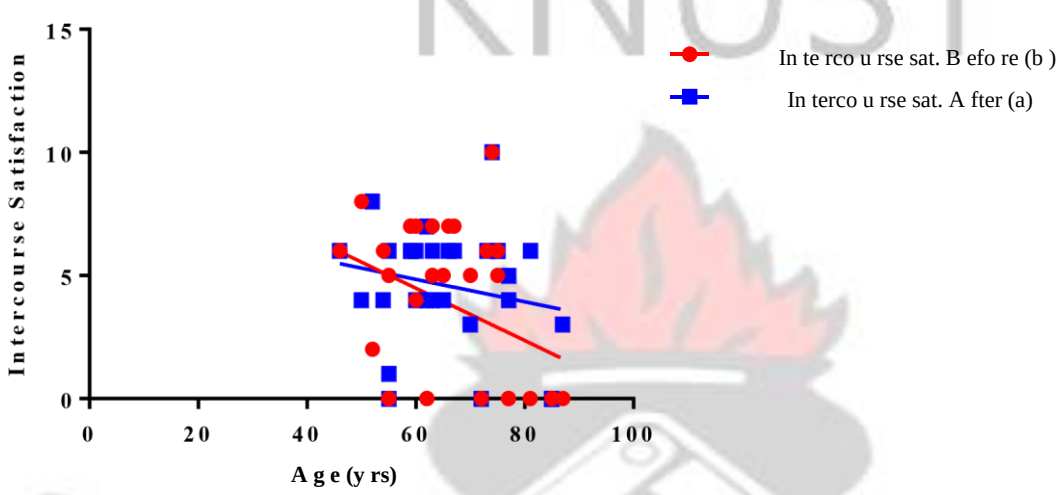


Figure 4.14: A relationship between age and intercourse satisfaction before and after treatment with CMERE, r(b)= -0.361, p-value (b)= 0.050 and r(a)= -0.205, p-value (a)= 0.278

4.3.6 Overall satisfaction and age relationship

A negative relationship was observed between age and overall satisfaction before treatment whiles a positive one occurred after treatment and this is shown in figure 4.15 with p-values (p= 0.048 and 0.901) respectively.

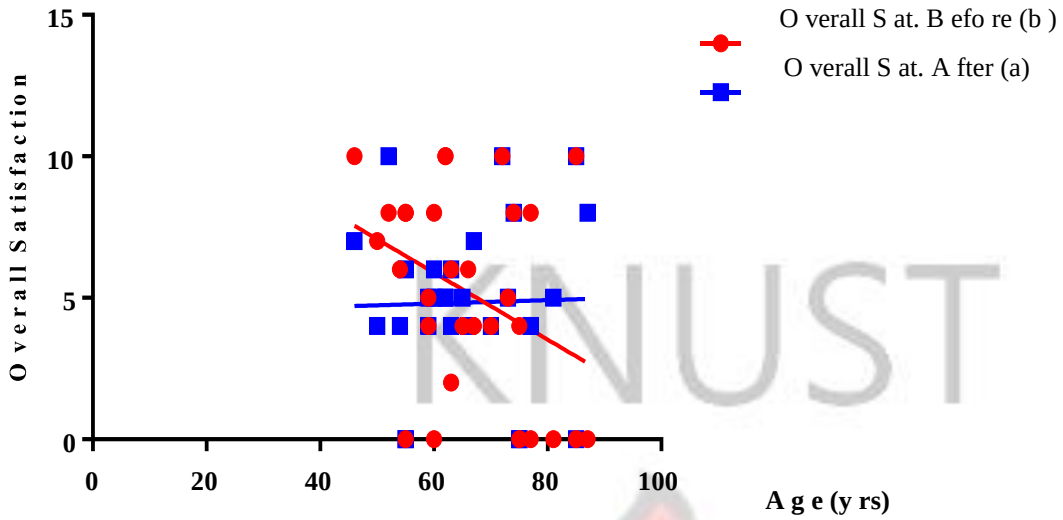


Figure 4.15: A relationship between age and the overall patient satisfaction before and after treatment with CMERE, r(b)= -0.3664, p-value (b)= 0.0484 and r(a)= 0.0241, p-value (a)= 0.9011

4.4 BIOCHEMICAL ANALYSIS

4.4.1 PSA and Prostate volume

Table 4.3 shows the serum levels of tPSA, fPSA, PSA ratio and the prostate volume before and after treatment. The change in means for tPSA, fPSA, and the prostate volume were statistically significant (p= 0.0015, 0.0448 and 0.0021) respectively. The change in PSA ratio was not significant (p= 0.8127).

Table 4.3*:* Levels of tPSA, fPSA, PSA ratio and prostate volume before and after treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value
Total PSA (ng/ml)	27.4±19.0	16.2±11.8	0.0015*
Free PSA (ng/ml)	6.1±4.8	3.9±2.9	0.0448*
PSA Ratio (%)	23.9±18.7	24.6±16.4	0.8127
Prostate Volume (ml)	102.5±48.6	61.8±41.6	0.0008*

*Significance at 95% Confidence Interval

4.4.2 Lipid Profile

The serum lipid profile is shown in table 4.4 with no statistical significance in any of the lipids after treatment.

