EFFECT OF METHYL JASMONATEON BENIGN PROSTATIC HYPERPLASIA INWISTAR RATS

BY

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CERTIFICATION

I declare that this research work was carried out by Olubukola Oyebimpe AKANNI under my mentorship, in Molecular Drug Metabolism and Toxicological Unit, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria.

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DEDICATION

This research work is dedicated to the Almighty God, the covenant keeping God, and all my Siblings.

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ABSTRACT

Benign ProstaticHyperplasia (BpH) is a progressive age-related disease. Currentdrugs used in the management of BpH have limited efficacy and many adverse effects, necessitating a continuous search for better options. Methyl Jasmonate (MeJa), a plant stress hormone, has been shown to be an anti-neoplastic agent via several mechanisms. However, its effect on BpH is unknown. This study was designed to assess the actions of MeJa in testosterone propionate (TP)-induced BpH in castrated rats.

Castration of rats was performed by removing both testes through the scrotum sac under ketamine anesthesia. The BpH was induced by daily intraperitoneal injections of Tp at 3 mg/kg for 28 days.Forty eight Wistar rats (170 – 250g) were grouped into eight groups of six animals each as follows: non-castrated control, castrated (CAS) control, CAS + Tp, CAS + Tp + MeJa (50 mg/kg), CAS + Tp + finasteride (10 mg/kg), CAS + MeJa, CAS + finasteride, CAS + TP + MeJa + finasteride. Biochemical indices [alkaline phosphatase (ALP), acid phosphatase (ACP)and zinclevels] were determined by standard methods using a spectrophotometer. Antioxidant parameters [Superoxide Dismutase (SOD), Catalase, reduced Glutathione (GSH),Glutathione Peroxidase (GPx) and Lipid Peroxidation (LPO)]as well as inflammatory markers [Nitric Oxide (NO) and myeloperoxidase] were determined by standard methods using a spectrophotometer. Testosterone level was determined using ELISA. Lipid profile [Total Cholesterol (TC), Triglycerides and High Density Lipoprotein-Cholesterol (HDL-C)] were also determined spectrophotometrically. Prostate Specific Antigen (PSA), ki67, inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2), p53, B-cell lymphoma (Bcl-2) and Bcl-2 associated X-protein (Bax) of the prostate were determined using immunohistochemistry. Histology of the prostate was performed using haematoxylin and eosinstaining. Data were analysed using ANOVA at $\alpha_{0.05}$.

Administration of Tp significantly increased prostate weight (0.59±0.08 gvs 0.18±0.06 g) and its organo-somatic weights (0.07±0.02 vs 0.19±0.03) relative to castrated control. In BpH rats, the activities of ACPs (total, non-prostatic, prostatic) and ALP were increased by 455.1%, 766.0%, 274.4% and 522.2%, respectively. The levels of zinc and LPO increased by 19.8% and 38.6%, respectively. Treatment of BpH rats with MeJa decreased the activity of non-prostatic ACP, zinc and LPO levels by 34.2%, 32.5% and 62.1%, respectively. The levels of serum testosterone, prostatic NO and activity of myeloperoxidase were increased in BpH by 151.8%, 28.6% and 25.0%, respectively. Relative to castrated control, serum triglyceride (345.24±14.31 vs

240.74±12.12mg/dL) and TC (381.11±22.80 vs 281.76±2.30mg/dL) were increased, while HDL-C was decreased in BpH (276.87±8.62 vs 313.79±14.38mg/dL).The activities of antioxidant enzymes namely SOD (8.09±0.55 vs 12.08±0.73U/mg protein),Catalase (9.17±1.00 vs 19.57±0.94U/mg protein), GPx (1109.12±180.82 vs 1562.23±194.50U/mg protein) and GSH level (156.31±23.84 vs 249.04±23.48U/mg protein) were reduced in BpH relative to control. The MeJa improved the inflammatory, antioxidant, lipid profile and attenuated the expression of PSA, Ki67, iNOS, COX-2, Bcl-2, p53, Bax and histological architecture of the prostate in BpH.

Methyl jasmonate attenuated testosterone propionate-induced benign prostatic hyperplasia in rats via induction of apoptosis, antioxidative and anti-inflammatory mechanisms.

Keywords: Prostate, Benign hyperplasia, Methyl jasmonate, Apoptosis,Inflammation.

Word count: 500

LIST OF ABBREVIATIONS

ABA:	Abscisic acid
ACP:	Acid Phosphatase
ACX:	Acyl-CoA Oxidase
AOC:	Allene oxide cyclase
AOS:	Allene Oxide Synthase
AR:	Androgen Receptor
ARIs	Alpha reductase inhibitors
AUA:	American Urological Association
AUR:	Acute Urinary Retention
Bax:	B cell lymphoma-2-associated X Protein
Bcl-2:	B cell lymphoma-2
BPH:	Benign Prostatic Hyperplasia
CCL3:	Chemokine (C-C motif) ligand 3
CDNB:	1-Chloro- 2, 4, - dinitrobenzene
COX-2:	Cyclooxygenase
CUA:	Canadian Urological Assosciation
DGDG:	Digalactosyldiacylglycerol
DHT:	Dihydrotestosterone
DNA:	Deoxyribonucleic acid
DPA:	Diphenylamine
DTNB:	$5^{!}$ $5^{!}$-Dithiobis-(2-nitrobenzoic acid)
EMT:	Epithelial-Mesenchymal Transition
GPx:	Glutathione Peroxidase
GSH:	Reduced Glutathione
HDL-C:	High-Density Lipoprotein Cholesterol
HPOT:	13-hydroperoxy-9,11,15-octadecatrienoic acid
IAPs:	Inhibitors of Apoptosis Proteins
iNOS:	Inducible Nitric Oxide Synthase
IPSS:	International Prostate Symptom Score
JMT:	Jasmonate Carboxyl Methyl Transferase
LH:	Luteinizing Hormone
LUTS:	Lower Urinary Tract Symptoms
MAPK:	Mitogen Activated Protein Kinase

MDA:	Malondialdehyde
MeJA:	Methyl Jasmonate
MGDG:	Monogalactosyldiacylglycerol
MPO:	Myeloperoxidase
MTOP:	Medical Therapy of Prostatic Symptoms
NF-KB:	Nuclear Factor kappa B
NIK:	NF-κB-inducing kinase
NO:	Nitric Oxide
OPDA:	12-Oxo-Phytodienoic Acid
OPR:	Oxo-phytodienoic Acid Reductase
PAP:	Prostate acidicphosphatase
PCa:	Prostate Cancer
PSA:	Prostate-Specific Antigen
PTPC:	Permeability Transition Pore Complex
PUFA:	Polyunsaturated Fatty Acids
PZ:	Peripheral Zone
ROS:	Reactive Oxygen Species
SA:	Salicylic acid
TBA:	Thiobabituric acid
TCA:	Trichloroacetic acid
TGF-β:	Transforming Growth Factor-β,
TP:	Testosterone Propionate
TRAF2:	Tumor Necrosis Factor-Associated Factor 2
TUIP:	Transurethral Incisionof the prostate
TURP:	Transurethral Resection of the prostate
TUVP:	Transurethral electrovaporization of prostate
TZ:	Transitional Zone
UTI:	Urinary Tract Infection
VEGF:	Vascular Endothelial Growth Factor
XIAP:	X-chromosome-linkedIAP

CHAPTER ONE

INTRODUCTION

Benign prostatic hyperplasia (BpH) is described as the increasein the prostatic mass which usually goes with lower urinary tract symptoms. This begins in the transition and periurethral zones of the prostate and signifies an inevitable inevitable phenomenon for the ageing male population (Untergasser*et al*., 2005).Once the prostate gets enlarged, it impinges on the urethra thereby causing difficulty in urination or blockageof urine stream from the bladder. Other lower urinary tracts symptoms that result from enlarged prostate are urine retention, dysuria, nocturia and weak urinary stream (Pais, 2010). In 2010, over two hundred million males were reported to have BpH globally; however this was only discovered from postmortem studies. Roehrborn and Rosen (2008) discovered that BpH development takes place after age forty and at fifty years about fifty percent of men will display symptoms that are related to BpH. Wu *et al.*(2015) also stated that the occurrence of BpH is alike globally and about fifty percent of men around sixty years presented BpH symptoms from histological examination of prostate tissue while nearly ninety percent of men of ninety years will have the disease.

Irrespective of the great influence of benign prostatic hyperplasia on male population universally, its cause still remainsunknown. Severalmechanismshave been suggestedto be implicated in BpH development and progression. Some of those mechanisms are age, hormonal alteration, metabolic disorder, and inflammation (Liu *et al*., 2007). In order for BpH to develop, the hormone (testosterone) is usually converted into dihydrotestosterone (its active metabolite)by 5-α reductase. Carson and Rittmaster (2003) discoveredin the prostate, that higher dihydrotestosterone level influences the developmentof epithelial and stromal cell of the prostate gland, bringing about hyperplasia. Hence, chemoprotective substances that hinder such process like finasteride and dutasteride were developed to manage the condition. Though those drugs are effective, their side effects could not be overlooked. Finasteride, for example,was found to reduce sexual drive, caused erectile dysfunction, nose blocks, enlargement of man's breast and serious myopathy(Carlin *et al.,* 1997; Bullock and Andriole, 2006). Therefore, to avoid these adverse effects, there is need to explore better drug products from plants that have minute or negligible side effects. Frequently used plant-based drugsreported to be effective in controllingLUTSlinked with benign prostatic hyperplasia include Saw palmetto (*Serenoa repens f. glauca* Arecaceae), African cherry(*Pygeum africanum Rosaceae),* Nettle(*Urtica dioica*) and

Pumpkin(*Cucurbita* pepo) whereas the frequently used functional foods are lycopene, isoflavones, and β-Sitosterol.

Methyl jasmonate, a cyclopentanone lipid, was originally detected injasmine (*Jasminum grandiflorum*) oil as its secondary metabolite(Demole *et al*., 1962). Nevertheless, itsnatural role or that of its free acid was not clear untill the role of jasmonic acid in the promotion of senescence in vegetal and that of MeJa in the inhibition of growth to *Vicia faba* were detected. Currently, the best described class of oxylipins in plants is jasmonic acid and its derivatives (JAs). They are one of the main hormones that control defense and development in plant. Other members of the jasmonate family besides methyl jasmonate are jasmonic acid (JA) and *cis*-jasmonate (Sembdner and Parthier, 1993). MeJa has been shown to be an anticancer agent via several mechanisms. *Invitro*, its anticancer property is connected tothe seizure of cell cycle in some malignant cells like melanocytic MDA-MB-435, breast MCF-7, cervical carcinoma, HeLa cells and human acute lymphoblastic leukemia Molt-4. Inspite of these anticancer actions of methyl jasmonatelittle is knownaboutits chemopreventive effectsin the management of BpH.