Table 4.4*:* Serum lipid levels before and after treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value
TC mmol/l	5.03±1.12	5.19±1.15	0.4233
TG mmol/l	1.15±0.43	1.22±0.56	0.5176
HDL mmol/l	0.76±0.33	0.88±0.32	0.0628
LDL mmol/l	3.74±1.03	3.75±1.02	0.9593

*Significance at 95% Confidence Interval

4.4.3 Apo proteins

Table 4.5 shows the levels of apo proteins in serum before and after treatment. The change in serum Apo A was significant (p= 0.0248) whiles that of Apo B and Apo B/A ratio were not.

Table 4.5: Serum Apo A1 and Apo B levels before and after treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value
APO A1 (g/l)	1.31±0.45	1.51±0.47	0.0248*
APO B (g/l)	0.58±0.37	0.49±0.13	0.2221
APO B/A ratio	2.81±1.47	3.24±1.27	0.0668

*Significance at 95% Confidence Interval

4.4.4 Renal function

The change in renal function is shown in table 4.6. There were no significant changes in all the individual assays.

Table 4.6: Renal function before and after CMERE treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value

Sodium (mmol/l)	142.8±6.6	142.2±6.6	0.6718
Potassium (mmol/l)	4.3±0.6	4.3±0.6	0.6094
Urea (mmol/l)	4.3±1.4	4.4±1.3	0.6644
Creatinine (µmol/l)	105.9±26.5	106.0±26.2	0.9807

*Significance at 95% Confidence Interval

4.4.5 Liver function

Table 4.7 shows the state of liver function before and after treatment. There were no significant changes in all the individual assays but for total and indirect bilirubin (p= 0.0008 and 0.0007) respectively.

Table 4.7: Liver function before and after CMERE treatment.

Parameter	Mean ± SD (Before)	Mean ± SD (After)	P-value
AST (U/L)	17.8±4.4	18.6±5.1	0.3908
ALT (U/L)	16.9±6.5	17.9±5.7	0.4645
GGT (U/L)	34.9±15.3	35.8±19.2	0.7296
ALK PHOS (U/L)	76.6±52.3	68.8±20.6	0.4411
TP (g/l)	74.4±6.9	75.3±9.0	0.4954
ALB (g/l)	44.2±4.8	46.0±7.2	0.0915
TBIL (µmol/l)	4.9±2.6	8.3±4.7	0.0008*
DBIL (µmol/l)	2.9±2.2	3.4±2.5	0.3469
IND BIL (µmol/l)	2.0±1.8	4.8±4.5	0.0007*

*Significance at 95% Confidence Interval

4.5 MOLECULAR ANALYSIS

4.5.1 Genotypes

4.5.1.1 Genotypes and PSA levels

Table 4.8 shows the individual genotypes and their PSA levels. Subjects with GSTT1(+)/GSTM1(+) showed a significant drop in PSA whiles the others did not.

Table 4.8: PSA levels in the various genotypes and their level of significance.

Groups	Mean ± SD Before (ng/ml)	Mean ± SD After (ng/ml)	P-value
GSTT1(+)/GSTM1(+) (n=16)	25.9±20.7	18.4±17.9	*0.025
GSTT1(-)/GSTM1(+) (n=8)	16.9±14.5	8.8±4.6	0.095
GSTT1(+)/GSTM1(-) (n=4)	31.3±19.6	12.9±9.1	0.151
GSTT1(-)/GSTM1(-) (n=2)	31.6±29.5	16.4±11.5	-
All Genotypes (n=30)	23.4±18.6	16.1±15.4	*0.0005

* Significance level <0.05

4.5.1.2 Genotypes and Apo A1 levels

Table 4.9 shows Apo A levels in the various genotypes. Although there were no significant changes in the individual genotypes, all the genotypes together showed a significant increase in Apo A1 levels.

Table 4.9: Apo A1 levels in the various genotypes.

Groups	Mean ± SD Before (g/l)	Mean ± SD After (g/l)	P-value
GSTT1(+)/GSTM1(+) (n=16)	1.2±0.4	1.4±0.6	0.169
GSTT1(-)/GSTM1(+) (n=8)	1.3±0.6	1.7±0.4	0.070
GSTT1(+)/GSTM1(-) (n=4)	1.4±0.3	1.4±0.5	0.989
GSTT1(-)/GSTM1(-) (n=2)	1.1±0.0	1.5±0.0	-
All Genotypes (n=30)	1.3±0.4	1.5±0.5	*0.025

*significance level <0.05

4.5.1.3 PCR Genotyping

Figure 4.16: shows the gel picture with the marker (DNA ladder) samples and controls.