1.1 Rationale of this study

Benign prostatic hyperplasia,a common disease of elderly men (Park *et al*., 2004), was identified for its contributiontochallenges of men and as at 2010, about two hundred million men wereknown to have BpH globally. In 2008, Roehrborn and Rosen discovered that BpH development takes place after age forty and at fifty years about fifty percent of menfolk will display symptoms that are related to BpH. Though BpH has a significant effect on the male population globally, yet, its origin and cause is still unclear. Sincedihydrotestosterone production waslinkedwith the development of BpH and also thechemoprotective substances that hinder such process are usually associated with adverse side effect,for example, finasteride was found to reduce sexual drive, causederectile dysfunction, nose blocks and enlargement of man's breast (Bullock and Andriole, 2006),therefore, the detection ofbetter drug products from plant that has minute or negligible side effects must be employed so as to bypass the adverse side effects.

The objectives of this study were therefore:

a) toexamine the effect of MeJa on:

- Prostate weight of BpH rats

- Antioxidant status of BpH rats
- Some trace elements in BpH rats
- Alkaline and acid phosphatase activities in BpH rats
- Proliferation markers in BpH rats

b) to evaluate other mechanism of action of MeJa and the possible synergistic role of finasteride and methyl jasmonate on:

- Prostate weight in BpH rats
- Inflammatory markers of BpH rats
- Markers of apoptosis in BpH rats
- Lipid profile levels of BpH rats
- Hormone levels
- Phase I xenobiotic-metabolizing enzymes in BpH rats

CHAPTER TWO

LITERATURE REVIEW

2.1 The Prostate

The prostate gland can be described as an organ of the reproductive system having the size of a walnut found at the bladder base responsible for the etiology of prostatitis, morbidity, prostate cancer (PCa) and BpH (Aaron *et al*., 2016).This organ is known to secrete an alkaline fluid (white or milky in appearance) which is approximately 30% of the semen and also contains some proteins**.** The prostatic secretion is involved in semen coagulation, gelation and liquefaction while proteins found in the prostatic secretion are implicated in the uncoating and coating of sperm and its relations with the mucus of the cervix **(**Lilja, 1985). The major secretory products found in the prostate gland of man are fibronectin, lactoferrin and seminogelin. Fibrinonectin and seminogelin have been implicated in the coagulation of semen after ejaculation **(**Lilja and Laurell, 1984).This coagulum of proteins from semen contains majorly polymerized gel and the enzyme involved in the gel formation is transglutaminase. Although the prostatic secretions are not totally necessary for fertility, yet, without the prostate fertility is compromised. Therefore, the role of prostate cannot be neglected in the reproductive success of several mammalian species.

2.2. Constituents of the Prostatic Fluid

2.2.1. Prostate Acidic Phosphatase (PAP)

Prostate acidic phosphatase is a constituent of seminal fluid produced in the prostate. PAP gene, located in chromosome 3q21q23, is known to code for a 100 kDa tyrosine and lipid phosphatase. Two kinds of PAP exist, a secreted and cellular form, with diverse biochemical properties. They are controlled by androgens and are greatly expressed in the healthy as well as diseased prostate (Li and Sharief, 1993). Since 1930, PAP has been recognized as indicator of prostate cancer (PCa) (Huggins and Hodges, 1941) and in progressive PCa, decreased cellular levels of PAP were observed. PAP is also known to dephosphorylate HER2, which in turn activates ERK/MAPK signaling. Though the molecular mechanisms that lead to reduced PAP levels in PCa are unclear, decreased levels of PAP and elevated tyrosine phosphorylation of HER2 still correlate with Gleason Score and PCa development.

2.2.2. Prostate Specific Antigens(PSA)

Prostate specific antigesn are proteins found in the seminal fluid. PSAs, 33kDa, are located on chromosome 19q13.41 andencode a single chain glycoprotein that serves as a protease. They are of the fifteen kallikrein family found in a group in the same chromosomal region. In BpH and highly differentiated PCa, elevated levels of PSA were reported but in PCa progression, decreased levels were observed (Hakalahti *et al*., 1993). The use of PSA as PCa marker arose in mid-1980s and was discovered in a European study that PSA reduced PCa death by twenty percent (Stamey *et al*., 1987).

2.2.3.Citrate

In healthy prostate, the amount of citrate found in the fluid is about 400-1500greater than the amount detected in the plasma.Although, the exact role of citrate in the semen is unknown, yet, it helps to sustain the osmotic/electrolytic equilibrium in the semen. Usually, the citrate is significant in the Kreb's cycle for the production of ATP. Nevertheless, a buildup of zinc in the PZ of the prostate impedes the activity of the enzyme (aconitase) that oxidizes citrate to isocitrate in the mitochondria. Hence, the cycle becomes shortened and the citrate is released into the prostatic fluid. Thus, the prostate cell epithelia are regarded as citrate-producing cells (Costello and Franklin, 2009).

2.2.4. Zinc

Testosterone and prolactin are hormones that stimulate the buildup of zinc in the prostate cells epithelia. But the actual model of zinc accumulation and how it is regulated in the prostate cells is not yet unraveled. In the prostatic fluid, zinc ions are essential for the thickening of the semen since they activate a conformationalchange in the semenogelin proteins that form a protein complex of the clotted mass. The usual amount of zinc in the semen fluid is around 2.4 mM and it is hundred fold more than in the plasma(Franklin *etal*., 2005).

2.2.5 Spermine

Spermine is a polyamine that has great affinity for nucleic acid, phosphate ions or phospholipids. The spermine phosphate is usually formed when the semen is left intact at 25°C. This is as a result of the hydrolysis of seminal phosphorylcholine, by acid phosphatase causing the discharge of inorganic phosphate ions which later reacts with spermine to form spermine phosphate. The oxidation of spermine to aldehyde product gives a special odour to semen (Folk *et al.,* 1980).

2.3. Anatomy of Prostate

McNeal (1984) described the prostate as a gland with three major zones that are different in their anatomical and histological features (Figure 2.1).The zones are the glandular areas (central and peripheral zones) and the non-glandular fibromuscular connective tissue that is found around the prostate. The central zone (CZ) is a piece of glandular tissue that makes up the largest percentage of the prostate base and encircles the ejaculatory canals while the other part is the peripheral zone (PZ). The PZ spreads backward to encircle, partially, the distal part of the urethra(McNeal, 1984). The ducts of the peripheral zone come out directly from the postero-lateral recesses of the wall of the urethra laterally.

The system comprises small, round or egg-shaped acinar assemblies that empty itself into the long thin ducts enclosed by a connective tissue of muscle bundles that are arranged loosely and interwoven randomly.The acini and ducts which are usually lined with simple columnar epithelium are the major spots where prostate cancer and prostatitis except BpH occur**.**

Another feature of the peripheral zone is the preprostatic sphincter which is a cylinder-shaped cover of smooth muscle that expands from the bladder base to the verumontanum.The CZ canal runs principally proximally behind the ejaculatory canals. The acini and canals in this zone are bigger and do not have a regular shape. The shape of the acini is polyhedral and the muscular connective tissue is far denser when compared with peripheral zone. Diseases rarely occur in the central zone. The transitional zone (TZ) surrounds the urethra inbetween the bladder and the verumontanum. Although it is an insignificant portion of the prostate, possibly five percent in the healthy organ,it is the major spot for BpH development.

Once this zone expands, the urethra will be compressed and the partial bladder outlet obstruction related to BpH may occur.The prostate in rodents, unlike humans, has four discrete lobular structures; the ventral, lateral, dorsal and anterior lobes (Sugimura *et al*., 1986). The lobes are found in pairs on the right and left sides and due to the variances in the branching morphogenesis, the final contour of the lobe is unique (Marker *et al*., 2003).In both mice and rats, the ventral lobes are found just beneath the urinary bladder on the ventral side of the urethra while the lateral lobes are found just beneath the seminal vesicles and coagulating glands, partly overlying the ventral lobes and blend with the dorsal lobe (Roy-Burman *et al*., 2004).

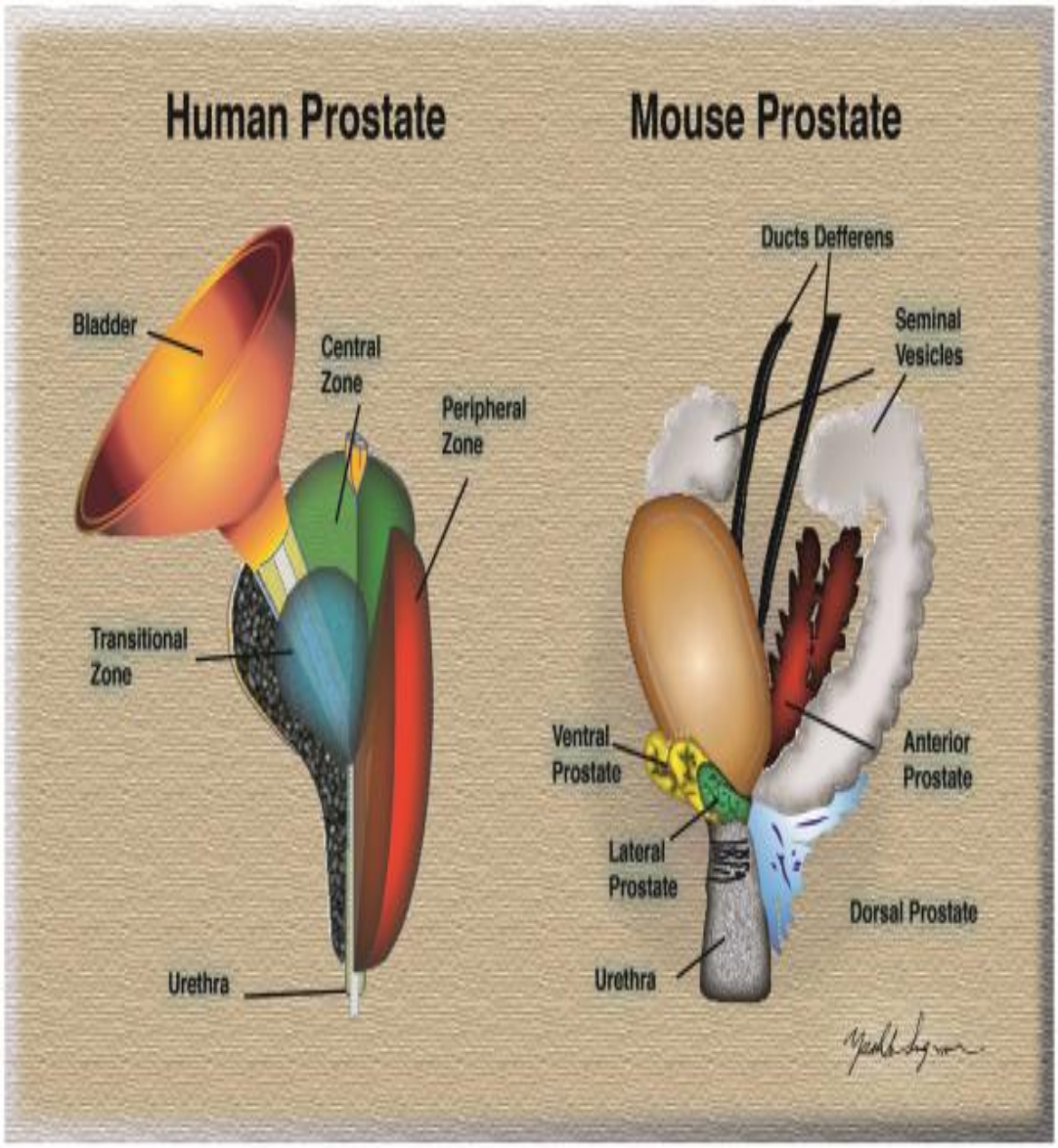


Figure2.1 The diagram of the prostate (Sugimura *et al*., 1986)

2.4. Benign Prostatic Hyperplasia

Benign prostatic hyperplasia, a known progressive ailment is regarded as the increase in the prostate mass and this usually goes with lower urinary tract symptoms. Itbeginsat the periurethral and transition regions of the prostate andsignifies an inevitable inevitable phenomenon for the ageing male population (Untergasser*et al*., 2005).Once the prostate getsenlarged (Figure 2.2), it impinges on the urethra thereby causing difficulty in urination or blockade of urine stream from the bladder. Other lower urinary tracts symptoms that result from enlarged prostate are urine retention, dysuria, nocturia and weak urinary stream (Pais, 2010). In spite of the hugeproblem of BpH on the well-being of the male populace, its pathogenesis ispartly known.Amongst the mechanisms implicated in the development of BpH are age, hormone, metabolic disorder and inflammation(Liu *et al*., 2007).

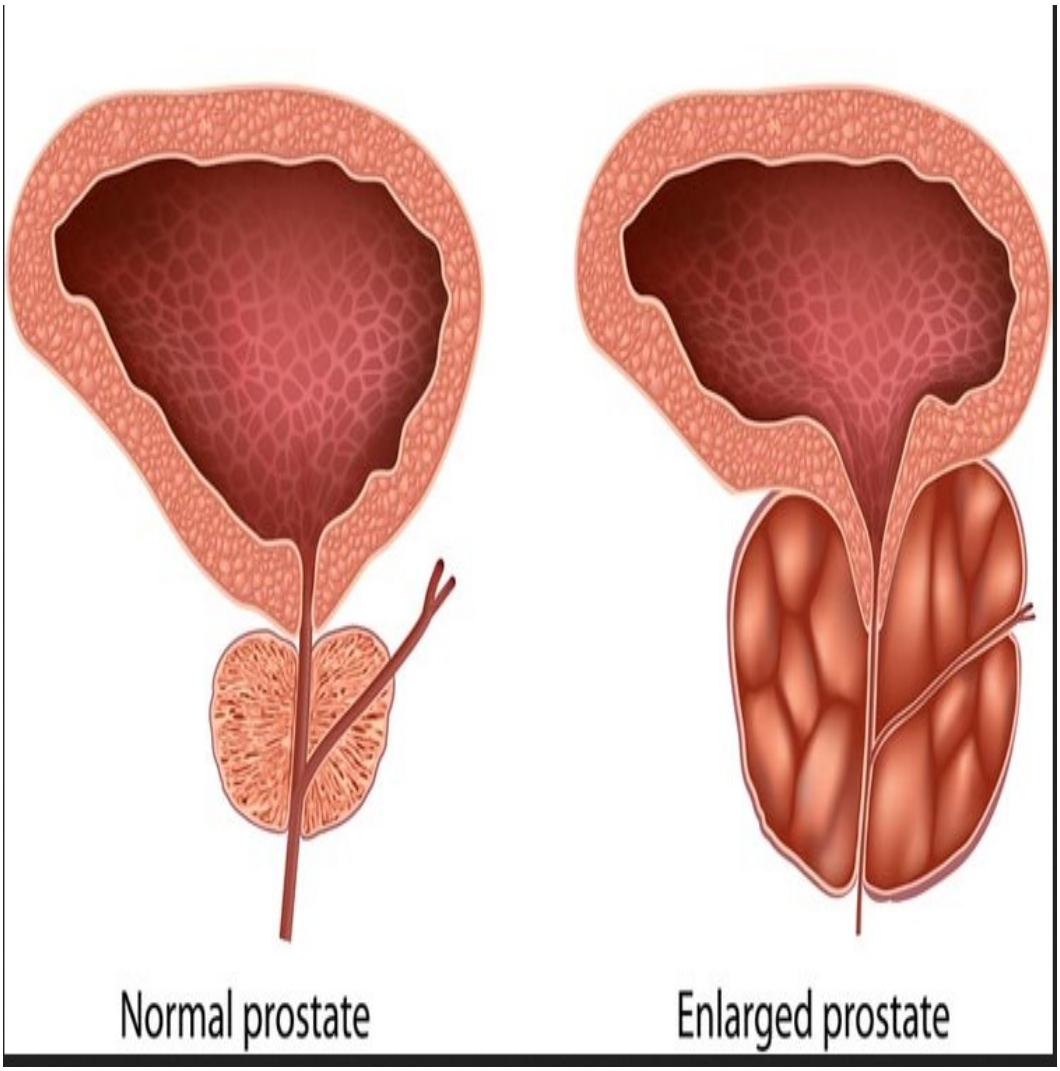


Figure2.2 The diagram of an enlarged and normal prostate (Marberger, 2013)

2.5. Pathophysiology of Benign Prostatic Hyperplasia

The understanding of pathology of BpH emanated from the research work of McNeal (1990). His study revealed that BpH starts freshly in the prostatic periurethral transition zone (TZ). This zone has 2 distinct lobules of tissue directly exteriortopreprostatic sphincter. On the side of thewall of the urethral lie the focal ducts of the TZ exactly where the urethral angulation merge with the verumontanum. The gland of the periurethral zone are found close to the source of the TZ ducts and they are restricted in the preprostatic sphincter and arranged side by side with the urethra axis. In the aging male population, lower urinary tracts symptoms (LUTS) was known to be as a result of enlarged prostate.

2.6. Features of Benign Prostatic Hyperplasia

Benign prostatic hyperplasia is known to be arealhyperplasticprocedure ashistologic studies reported an increased cell number(McNeal, 1990).In BpH, most periurethralnodulesare purely stromal in character (McNeal, 1990) and those small stromal nodules resemble an embryonic mesenchyme,with an abundance of pale ground substance andminimal collagen. It is unclear whether these early stromalnodules contain mainly fibroblast-like cell or whether differentiationtoward a smooth muscle cell type is occurring (McNeal, 1990).

The connective tissueobservedoriginallycomprisesmainly of well-developed smooth muscle that is different from those of theuninvolved TZ tissue. These glandular nodulesare actually made from newly formed small ductbranches that develop from previous ducts, resulting incompletelynew ductal system inside the node. This kind of newgland development is relativelyuncommon outside of the development of embryo.The proliferative procedure usually causes a tight packing ofglands in a particularregion, together with elevation in theheight epitheliumlining(McNeal, 1990). Also, theincrease seen in the TZ dimensionsrelative to agingseemsto be linkedto both rise in the number of nodules and zone size.

McNeal also considers that in the first twenty years of the development of BpH,itcan be primarilycategorizedby means of higher growth of the nodules, while the followingdevelopment of each nascent lump is usuallynot fast(McNeal, 1990). Thefollowingstage of developmental processensues wherethere exists a remarkablerise in big nodules. In the initialstage, the glandular nodules have a tendency to grow bigger than the nodules for the connective tissue while in the next stage, once the size of individualnodules increases, the large glandular nodulesplainlyprevail.

2.7. Prevalence of Benign Prostatic Hyperplasia

Berry and other researchers (Berry *et al*., 1984) described fivepost-mortem studies focusing on the prevalence of histologic BpH due to age. Men of less than thirty years of age do not display histologic BpH while nearly half of men around sixty years displayed histologic features of BpH. Nearlyall males in their 90s have histologic BpH. Anassessment of the literature revealed that the occurrence of histologic BpH is alikeworldwide.The prevalence of BpH varies and depends on the criteria as wellas research settings. Many individuals with histological BpH areasymptomatic making clinical BpH the appropriate terminologywhen conducting research on BpH in the community (Oyewole *et al*., 2017)**.** Several community-based epidemiological studies have documentedthe prevalence of BpH ranging from 30 to 50% and 18.1 to 25.3%in hospital-based and community-based settings, respectively (Garraway*et al*., 1991; Huh *et al*., 2012)**.**However, such studies are relatively scarce in sub-Saharan Africanswhere almost all the existing reports are hospital-based settings.

Secondly, there are differences in the reported prevalence of LUTSand BpH among countries, possibly arising from cultural or linguistic differences. As thepopulation of ageing men increases, BpH has become an importanttopic of public health concern. The only community-based study inNigeria utilized the International Prostate Symptom Score (IPSS)as the only tool to determine the prevalence of BpH (Ezeanyika*et al.,* 2006)**.** Anotherstudy conducted amongst Ghanaian men utilized IPSS and prostaticenlargement by digital rectal examination **(**Chokkalingam*et al*., 2012)**.**The burden of LUTS and BpH is increasing in Nigeria and among Nigerian men irritative symptoms were more prevalent than obstructive symptoms. According to Oyewole *et al.* (2017), the overall prevalence of BpH in their study was 23.7% or237 per 1000 men and the age-adjusted prevalence increases withincreasing age (Figure 2.3).