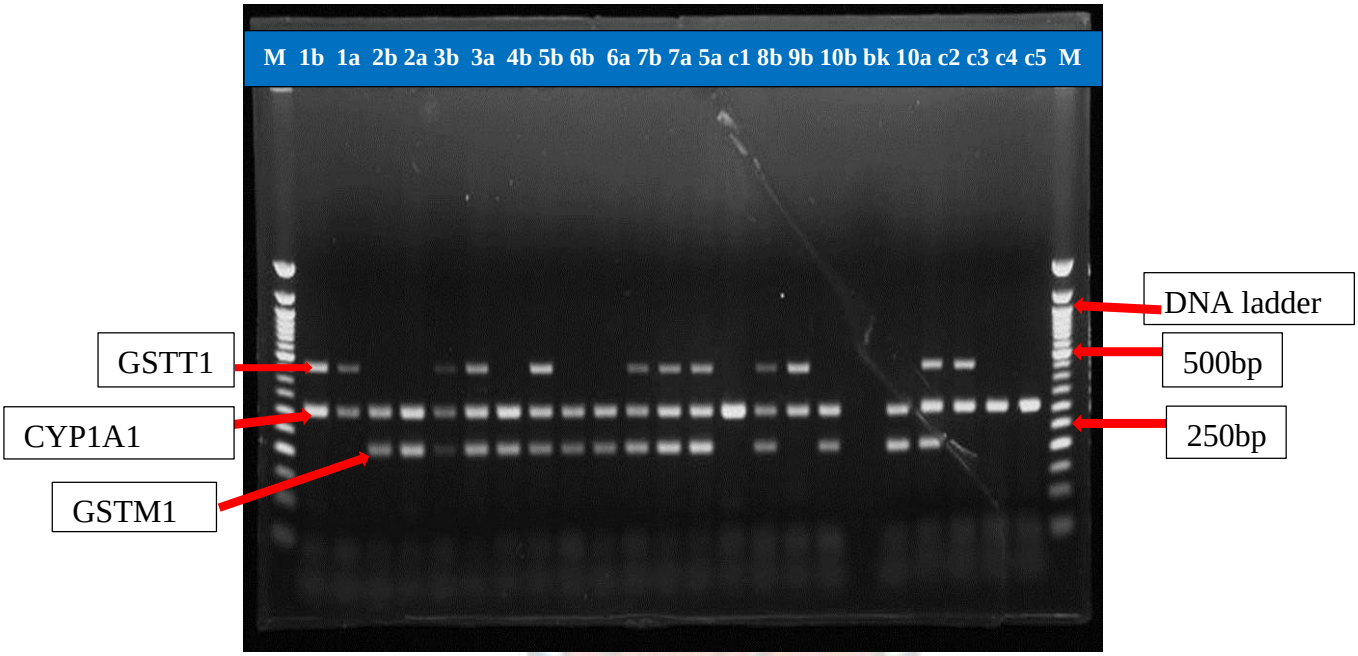


Figure 4.16: Agarose gel demonstrating multiplex PCR genotyping of genomic DNA samples for detection of GSTM1 and GSTT1 gene deletion. The absence of a 215bp band indicates the GSTM1 null genotype; the absence of a 480bp band indicates the GSTT1 null genotype; CYP1A1 was coamplified in all samples as well as five positive controls and one negative control with 50bp DNA ladder at the extrime ends. **Key:** bk is negative control, c is positive control and M is DNA ladder.

4.5.1.4 The various genotype distribution

The differences in genotype status (Figure 4.17).

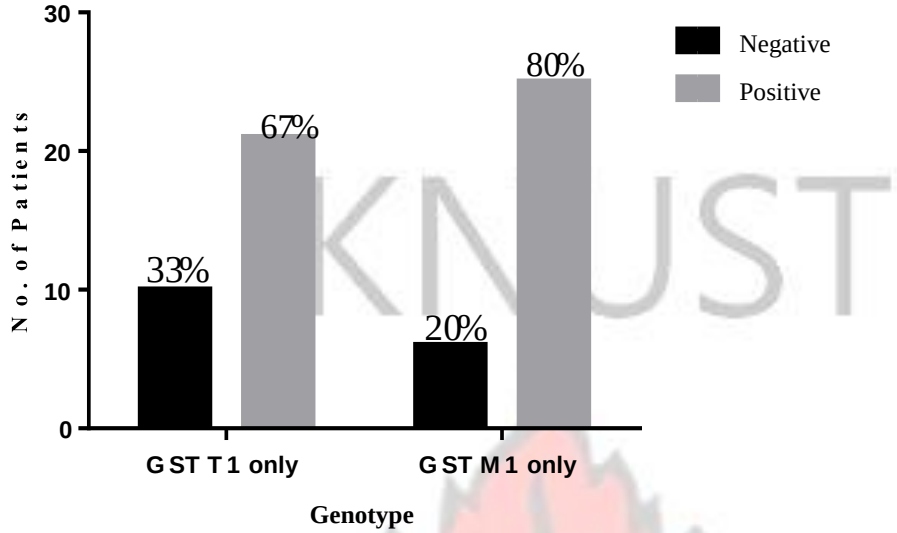


Figure 4.17: The genotypic distribution of GSTT1 and GSTM1 among the subjects.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.2 DISCUSSION

This study examined prostate symptom and erectile function using the international prostate symptom score (IPSS) and the international index of erectile function (IIEF) questionnaires to ascertain the state of the prostate before and after treatment with 60 mg daily ethanolic extract of *Croton membranaceus*. Thirty (30) diagnosed BPH patients with age ranging between 40 and 90 years (mean age 66±11 years) participated in the study. General biochemistry of the subjects on liver function, renal function, lipid profile and Apo A1 and Apo B proteins were performed to determine the effect of *Cm* on the various organs. The total and free PSA levels and the prostate volume were also determined.

Serenoa repens an alternative medicine which is also called the dwarf American palm has been reported to improved IPSS by 37% compared with 39% in finasteride when it's pharmacologic and therapeutic efficacy was reviewed in BPH (Plosker and Brogden 1996). Its combination separately with *Urtica dioica* and *Cucurbita pepo* showed a decrease in IPSS and increase in urinary flow with a reduction in residual urine volume respectively (Azimi *et al.,* 2012). In the current study however, the IPSS value improved significantly with a p-value of 0.0005 and mean values of 15.1±8.7 and 10.3±4.7 before and after treatment (Table 4.1), respectively. Urinary symptoms among the participant improved after the *Cm* treatment. Eleven (37%) of the patients were having severe urinary symptoms, twelve (40%) were with moderate symptoms and seven (23%) were also with mild symptom. After treatment, there was no patient with severe urinary symptom (0%). The number of patients with moderate symptom had increase to 17 (57%) and that of mild symptoms had also increased to 13 (43%) mean (Figure 4.9). A negative relationship was observed in QoL and age both before and after treatment with p-values showing no significance (Figure 4.2). QoL of the patients also improved significantly (p=0.0001) and mean values of 3.3±1.6 and 1.7±0.9 were obtained before and after treatment (Table 4.1).

Studies conducted to investigate the relationship between age, PSA and prostate volume, indicated that prostate volume and serum PSA concentration were significantly correlated and increase with advanced age. Prostate volume, serum PSA and IPSS were also observed to correlate significantly with age (Vesely *et al.,* 2003). In this study, positive relationship was observed between IPSS and age both before and after treatment. Although p-values were not significant in both cases, there was a change in the r-values showing improvement (Figure 4.1). On the other hand, there was a positive relationship between QoL and IPSS with p=0.0002 (Figure 4.3) which actually disappeared after

treatment p=0.747 (Figure 4.4) indicating that although QoL and IPSS may worsen, the situation improved with *CM*ERE treatment. tPSA and IPSS showed a negative relationship before and after treatment with insignificant p-values in both cases (Figure 4.7 and Figure 4.8). Although there was a negative relationship between tPSA and QoL before and after treatment, a significant p-value (0.035) which was observed before treatment, disappeared after treatment p=0.219 (Figure 4.5 and Figure 4.6) and this is indicative of improvement.

Erectile dysfunction (ED) is one of the major health concerns affecting the quality of life among males. However, alternative medicine can be beneficial for the treatment of ED. Cappra, a traditional medicine is effective for mild and mild to moderate ED in men and it showed a significant change in all the domains of ED using IIEF to assess the efficacy of the medicine (Punyawudho *et al*., 2012). Also Korean ginseng berry extract has shown a significant improvement in men with erectile dysfunction (Choi *et al.,* 2013). This study therefore did not show a significant change in the domains of ED but rather showed slight changes in the means (Table 4.1). However, there was an improvement in all the domains for patients with severe dysfunction except for sexual desire which showed a slight increase in the number of patients after treatment. Whiles the number of patients with moderate ED increased across all the domains that of mild to moderate dysfunction just increased for three of the domains and decreased for two of the domains. Patients with mild dysfunction decreased across all the domains as well as that of no dysfunction after treatment (Figure 4.10).

The relationship between the individual domains and age was negative for erectile function, orgasmic function and intercourse satisfaction both before and after treatment with insignificant p-values (Figure 4.11, Figure 4.12 and Figure 4.14), respectively.

Unlike the other domains, sexual desire showed a positive relationship after treatment but all the p-values were not statistically significant (Figure 4.13). The overall satisfaction showed slight significance with p-value 0.048 as baseline data before treatment started. This disappeared after treatment with p-value 0.901 and the negative relationship of which initially occurred changed to a positive one (Figure 4.15).

In general, it has been reported that *Cm* ingestion has no general acute toxicity (Asare *et al*., 2011). This study agrees with the said report. There were no significant difference in renal function test (Table 4.6), liver function test (Table 4.7) and the lipid profile (Table 4.3) test which were performed before and after *Cm* treatment except total and indirect bilirubin which showed a significant increase after treatment but was within the reference intervals. The significant increase may be as a result of the function of bilirubin as an antioxidant (Nag *et al*., 2009). Apo A1 which is the major protein transporter of High Density Lipoprotein (HDL) reflects the anti-atherogenic potential in

HDL particles, the higher the value the better protection of cardiovascular (CV) risk (Goran, 2012). This anti-atherogenic potential for *Cm* was previously reported (Afriyie *et al*., 2013).