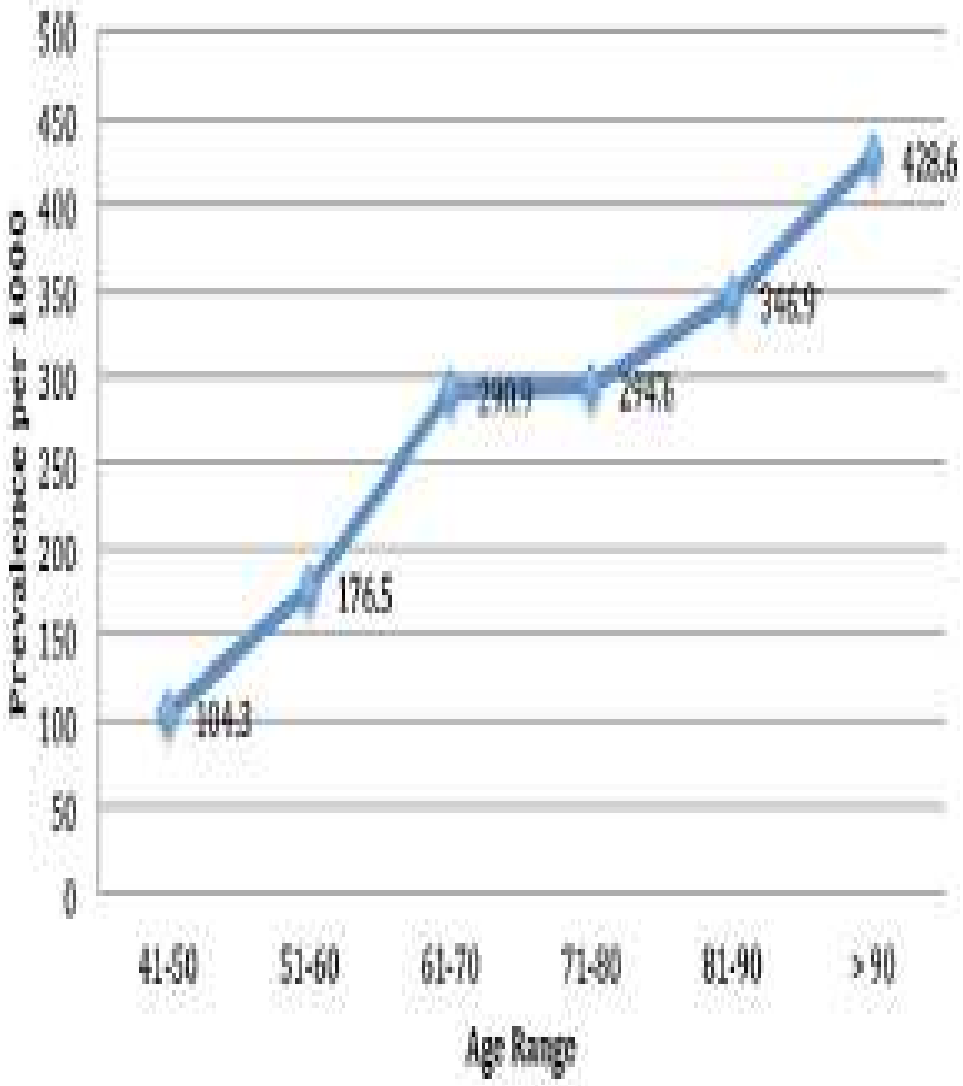


Figure2.3 Age-dependent prevalences of clinical BpH per 1000 men (Oyewole *et al.,* 2017)

2.8. Benign Prostatic Hyperplasia and its Symptoms

The following are the common symptoms found inmen with BpH:

Obstructive symptoms:weak stream, hesitancy, prolonged voiding of urine and straining.

Irritative symptoms:nocturia, urges incontinence, urgency, frequencyand voiding, small volumes of urine

Post micturition symptoms:post void dribbling and inability to empty the bladder (Chughtai *et al*., 2011).

2.9. Etiology of Benign Prostatic Hyperplasia

The exact molecular source of benign prostatic hyperplasia is multifaceted and unidentified, but manymodelshave beensuggested (Figure 4). Among the well-known models of the causes and origin of BpH suggestedby reseachers is the reawakening of the embryo modelof McNeal, 1978. Hepostulated that unusual growth of the prostateconnotes an awakening of androgen-controlled progressive change.From hisdetailed study of sixty three autopsy prostates, McNeal discovered that BpH nodescame from the TZ and referred to it as the "special blending of prostate glands with sphincteric connective tissue". Cunha *et al.* (2004) affirmed that some factors that are necessary for enlargementof prostate gland are androgen regulation and paracrine interactions and then established the significant effects of thecollaborationsamong the prostatic stromal and epithelial cell. Other mechanisms connected with the progressionof BpHin additionto hormonal alterations are aging, metabolic disorder, and inflammation,physical activity, diet, family history, genetics and geography (Liu *et al*., 2007).

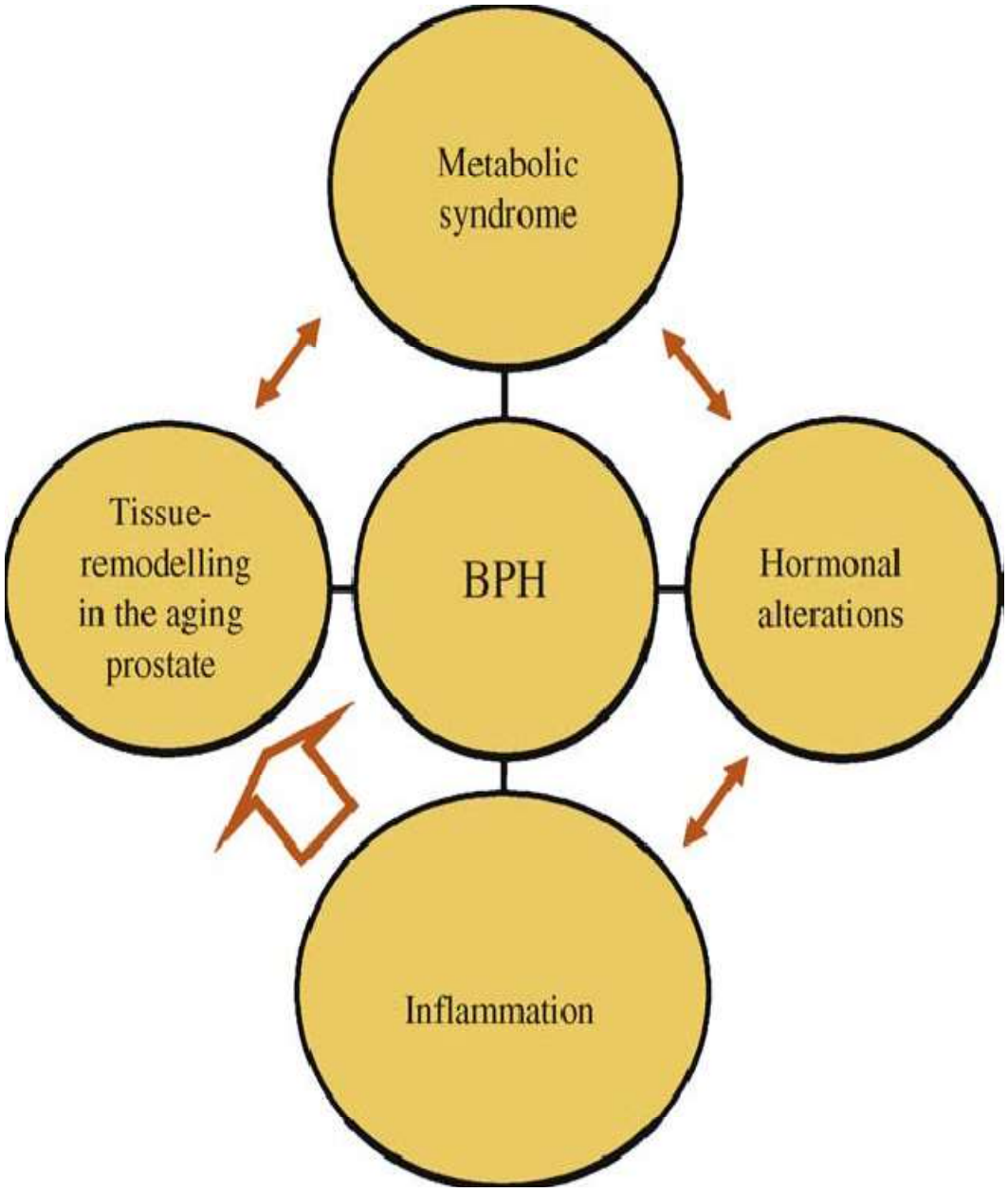


Figure2.4 Relationship between BpH and its risk factors (Briganti *et al*., 2009)

2.9.1 Benign Prostatic Hyperplasia and Age

The occurrence of BpH riseswith age and report from autopsy studies revealed a histological prevalence of 8%, 50% and 80% in men of forty, sixty and ninety years of age, respectively (Barry*et al*., 1995). One risk factor for BpH development reported by several studies from Asia, US and Europe isold age.Another study also demonstrated that the prostatic volume increases with age and statistics from the studypostulates that the rate of prostatic growth is two to two and a half percent per year in elderly men.Despite that the volume of the prostateis not directly linked with the severity of the symptom, prostate growth is still regarded as a featureof LUTS and higherprostatic volumes are connected with high risk of clinical progression in BpH, holding of urine and requirement forsurgical treatment (Roehrborn, 2008).

In addition, it has been reported that BpH may develop between ages twenty-five to thirty and frequency is almost 10% in such age bracket. As a man ages, the frequency of histologically detectable BpH risesgradually, andaround60 years, the prevalence will be above fifty percent while ateighty-five years, almost 90% of men will have this condition.Even though each microscopic BpH is a potential macroscopic BpH nodule, the rate of development may not be similar (Hinma, 1986). Berry *et al.* (1984), in their autopsy studies, linked the change in wet weight of healthy prostate and enlarged prostate as a function of age. Average prostatic weight lacking noticeable histological BpH was 20 ± 6 g around thirty years of age and did not increase afterward. However, all through adulthood, a steady growth was observed in the weight of hyperplastic prostate. The average weight of all hyperplastic prostate, irrespective of time of life, was 33 ± 16 g which is sixty percent higher than the unaffected gland ($p<0.01$).

In addition, in elderly male, tissue-remodeling process is known to occur inthe prostate's transition zone. The mainchanges occur in the cells forming the basic layer of the skin where the intracellular metabolism is transformed and the prostate become enlarged and hypertrophic. Manifestation of corpora amylacea and prostatic calculi also complemented BpH progress. Afterwards, changes in the secretion of luminal cells, the existence of prostatic calculi and corporal amylacea causes more calcification and the clogged ducts also become noticeable. This tissue restructuring cause modifications of extremely specified cells accountable for prostate tissue function and homeostasis (Briganti *et al*., 2009).

2.9.2. Benign Prostatic Hyperplasia and Androgens

Androgens are crucialin the growth and upkeep of BpH. White (1895) in “The consequences of dual gelding in prostate’s hypertrophy”, exanded his models of the prostate’s dependency on an unidentifiedsource of energy from the testes. He recommended two keyideas about the testes: necessary for reproduction of the species and for maintenance of male features. Basically, it has beenhypothesized that many alteration of the functions of gonadsmatched with increased prostate size as an “effect of misdirected energy”. The determination to manage enlarged prostates bycastration confirmed the significant effect of androgens in the management of BpH. Though the model had success rates of about 80%, it failed todifferentiate BpH patients from prostate cancerpatients(Coffey and Walsh, 1990).

Huggins and Hodges illustrated that androgen is crucialin prostate diseases. Theyalso demonstrated that castration effectively cures metastatic prostate cancer andbecause of this discovery, Huggins and Peyton Rous received a Nobel Prize in 1966 (Huggins and Hodges, 2002). Testosterone is known to promote androgen-dependent activities in the seminiferous epithelium and brain while within the prostatic tissue, it is converted to dihydrotestosterone (DHT)to promote hyperplasia (Figure2.5) (McConnell, 1995). Generally, about 90% of total prostatic androgen exists as DHT, primarily formed from testicular androgens. Two isoforms of 5α-reductase enzymes exist, each with a distinct genetic code. The type I 5α-reductase, predominates in extra-prostatic tissue likeliver.The prostate is known to house type 2 5α-reductase and isaccountable for the symptomsdetected in the 5α-reductase deficiency syndrome (Imperato-McGinley *et al.,* 1975).