However, a Significant (p=0.0248) increase in Apo A1 which is an indication of increase in HDL was also observed in this study with mean values of 1.31±0.45g/l and 1.51±0.47 g/l before and after treatment (Table 4.5) respectively. Apo B the major protein involved in the transport of Very Low Density (VLDL), Intermediate Density (IDL) and Low Density Lipoproteins (LDL) to tissues indicating atherogenic potential (the higher the level, the higher the cardiovascular (CV) risk) was observed to have decreased after treatment with a mean value of 0.49±0.13 g/l (Table 4.5). Furthermore, the apoB/apoA1 ratio (apo-ratio) indicates the balance between atherogenic and anti-atherogenic particles,

higher values shows higher CV risk (Goran, 2012). However, Apo B and the apoB/apo A1 ratio did not show any significance but the ratio showed a slight increase in the means after treatment (Table 4.5).

Animal studies using normal Sprague-Dawley (S-D) rats demonstrated shrinking of the prostate and reduction in epithelial cells upon *Cm* administration with a significant PSA ($P < 0.01$) reduction (Afriyie *et al.*, 2013). Further, BPH models (S-D rats) also demonstrated prostate volume and prostatic index reduction in *Cm* treated rats alongside a finasteride positive control (Afriyie *et al.*, 2013).

The results of this investigation agrees with this documented report. This study clearly indicated a significant decrease in PSA level by 40.87% after the patients were treated with *Cm* (p= 0.0015) with a mean of (27.4±19.0) ng/ml before the treatment which dropped to (16.2±11.8) ng/ml after treatment (Table 4.3). Furthermore, the prostate volume also decreased significantly by 39.71% (p-value 0.0008). The mean prostate volumes were (107.0±49.2) ml before and (65.9±41.8) ml after, which indicates a significant change in the prostate volume after treatment (Table 4.3).

Other studies have shown that PSA ratio is clinically useful for the detection of prostate cancers in men with total PSA between 4.1 and 10 ng/ml in conjunction with other clinical observations such as digital rectal examination (DRE) (Ito *et al*., 2003). In this study fPSA was also found to be significant with a p-value 0.0448. However, the ratio was not significant with a p-value 0.8127 (Table 3). Collectively, the free/total PSA ratio can be used as an additional marker for prognosis of hormone treatment (Kim *et al*, 2012).

The deletion of GSTM1and GSTT1 genes (isoenzymes of GST) leads to complete lack of the enzymes activity. Absence of these enzymes due to homozygous deletions have been implicated in poor elimination of carcinogenic substances in the body, making individuals with these deletions susceptible to oxidative injury. Studies have reported a positive association of GSTM1or GSTT1 polymorphism with increased risk of BPH (Kumar *et al*., 2011). A historical case-controlled study has indicated that most individuals are both GSTT1 and GSTM1 positive (Asare *et al.,* 2015a unpublished data). In this study also, thirty-three percent (33%) and 67% of the subjects were GSTT1 negative and GSTT1 positive respectively whiles 20% of the subjects were GSTM1 negative and 80% GSTM1 positive respectively showing a similar trend (Figure 4.17) as observed in the case-controlled study (Asare *et al.,* 2015b unpublished data).

Furthermore, the increase in Apo A levels was significant among the entire genotypes (p= 0.025) but did not show significance in the individual genotypes (Table 4.9). There was also a significant reduction of PSA levels in both GSTT1/GSTM1 positive subjects whiles those with GSTT1(-)/GSTM1(+) and GSTT1(+)/GSTM1(-) were not significant (Table 4.8). It is therefore possible that treatment response is dependent on the presence of these genotypes.

5.3 CONCLUSIONS

The results obtained from this study shows that *Cm* is safe and a highly desirable option for men with BPH. IPSS and QoL improved significantly. However, there was no significant change in IIEF meaning *CM*ERE has no negative effect on sexual function. Also, there was a significant reduction of PSA levels in both GSTT1 and GSTM1 positive subjects whiles those with GSTT1(-)/GSTM1(+) and GSTT1(+)/GSTM1(-) were not significant suggesting that treatment response could be influenced by genetic predisposition. Apo A1 levels increased significantly among the entire genotypes. The

therapy also resulted in a significant reduction of PSA in BPH patients as well as reducing the prostate volume significantly resulting in LUTS improvement.

5.4 LIMITATION AND RECOMMENDATION

Although a lot of subjects were recruited during the baseline study, most of them dropped out during the follow-up. Also, a placebo was not used in this study.

It is therefore recommended that the sample size should be increased in further studies for a broader view to be observed. Furthermore, the effect of *Cm* on total and indirect bilirubin must be investigated as well as the mechanism of its action.

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APPENDICES

APPENDIX I

MATERIALS

Equipments and Laboratory wares

Ultrasound system (digital colour dopper) Model SS1-6000 Serial number: 15213688, sonoscape company Ltd. China.

Chemistry system analyser, Model: vitros 5,1, FS Serial number:005c by J&J company Rochchester New York.

Plate reader: Labsystems Multiskan MS 352 serial number 35200 by Labsystems Finland..