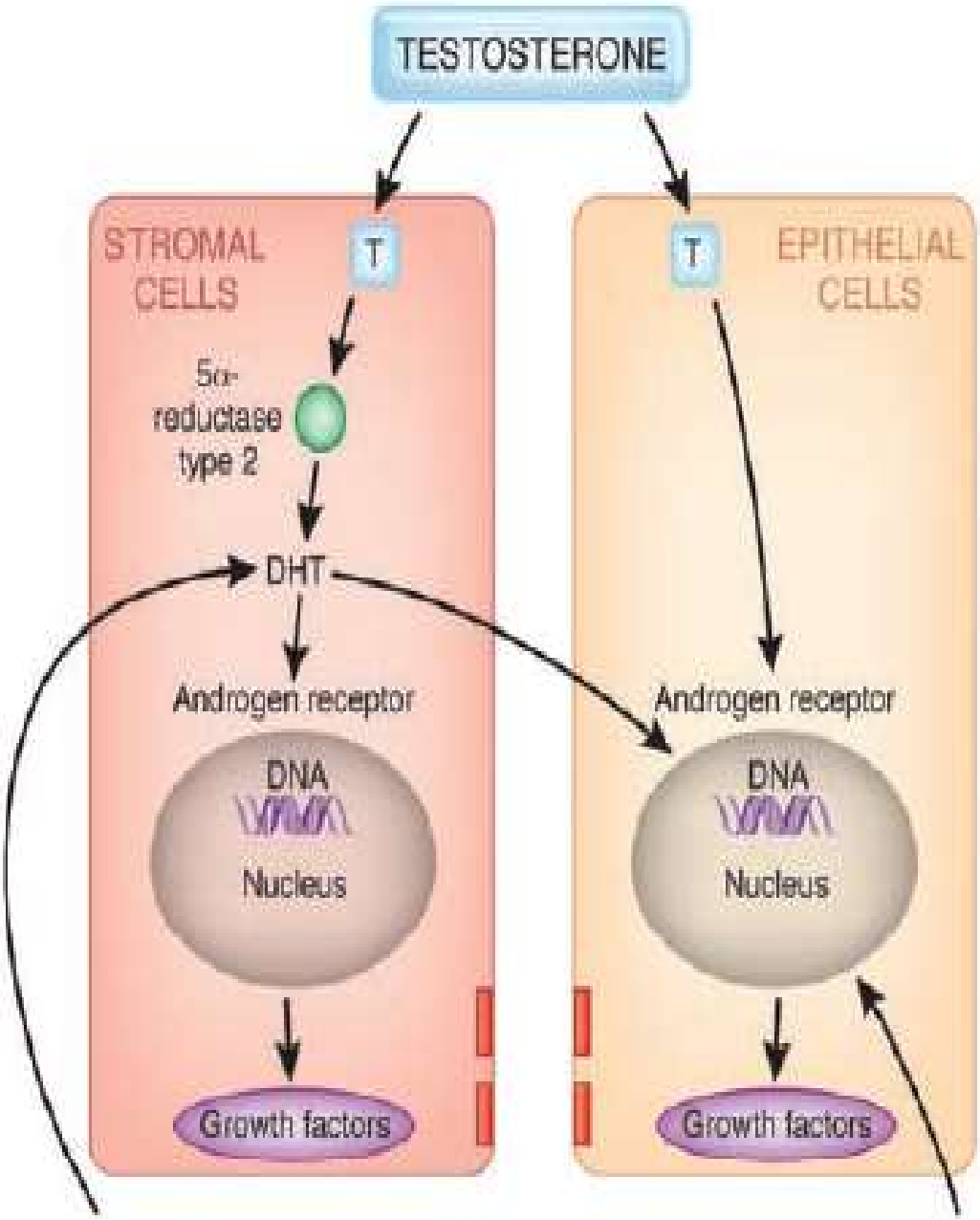


Figure2.5 Production of DHT from testosterone by type 1 and 2 5α-reductase (McConnell, 1995)

2.9.3. Benign Prostatic Hyperplasia and Metabolic Syndrome

Metabolic syndrome is a multifaceted and global epidemic condition that hasgreat socioeconomic influence because of its relationship with high rate of morbidity and mortality (Hammarsten *et al.,* 1998). It is a progressive disease linked with faulty insulin-mediated glucose uptake andis graduallybecoming a major challenge worldwide. Italso includes a collection of aberrations including hypertension, obesity, insulin resistance and dyslipidemia, impaired glucose metabolism and development of hyperinsulinemia. Recently, in the examination of the connectionbetween BpH and metabolic syndrome, Hammarsten*et al*., 1998established that low quantity of high-density lipoprotein cholesterol, hypertension, noninsulin-dependent diabetes mellitus and obesity are risk factors connected withbenign prostatic hyperplasia. Also, another studyestablished that in BpH patients with metabolic syndrome, theserum glucose, prostate-specific antigen, median body weight, body mass index and triglyceridelevels was significantly elevated while high density lipoprotein cholesterol levels in the serum was decreased significantly relative to BpH patients without metabolic syndrome(Hammarsten and Hogstedt, 2001).

BpH and diabetes are identified to be linked with lowstream of urine and a highretention of urine.Even though the means by which diabetes is linked withbenign prostatic hyperplasiaremains unknown, literaturespreviously revealed that damage caused by type II diabetes can supportbenign prostatic hyperplasia (Hammarsten *et al*., 1998). Another metabolic syndrome that was implicated in BpH is hypertension. Arterial hypertension takes place in nearlytwenty-five percentage of people with benign prostatic hyperplasia. Epidemiologic studies also showed that men that are hypertensive may possibly have BpH and go throughsurgical operation than unaffected men (Man in"t Veld, 1998; McVary, 2006).

2.9.4. Benign Prostatic Hyperplasia and Inflammation

Many studies havelinked benign prostatic hyperplasiadevelopment with inflammation. In BpH patients, inflammatory infiltrates are foundin prostate gland and the inflammatory injury observed has a part toplay in the production of cytokines. This proinflammatory microenvironment is associated with overproduction of stroma cells in BpH together with tissue reordering as well as limited oxygen. This process is initiated by high oxygen requirement by cells that are undergoing proliferationand this supports long-lasting inflammation as basis of oxidative injurybringing aboutdamageto the tissue in the infiltrating area (Rahman, 2016).Some articles reported that the

prostate is typicallycolonized by few macrophages,mast cells and T and B lymphocytes.The reaction of the prostateto the cells immunity is known to be facilitatedprincipally by cytotoxic and regulatory T cells in the epithelium and stroma respectively.

2.10. Benign Prostatic Hyperplasia and Apoptosis

Apoptosis, program cell death, is a gene-regulated cellular suicide model in cell growth and homeostasis. The description of the pattern of cell death inBpH and cancer cells by analyzing the appearanceof gene that controls apoptosis could yield significant prognostic evidence and make availablefresh therapeutic targets, as the irregularmanifestation of such molecules can lead to resistance to apoptosis (Bruckheimer and Kyprianou, 2000). It is identified that unaffected cells develop via a slightbalance between the initiation and the inhibition of definite pathways of apoptosis. Amongstthe genes involved are those that encodeBcell lymphoma-2 associated X protein (Bax) and Bcl-2 Specifically, upregulation of Bax expression suggest cells that are going through apoptosis, while those that overexpressed Bcl-2 often go through carcinogenesis.

In the prostatic tissues, Bcl-2 expression is restricted to the base ofthe cell's epithelia and the expression is unaffected even when androgen is withdrawn. Studies also suggest that the Bax/Bcl-2 is significant inregulating the hormone involved in cell death and this also determines the fate of the cell. Indeed, during apoptosis, an increase in this ratio has been observed when androgen is withdrawn (Iacopino*et al*., 2006).Thismechanism of cell death in the prostate cells can beregulated by transcription factors, for example, transforming growth factor β1 (TGFβ1) which controlsthe production and degradation of extracellular matrix, differentiation in cells and cell proliferation.Also, in prostates epithelial cells, proliferative activity is controlledthrough several signaling pathways such as glycoprotein Dickkopf-related protein 3.

2.11. Benign Prostatic Hyperplasia andOxidative Stress

The disparitybetween the creation and detoxification of reactive oxygen species (ROS)resultsin oxidative damage and thus may lead to tissue destruction. In inflammatory cells, ROS production increased and the protective role of antioxidantsare usually overwhelmed (Kullisaar *et al*., 2012). Hence, oxidative stress may be due to depletion of antioxidant defenses or due to overproduction of oxidant. Oxidative stress and tissue damage in the prostate can cause cell proliferation which

can eventually lead to prostatic growth. Oxidative stress can also induce protein structure and function damage andgenomic and vascular tissue injury. Oxidative stress can alsotriggerNF-kB via the tumor necrosis factor-α, the nuclear factor kappa-B-inducing kinase (NIK) transduction and activator protein-1 pathway. The nuclear factor kappa formed is a key transcriptional regulator in inflammatory processes that targets genes whichregulatecell proliferation, immune response, cell migration, apoptosis and inflammation(Hamid *et al*., 2011).

The movement of nuclear factor kappa to the nucleus triggers target genes implicated in tumorigenesis and itsdysregulation is suggested as a well-recognized molecular mechanism resulting in chronic inflammation and cancer. Nuclear factor kappa can also be initiated by exposing theprostate cell epithelia to proteins that can induce inflammation and such occurrence can also cause the induction of local assembly of pro-inflammatory proteins in the epithelial cells (Wong *et al*., 2009).ROS may also stimulate thedevelopment of adducts in deoxyribonucleic acids by instigating peroxidation of lipid, with subsequent generation of metabolites likealkoxyl and peroxyl radical and aldehydes like malondialdehyde. Lipid peroxidation was elevatedin BpH patients relative to controls.

2.12. Androgens

These are steroid hormones mostly implicated in the progression of male characteristic featuresall through embryogenesis, sexual development at adolescence, maintenanceof male reproductive role, formation and development of spermatozoa in the testes spermatogenesis as well as sexual activitiesin the period of adulthood (Marilia *et al*., 2009). They also have effect on several activities occurring in tissueslike skeletal muscle and bone which are not involved in reproduction in male and female. In the male foetus, they promote the growth of ejaculatory duct, vas deferens, epididymis, seminal vesicles, scrotum, urethra and penis (Wilson *et al*., 1981). Atadolescence, their effect isrevealed by the development of testes,bulbourethral, seminal vesicles and prostateearlier than the commencement of secretory activity.

In addition, the secondary sexual featuresshown at puberty are described as features with resultant androgenic and anabolic effects. Anabolic effects is involved in the growth of bone and skeletal muscle and it also stimulate linear growth which ultimately stopped because of the cessation of the epiphysis whereas the androgenic effects involve the broadening of the larynx which makes the voice deeper, cause hair to grow at terminal regions (face and pubicregion as well as in other regions which is dependent

on many factors), rise in sebaceous gland activity (which can cause acne) and central nervous system effects (amplifiedviolent behavior and lipido) (Marilia *et al*., 2009).