Mastercycler ABI 2720 thermocycler Applied Biosystems UK

Sample tubes containing ethylene diethyl tetra acetic acid (EDTA) anti-coagulant and gel separator tubes.

Autovortex mixer model SA6, Stuart Scientific, United Kingdom.

Two milliliter hypodermic syringes with 21G needles

Tourniquet

Automatic pipettes of various sizes (100-1000µl variable and 10-100µl variable).

Reagents

Reagent kits for the general biochemical test were obtained from Johnson & Johnson Company limited the suppliers of the chemistry systems analyzer New York.

Total and free PSA kits: Monobind Inc. (AccuBind) ELISA Microwells, Lake Forest CA 92630 USA.

Buffy coat preparation: Whole blood was collected into an EDTA tube ,equal volumes of phosphate buffered saline (PBS) with 2% fetal bovine serum (FBS) and fresh whole blood (dilution), centrifuged at room temperature for ten minutes at 2000rpm and the concentrated leucocyte band separated from the plasma and red cells.

APPENDIX II

International prostate symptom score (IPSS)

Name: Date:

	Not at all	Less than 1	Less than half	About half the	More than half	Almost always	**Your score**
Incomplete emptying Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
Frequency Over the past month, how often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
Intermittency Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	

Urgency Over the last month, how difficult have you found it to postpone urination?	0	1	2	3	4	5	
Weak stream Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
Straining Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	

	None	1 time	2 times	3 times	4 times	5 or more times	**Your score**
Nocturia Over the past month, many times did you most typically get up to urinate from the time you went	0	1	2	3	4	5	
to bed until the time you got up in the morning?							

Total IPSS score	

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed – about equally satisfied and dissatisfied	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?	0	1	2	3	4	5	6

Total score: 0-7 mildly symptomatic; 8-19 moderately symptomatic; 20-35 severely symptomatic.

International Index of Erectile Function Questionnaire (IIEF)
Source: Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A.

IIEF: International Index of Erectile Function Questionnaire

Investigator: _____

Date of Visit:_____

Please use an X where applicable and be sure to initial and date all corrections

Score 0 if not done _____ Subject questionnaire Section 1

Instructions: These questions ask about the effects your erection problems have had on your sex life, over the past 4 weeks. Please answer the following questions as honestly and clearly as possible. In answering these questions, the following definitions apply:

Definitions:

Sexual activity includes intercourse, caressing, foreplay and masturbation

Sexual intercourse is defined as vaginal penetration of the partner (you entered the partner)

Sexual stimulation includes situations like foreplay with a partner, looking at erotic pictures, etc.

Ejaculate is defined as the ejection of semen from the penis (or the feeling of this) **Mark**

ONLY one circle per question:

1. Over the past 4 weeks, how often were you able to get an erection during sexual activity?

0 No sexual activity
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

2. Over the past 4 weeks, when you had erections with sexual stimulation, how often were your erections hard enough for penetration?

0 No sexual stimulation
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

Questions 3, 4 and 5 will ask about erections you may have had during sexual intercourse.

3. Over the past 4 weeks, when you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?

0 Did not attempt intercourse
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

4. Over the past 4 weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?

0 Did not attempt intercourse
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

5. Over the past 4 weeks, during sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?

0 Did not attempt intercourse
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time) 0 A few times (much less than half the time)
0 Almost never or never

6. Over the past 4 weeks, how many times have you attempted sexual intercourse?

0 No attempts
0 1-2 attempts
0 3-4 attempts
0 5-6 attempts
0 7-10 attempts
0 11 or more attempts

7. Over the past 4 weeks, when you attempted sexual intercourse how often was it satisfactory for you?

0 Did not attempt intercourse
0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time) 0
Almost never or never

8. Over the past 4 weeks, how much have you enjoyed sexual intercourse?

0 No intercourse
0 Very highly enjoyable
0 Highly enjoyable
0 Fairly enjoyable
0 Not very enjoyable
0 Not enjoyable

9. Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you ejaculate?

0 Did not attempt intercourse
0 Almost always or always
0 Most times (more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

10. Over the past 4 weeks, when you had sexual stimulation or intercourse how often did you have the feeling of orgasm or climax (with or without ejaculation)?

0 No sexual stimulation or intercourse

0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time)
0 Almost never or never

Questions 11 and 12 ask about sexual desire. Let's define sexual desire as a feeling that may include wanting to have a sexual experience (for example, masturbation or intercourse), thinking about having sex or feeling frustrated due to a lack of sex.

11. Over the past 4 weeks, how often have you felt sexual desire?

0 Almost always or always
0 Most times (much more than half the time)
0 Sometimes (about half the time)
0 A few times (much less than half the time) 0
Almost never or never

12. Over the past 4 weeks, how would you rate your level of sexual desire?

0 Very high
0 High
0 Moderate
0 Low
0 Very low or none at all

13. Over the past 4 weeks, how satisfied have you been with you overall sex life?

0 Very satisfied
0 Moderately satisfied
0 About equally satisfied and dissatisfied
0 Moderately dissatisfied
0 Very dissatisfied

14. Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner?

0 Very satisfied
0 Moderately satisfied
0 About equally satisfied and dissatisfied
0 Moderately dissatisfied
0 Very dissatisfied

15. Over the past 4 weeks, how do you rate your confidence that you can get and keep your **erection**?

0 Very high
0 High

0 Moderate
0 Low
0 Very low

Scoring Algorithm for IIEF

All items are scored in 5 domains as follows:

Domain	Items	Range	Score Max Score
Erectile Function	1, 2, 3, 4, 5, 15	0-5	30
Orgasmic Function	9, 10	0-5	10
Sexual Desire	11, 12	0-5	10
Intercourse Satisfaction	6, 7, 8	0-5	15
Overall Satisfaction	13, 14	0-5	10

Clinical Interpretation

I. Erectile function total scores can be interpreted as follows:

Score	Interpretation
0-6	Severe dysfunction
7-12	Moderate dysfunction
13-18	Mild to moderate dysfunction
19-24	Mild dysfunction
25-30	No dysfunction

II. Orgasmic function total scores can be interpreted as follows:

Score	Interpretation
0-2	Severe dysfunction

3-4	Moderate dysfunction
5-6	Mild to moderate dysfunction
7-8	Mild dysfunction
9-10	No dysfunction

III. Sexual desire total scores can be interpreted as follows:

Score	**Interpretation**
0-2	Severe dysfunction
3-4	Moderate dysfunction
5-6	Mild to moderate dysfunction
7-8	Mild dysfunction
9-10	No dysfunction

IV. Intercourse satisfaction total scores can be interpreted as follows:

Score	**Interpretation**
0-3	Severe dysfunction
4-6	Moderate dysfunction

7-9	Mild to moderate dysfunction
10-12	Mild dysfunction
13-15	No dysfunction

V. Overall satisfaction total scores can be interpreted as follows:

Score	**Interpretation**
0-2	Severe dysfunction
3-4	Moderate dysfunction
5-6	Mild to moderate dysfunction
7-8	Mild dysfunction
9-10	No dysfunction

Ethical Clearance

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Your Ref. No.

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RECEIVED
02 NOV 2012

24th October, 2012

Dr. George Awuku Asare,
Dept. of Med. Lab. Sci.,
SAHS.
Korle Bu.

Dear Dr. Asare,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET./SAHS/PSM/ML/09/AA/26A/2012-2013.

Following a meeting of the Ethics and Protocol Review Committee of the School of Allied Health Sciences held on Tuesday 23rd October, 2012, I write on behalf of the Committee to approve your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: **"Observational Study: The effect of the aqueous root extract of Croton membranaceus on the treatment of Benign Prostate Hyperplasia and Prostate cancer".**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

Please note that any significant modification of the research must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this research. You will therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethical identification number in all future correspondence in relation to this protocol.

Thank you.

Yours sincerely,

Dr. Samuel Opoku
(Ag. Chairman, Ethics and Protocol Review Committee)

cc Dean
Senior Assistant Registrar

CONSENT FORM

Title of Research: The effect of the aqueous root extract of *Croton membranaceus* on the treatment of Benign Prostate Hyperplasia.

Name of Candidate ……..

Address ……

Declaration

I ……. wish to state that the procedures involved in this research have been fully explained to my understanding and agree to participate in this study. I was not coerced or induced and I have freely consented to be a participant.

Signed …… Date ……

Or

Patient's thumbprint

Date ……..

Investigator's statement

I confirm that I have carefully explained the nature, demands and foreseeable risk of the proposed study to the volunteer,

Signed …… Date ……

Raw Data

Age (yrs)	Before TIPSS	E/F	O/F	S/F	I/S	O/S	QoL
46	6	16	5	8	6	10	2

50	24	12	7	8	8	7	4
52	11	11	5	6	2	8	3
54	22	13	4	6	6	6	4
55	6	3	0	6	0	0	4
55	10	12	1	8	0	8	2
55	17	13	2	4	5	8	6
59	18	9	3	3	7	5	3
59	7	7	6	6	7	4	3
60	19	4	0	4	7	0	2
60	27	10	2	8	4	8	4
62	27	25	0	8	0	10	4
62	26	5	0	5	0	10	5
63	8	7	2	3	5	2	3
63	2	17	4	6	7	6	2
65	5	12	4	5	5	4	2
66	2	17	4	6	7	6	2
67	9	6	2	6	7	4	2
70	28	12	4	6	5	4	5
72	23	4	0	8	0	10	5
73	22	22	2	4	6	5	5
74	23	15	5	9	10	8	3
75	17	8	3	3	6	0	0
75	28	13	4	6	5	4	5
77	8	3	0	8	0	0	3
77	10	12	0	8	0	8	2
81	14	8	0	9	0	0	3
85	13	5	10	10	0	10	5
85	21	0	0	0	0	0	6
87	0	0	0	0	0	0	0

AFTER

Age (yrs)	TIPSS	E/F	O/F	S/F	I/S	O/S	QoL
46	7	8	3	5	6	7	2
50	16	13	3	6	4	4	2
52	10	7	4	0	8	10	3
54	16	14	3	6	4	4	2
55	4	3	0	6	0	0	2
55	4	3	0	6	1	0	2
55	9	6	13	6	6	6	2
59	14	16	4	4	6	5	3
59	6	7	4	6	6	4	1
60	12	13	3	4	4	6	0
60	10	8	3	5	6	5	2