2.12.1. Testosterone

Testosterone, mostly in the testis, is the main androgenic steroid formed in males. However, in women testosterone is less produced in the ovary and adrenal gland. Testosterone was isolated by David *et al.*(1935) from bull's testis nevertheless; they were unable to detect the structure of testosterone. The structural elucidation by synthesis was achieved by the joint effort of Butenandt and Hanisch, 1935 and Ruzicka and Wettstein, 1935.As soon as the hormone was detected, they discovered that it wasnot active via oral administration or by parenteral injection, because of its quick absorptioninto the blood system and its hepatic metabolism. In order to avoid this first-pass effect, itis usually administered as an ester or chemically modified form such as testosterone propionate, testosterone enanthate etc.

2.12.2. Androgen Receptor Roles in BpH Development

Since 1895, the reliance on hormone in BpH development, the level of the androgen or the activity of its receptor (AR) in BpH patients is gaining interest.In the 1980s, a study discovered slightalteration in AR expressed in prostatic tissues excised from bothBpH patients and healthy control (Elhilali *et al*., 1983). Nevertheless, the level of serum testosterone and DHT could vary in respect to age, i.e. low serum testosterone level could be found in healthy elderly males as compared to that in healthy younger males. On the contrary, serum dihydrotestosterone levels are greater in BpHrelative to healthy maleof similar age.

Tang *et al*. (2017) found no difference in AR expression betweenthe peripheral zone (PZ) and the transitional zone (TZ) inBPH. In other studies, although nucleic AR expression wasdetected in both epithelial and stromal cells of hyperplasticnodules, higher nuclear AR expression was detected inprostate epithelial cells than in stromal cells (Kyprianou and Davies, 1986; Peters and Barrack, 1987). Othersfound higher 5α-reductase activity in stromal cells than inepithelial cells, with AR distributed evenly between epithelial and stromal cells (Krieg *et al*., 1983; Tunn *et al*., 1986).Importantly, the primitive BpHnodules found in the periurethral area of the TZ had higherconcentrations of androgens and higher nuclear AR expressionthan those in other prostate regions. These findingssuggest that androgen/AR signaling may play important rolesin promoting the proliferation of epithelial and stromal cellsin the periurethral area

of the TZ, thus leading to developmentof BpH with urinary obstruction (Figure 2.6) (Monti*et al*., 1989).

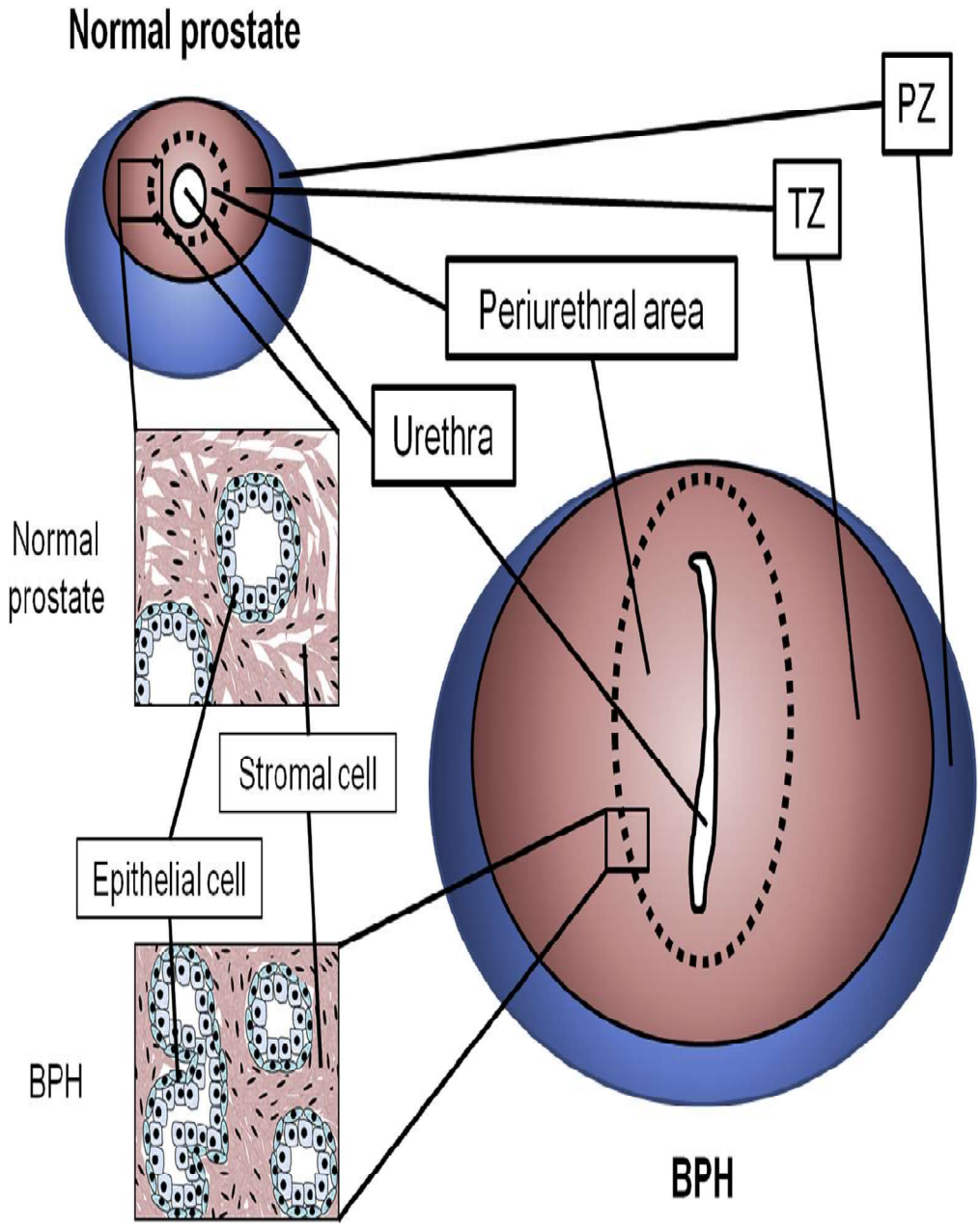


Figure2.6 Schematic of prostate structure (Monti *et al*., 1998)

2.12.3. Androgen Receptor in Prostate Epithelial Cell Growth and Enhanced Epithelial-Mesenchymal Transition in BpH Development

Epithelial-Mesenchymal Transition (EMT)is a physicalpractice where the cell's epithelia obtain the mesenchymal cells' motile features.It is also associated primarily with tumor progression, migration, invasion and metastasis. Malignant cells that are going through EMT have the ability to invade the neighboring stromal cells and form a conduciveenvironment needed for both progression and metastasis of tumor cells (Iwatsuki *et al*., 2010). In addition, EMT promotes BpH developmentand the evidence that the cell proliferatewas found in the connective tissues of BpH patient by the strong expression of Ki67 and PCNAin the cell's epithelia (Alonso-Magdalena *et al*., 2009) (Figure2.7).

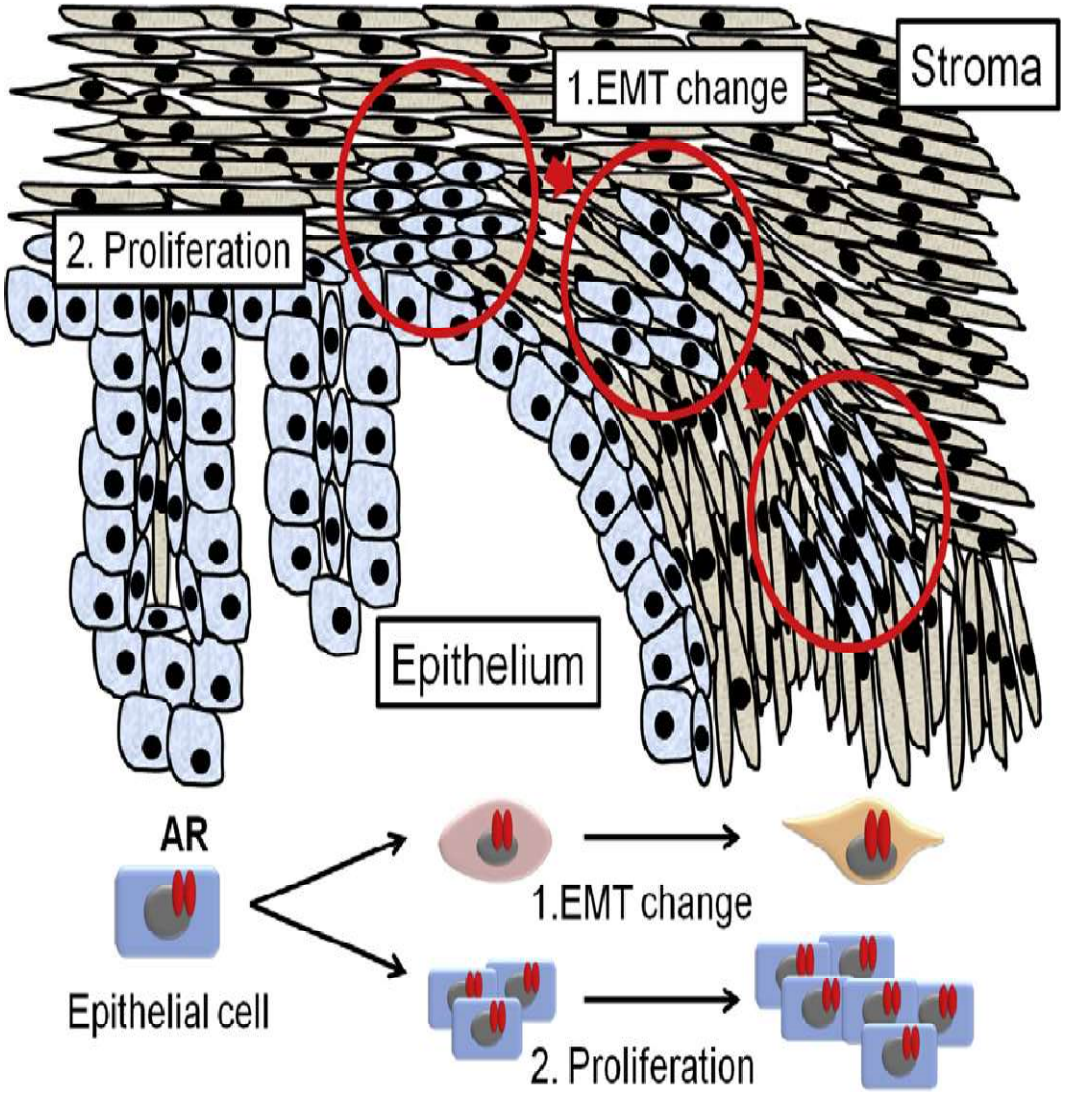


Figure2.7 Diagram of EMT and proliferation in development of BpH (Izumi *et al*., 2013)

2.12.4. Androgen Receptor and Prostate Stromal Cell Growth in BpH Development

Jiang *et al.*(1990) reported that BpH contain stromal cells (about 88%) and epithelial cells (9%), hence it is sensible to consider that stromal cells are involved in BpH.Jiang *et al*. (2011) observedvariancesamongst connective tissue of the TZ and PZ in lieu ofandrogen receptor,the expression of cytokines andthe hormone response. Theyalso confirmed that stromal cells in the PZ supportPCa epithelial cell growth. Nevertheless, there is a dearth of information on whethera stromal cell of the transition zone is involved in BpH development. In another study, high concentration of DHT wasobserved in the periurethral area of the TZ of the BpH subject while the basic fibroblast growth factor expressed bycells of the connective tissues was decreased in BpH patients after treatment with finasteride (Sáez *et al*., 1999). These results therefore show that stromal cells in the TZ significantly influence BpH development by modulatingthe ratio ofandrogen and androgenreceptor signaling including the expression of cytokines and growth factors.