62	17	12	4	5	7	5	3
62	6	14	2	4	4	5	2
63	6	12	3	4	4	4	1
63	2	13	4	6	6	6	2
65	6	14	2	5	4	5	2
66	6	9	4	6	6	4	1
67	7	8	2	5	6	7	2
70	16	13	3	6	3	4	2
72	7	4	0	8	0	10	3
73	18	11	4	5	6	5	3
74	16	15	5	9	10	8	1
75	12	8	3	3	6	0	0
75	14	4	3	1	6	0	2
77	7	13	2	4	4	4	0
77	11	13	2	4	5	4	1
81	13	10	4	4	6	5	0
85	6	8	0	9	0	10	2
85	18	0	0	0	0	0	1
87	13	11	1	7	3	8	2

TPSA (ng/ml) B/4	**TPSA (After)**	**FPSA (B/4)**	**FPSA (After)**	**(FPSA/TPSA)*100 B/4**	**(FPSA/TPSA)*100 After**
15.4	3.9	1.6	1.5	10.39	38.46
3	2.2	1.2	1	40.00	45.45
7.2	4.8	1.7	1.4	23.61	29.17
51.5	54	8.2	2.1	15.92	3.89
1.3	1	1.3	1.1	0	0
11.1	11.3	2	2.1	18.02	18.58
3.5	3.5	1.2	1.4	34.29	40.00
18.8	6.2	3.1	1.1	16.49	17.74
49.8	45.4	5.1	4.1	10.24	9.03
17.5	16.3	2.1	2.2	12.00	13.50
20.2	12.2	4.2	3.1	20.79	25.41
53	7.8	9.3	1.1	17.55	14.10
3	2	1.2	1.2	40.00	60.00
55.1	51.5	9.3	8.3	16.88	16.12
52	52.1	8.1	9.3	15.58	17.85
52.5	24.5	9.3	3.2	17.71	13.06
16.3	12.7	2.3	2.2	14.11	17.32
42.2	26.2	2.9	1.6	6.87	6.11
12.8	12.7	2	2.6	15.63	20.47
19.1	13.2	3.5	4	18.32	30.30
24.4	17.5	2.7	2.1	11.07	12.00
20.9	11.2	1.9	2.1	9.09	18.75

100	24.5	3.2	8.1	3.20	33.06
22.1	7.25	52.1	24.4	0	0
25.6	17.5	4.5	3.2	17.58	18.29
11.5	3.9	9.5	2.1	82.61	53.85
75.5	24.5	1.8	1.2	2.38	4.90
7.4	6.1	9.3	7.3	125.68	119.67
19.5	0.5	8.2	9.5	0	0

Apo A (g/l) B/4	Apo A (g/l) A/F	Apo B (g/l) B/4	Apo B (g/l) A/F	Ratio B/4	Ratio A/F
1.28	1.78	0.36	0.53	3.6	3.4
0.49	0.89	0.46	0.56	1.1	1.6
1.89	1.45	0.46	0.45	4.1	3.2
0.96	1.05	2.15	0.5	0.4	2.1
1.05	0.52	1.34	0.5	0.8	1.04
1.14	1	0.76	0.82	1.5	1.2
0.96	1.64	0.45	0.37	2.1	4.4
1.09	2	0.3	0.49	3.6	4.1
1.02	1.37	0.37	0.38	2.8	3.6
1.08	1.41	0.54	0.68	2	2.1
0.07	1.09	0.44	0.47	0.2	2.3
1.63	1.58	0.53	0.29	3.1	5.4
1.05	1.77	0.6	0.6	1.8	3.0
0.9	0.83	0.42	0.43	2.1	1.9
1.04	1.44	0.47	0.45	2.2	3.2
1.12	1.51	0.21	0.34	5.3	4.4
1.07	1.08	0.57	0.47	1.9	2.3
1.67	0.93	0.28	0.39	6.0	2.4
1.68	1.81	0.42	0.35	4	5.2
1.77	2.17	0.36	0.38	4.9	5.7
1.56	1.35	0.42	0.44	3.7	3.1
2.03	2.05	0.54	0.68	3.8	3.0
1.17	2.16	0.75	0.72	1.6	3
1.65	1.3	0.8	0.47	2.1	2.8
1.62	1.85	0.45	0.35	3.6	5.3
1.78	2.25	0.36	0.41	4.9	5.5
1.56	1.35	0.44	0.46	3.5	2.9
2.05	2.07	0.52	0.68	3.9	3.0
1.25	2.36	0.75	0.72	1.7	3.3
1.65	1.38	0.82	0.52	2.0	2.7

Prostate Vol. (ml)B/4	Pros. Vol. (ml) After
104	51
94.5	76.8
30.1	24
79.8	21
148.5	87
52.4	43.03
198.4	169
150.2	61.1
61.7	24.8
105.4	60.6