2.13. Diagnosis of BpH

2.13.1 Family History

Men that are at risk of developing BpH are those who have blood relation, either father or brother. Hence, a medical history of patients should be known in orderto plainly establish the symptoms and its severity and also to differentiate between benign prostate hyperplasia and other conditions, such as prostatitis (Edward, 2008).

2.13.2. Physical Examination/digital examination of the rectum

This is the physical investigation of the prostate to recognize an enlarged prostate. It is a fast and painless examination wherein the health care giver (doctor) puts a greased gloved limbto the rectumof the patient and touches the prostate so as to evaluate the prostate size and also to identify lumps or tenderness (Kapoor, 2012). These test help to exclude prostate cancer or problems with the muscles in the rectum which can also cause such symptoms, but it can underrate the size of the prostate. Hence, it cannot be the only way to detecteither PCa or BpH. The physician may also check for signs of kidney or bladder abnormalities by pressing and palpating the abdomen and sides of the subject.

2.13.3 Prostate Specific Antigen Test

Prostate specific antigen (PSA)is arecognized prostate cancer biomarker andbut may also be a biomarker of BpH and also offerssignificantevidence of its progression.Men

who have enlarged prostate glands also havehigh PSAlevels in the serum and they are more predisposing to developing BpH (Roehrborn, 1999). Hence, the more the levels of PSA, the higher the possibility of developing BpH. Also, PSA in the serum reflectepithelial cell volume, because as the volume of the epithelial cells increases, the level of PSA in the serum also increases.

2.14. Management or treatment of BpH

The objectives of BpH management are to improve urine flow and to decrease symptoms. BpH can therefore be managed by: watchful waiting, lifestyle, medications, surgery and phytotherapy (Nickel, 2005).

2.14.1. Lifestyle and Watchful waiting

This is a technique that includesmonitoring of the patient by the heath care giver without real treatment. Watchful waiting together with alterations in lifestyle of malespredisposed to BpH is most appropriate for male with mild-moderate LUTS and no bothersome symptoms although the level of symptoms accepted by an individual differs. Lifestyle changes proposed are reduction in the volume of fluid taken before going to bed, timed voiding (bladder retraining), avoidance of irritable beverages or foods (alcohol or caffeine), close monitoring of the use of some drugslike decongestants, diuretics, antidepressants and antihistamines, pelvic floor exercises, and treatment for constipation (Nickel, 2005).

2.14.2. Pharmacotherapy

The 5α-reductase inhibitors and the alpha-blockers are the drugs currently use in the treatment of BpH (Nickel, 2005). Pharmacologic treatmentsgive satisfactory symptom relief though they may not be as efficient as surgical treatments.

2.14.2.1. Alpha-blockers

Alpha-blockers are such drugs that cause thebladder neck and smooth muscle of the prostate to relax by inhibiting sympathetic stimulus facilitated by α1-adrenoceptor. Five major α-blockers are recognized in the management of LUTS as a result of BpH.Terazosin and doxazosin (second-generation drugs) and Silodosin, Alfuzosin and Tamsulosin (third-generation drugs) (MacDonald, 2005). Although there are slightvariances in the adverse effects of these drugs, they are believed to be clinically efficient and give fast symptom relief. Also, α-blockers did not offerlifelong decrease in the menace of seriousretaining of urine or surgery associated with BpH although they improve symptoms. The primary side effects observed with alpha-blockers are fatigue, orthostatic low blood pressure, fatigue, vertigo and blocking of nose. The

menace of vertigoremains small with tamsulosin and alfuzosin relative to the second-generation drugs. Tamsulosin have low likelihood of orthostatic low blood pressure together with a greaterlevel of ejaculatory dysfunction and can result in erectile dysfunction or reduced libido(Giuliano, 2006).

2.14.2.2. 5-alpha reductase inhibitors (5-ARIs)

These drugs inhibit 5-alpha reductase. The frequently used 5-ARIs are dutasteride and finasteride and they represents the main hormonal therapy that iseffective and suitable for benign prostatic hyperplasia treatment. A decline in DHT level can cause apoptosis and atrophy of the prostatic epithelial. The basis for employing 5-ARIs to manage BpH is, thus, to reducesignificantly the DHT levelsso as toreduce the prostate size. Finasteride, a member of the class, lessens serum DHT after 24 weeks of administration but not to castration levels. Dutasteride however, decreases the levels of serum DHT much more since it canhinderthe two isoforms of the 5-alpha reductase (Gittens, 2008).

2.14.2.3. Combination therapy

The management of BpH with the mixture of a 5-ARI and α-blocker has been commended.This is because, as the alpha-blocker gives fast relief, the 5-ARI will provide a long-lasting relief of symptoms. A study indicated that the risk of progression decreased by sixty-six percent via combination therapy (doxazosin and finasteride) relative to the inactive substance ($p < 0.001$) and up to a higherlevel than any of the drug by (39% for doxazosin and 34% for finasteride) (McConnell *et al*., 2003).

2.14.3. Surgery

The treatment of BpH with surgery is required, and recommendationby urologist is necessaryonly if the use of drugs failed or, if there are withholding of urine, renal failure, bladder calculi or continuinginfections in the urinary regionbecause of benign prostatic obstruction (Levy and Samraj, 2007).Most of the surgeries performed areopen or laser prostatectomy, transurethral incision or resection of the prostate (TUIP or TURP)and electrovaporization.Generally, TURP is the best of prostate interventions especially for BpH patients with resistance to pharmacotherapy. Transurethral resection of the prostate is performed by removing the internal part of the prostate, where BpH develops and this decrease the related symptoms in about 88% of patient. The most commondifficulties of the practice are inability to empty the bladder, clot retention and secondary infection. Hemorrhagetakes place in only one percent of patient whilelong-term complications include partial incontinence, retrograde ejaculation, impotence and total incontinence (Levy and Samraj, 2007).

2.14.4. Phytotherapy

Phytotherapy is a system of medicine where plant extracts and products are used to procure natural remedies for many disorders. Phytochemicals are recently used in the management of BpH. The commonly described and studied chemoprotective agent in BpH, *Serenoa repens*, slightly reduced nocturia, increasedthe maximal flow of urine and improved IPSS in BpH patients with results equivalent to that of tamsolusin (Hizli*et al*., 2007). *Pygeum africanum* is another common alternative cure for BpH and is known to offer minimum aid from the urinary challengestriggered by prostate enlargement. Nevertheless, the shortcomings of those studies are short duration, varied doses and preparations including small size. Other phytotherapeutic agents are pumpkin (*Cucurbita pepo*), Nettle (*Urtica dioica*), Rye grass pollen (*Secale cereal*), Soy isoflavones (*Glycine max*) etc and the frequently used nutraceuticals are isoflavones, β-sitosterol and lycopene.

2.15. Methyl jasmonate (cyclopentaneaceticacid, 3-oxo-2-(2-penten-1-yl)-, methyl ester).

Methyl jasmonate (MeJa), a cyclopentanone lipid naturally found among the jasmonates (JAs) was originally detected in*Jasminum grandiflorum*oil (Demole *et al*., 1962). Nevertheless, its biological roleor that of its free acid was not cleartillwhen the effect of jasmonic acid in the promotion of senescence in plant and that of MeJa itself in the inhibition of growth to *Vicia faba* were detected. Currently, the best described class of oxylipins in plants is jasmonic acid and its derivatives (JAs). They are one of the main hormones that control defense and development in plant. Other members of the jasmonates family besides methyl jasmonate (Figure 2.8) are jasmonic acid (JA) and *cis*-jasmonate (Sembdner and Parthier, 1993).

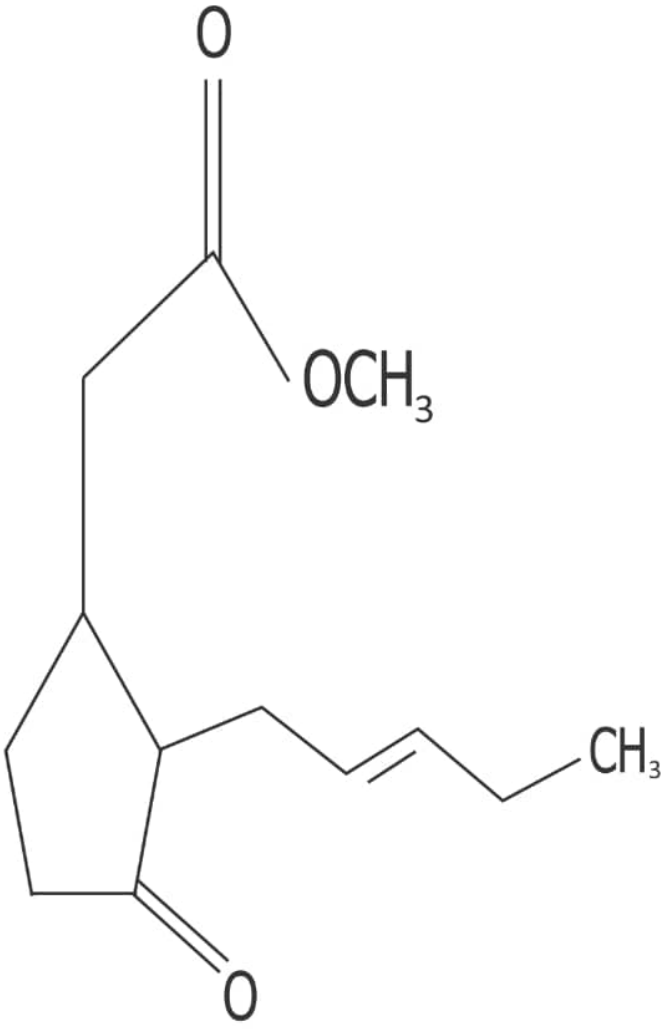


Figure2.8 Chemical structure of methyl jasmonate (Cesari *et al*., 2014)

2.15.1. Biosynthesis of methyl jasmonate

Vick and Zimmerman in 1984 elucidated the stages of the jasmonates biosynthetic sequence of reactions in full and since then, the signaling and biosynthetic pathways of the jasmonate family are explored with keen interest, particularly in the dicot like*Solanum lycopersicum, Arabidopsis thaliana*and to a smaller degree in few monocots. α-linolenic acid is a known precursor of jasmonic acid located in themembranes of the chloroplast. The α-linolenic acids (α-LeA) are related in structure and biogenesis to prostaglandins and are of great significance in signaling molecules of invertebrates and vertebrates. Wounding or pathogen attacks in plant prompts the production of α-LeA from chloroplast membranes lipids via the activity ofphospholipase A (Cheong and Choi, 2003).

When introduced into the interior of the chloroplast, oxygen molecule is incorporated into α-LeA by 13-lipoxygenase (LOX), which happen to be the first stageof the synthetic pathway, producing 13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HPOT) (Figure 2.9). The product formed is oxidized via the activity ofanother enzyme named allene oxide synthase (AOS) forming an allene oxide which is relativelyunsteady. The enzyme, allene oxide cyclase, thenconverts the product formed in the latter reaction to 12-oxyphytodienoic acid (12-OPDA). At the last phase in the production of jasmonic acid, the product (12-OPDA) go through 3 rounds of β-oxidation in the interior of the peroxysomes and after it is formed, it will undergo diverse metabolic reactions yielding distinct jasmonates. One of such reaction is the methylation of jasmonic acids to produce methyl jasmonate in the cytoplasm (a reaction catalyzed by methyltransferase) (Seo *et al*., 2001).

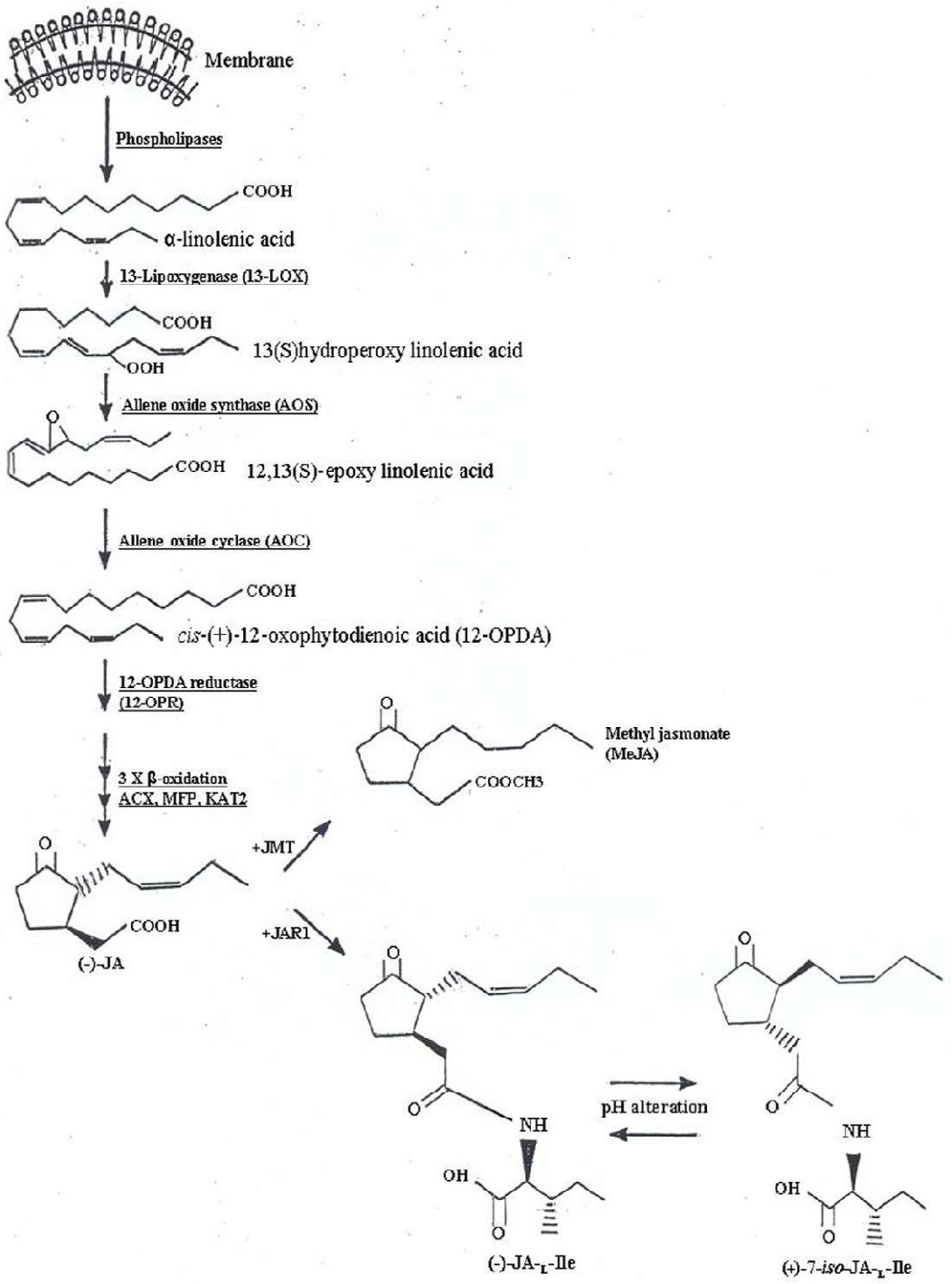


Figure2.9 The jasmonic acid biosynthetic pathway together with all the intermediate oxylipins (Browse, 2009 and Wasternack, 2007)

2.15.2. Regulation of Jasmonic Acid Biosynthesis

The amount of jasmonic acid synthesized in plants is dependent on several factors like tissues, diverse environmental stimuli and growingphase. A sizeable amount of jasmonates are detected in the chloroplasts of illuminedfloras, skin ofemerging fruit and florets.The amounts of jasmonate also rise swiftlydue to mechanical agitations likewinding of tendril and pathogenic attack in plants that result in wounding (Creelman and Mullet, 1995). Numerousapproachesare employedin plants to controljasmonates activities. Thisis by regulating the expression of the enzymes (AOS, AOC, LOX, OPR3, JMT) implicated in the biosynthesis pathway of jasmonate, availability of substrate and accumulation of intermediates for future use in the synthetic pathway.

2.15.3.Impact of Jasmonates (JAs) in plant development and defense

Many processes are regulated by jasmonates in plants but the best understood among them is its importance in wound response. JAs biosynthesis is promptly stimulated following mechanical wounding or by herbivores leading to the manifestation of the proper response genes. Precisely, in dicotsplant, insect attackcreatesprotective substances that hinder the digestion of plant part in the alimentary canal of the insect. MeJa,present in leaves, can move in the air to neighboring plants and up-regulate levels of transcripts related to wound response which then makes those plants to prepare for their defenses in case of herbivore (Farmer and Ryan, 1990). Besides its part in defense, JAs also have the ability to cause mitochondrial death by causing the buildup of ROS which are known to distort mitochondria membranes and reduce the integrity of the cell by triggering apoptotic cell death (Reinbothe *et al*., 2009).

2.15.4. Biologicaleffects of Jasmonates in Fruit and Seed Ripening

Mutants in JAs synthesis in Arabidopsis usually present with male sterility, normally as a result of the delay in development processes. Remarkably, the genes that are responsible for male fertility in *Arabidopsis thaliana*are also responsible for female fertility in tomatoes. JAs mutant *jar1* of tomato yields lesser fruits when comparedwithwild type and the immature seeds of the transformed fruitlets failed to reach maturity. This is an indication that JAs signal is significant in the initialphase of fruit progression and seed ripening in tomato. In apples andsweet cherries, endogenous JAsaccruedas the fruits and seeds ripensand this suggest that JAsaresignificant in the development of fruit and seed (Kondo *et al*., 2000).

2.15.5.The effects of Jasmonates in Leaf senescence

The process of aging in leaf includes age-related cell death that is also relying on the effect of endogenous and ecological factors. A number of phytohormones including jasmonic acid, cytokinins, ethylene, abscisic acid and salicylic acid were involved in leaf senescence program. During the biosynthesis of jasmonate mutant *kat2* and signaling mutant *coi1*, the yellowing phenotype was seen to be delayed during natural senescence and when the detached leaves were incubated in the dark.

2.15.6. Physiological roles of Jasmonates in accumulation of storage protein

Increased biosynthesis of jasmonic acidsencouraged the buildup of storage proteins because genes codingfor plant growth proteins are receptive to jasmonic acids. Precisely, tuberonic acid, derived from jasmonic acid, initiates tuber formation (Hause, 2009).

2.16. Jasmonate and its anti-cancer mechanisms

Three models have been suggested toelucidate the suppression of tumor byjasmonates. The modelsmay either take place in tandem,by affecting several kinds of malignant cells, or becomedynamicin diverseperiod and concentration since the models are not mutually exclusive. The mechanisms are:

2.16.1.The bio-energetic mechanism

Program cell death and tissue death may come from mitochondrial agitation since mitochondria are commonlyknown as crucial to life and death verdicts in cells. Nevertheless, in recent time, a newtheory was developedwhich mightmake way for novel drugs against cancer cells (Constantini *et al*., 2000). Based on this theory, substances that hinder the normal function of mitochondria will also by-pass pre-mitochondrial mutations. The mutations will then make the transformed cell to resistanti-cancer agents that stimulate cell death in the mitochondria. Studies revealed that jasmonates agitated mitochondria in intact human hepatoma and leukemia cells. Once the mitochondria were removed, the effect of MeJawas evaluated by examining the markers of apoptosis like cytochrome c and mitochondrial swelling. Thoughthe isolated mitochondria from themalignant cells were agitated by MeJa, the fibroblasts that are not transformed were unaffected and this is indicative of jasmonates selectivityfor transformed cells (Rotem *et al*., 2005).

2.16.2.The re-differentiation mechanism

Recently, oncologists are interested in the induction of re-differentiation as anapproach to ‘normalize’ malignant cells that are yet to differentiate.Basically, re-differentiation happens to be a procedure where the genome of malignant cells is changed to a well differentiated genotype and phenotype. The rate at which the transformedcells multiply is slow and such cell may lose itsinitial malignant characteristics. Infact,severaltherapeutic agents were proposed to elicit their effects by this mechanism, retinoids being the best example (Ishii *et al*., 2004).Also, methyl jasmonate stimulate manyindicators of differentiation such as expression of both CD14 (surface antigen that is specific for monocytes), CD15 (antigen specific for granulocyte) andNBTreduction.

2.16.3.Mechanism mediated by reactive oxygen species

The major concern of many scientists has been on the effects of several reactive oxygen species (hydoxyl radical, H_2O_2, superoxide ion and singlet oxygen) on the cell and that is why the probableassociation of ROS in induction of apoptosis by MeJa was evaluated and reported by(Kim *et al*., 2004).Antioxidants like catalase or N-acetyl cysteine can block death in A549human lung adenocarinoma cells which were induced by methyl jasmonate. Methyl jasmonate also triggered Bcl-Xs and Bax expression whilethat ofBcl-XL and Bcl-2 is unaffected. The expressions of Bcl-2 family members modified by methyl jasmonatecan also be prevented by catalase. Hence, methyl jasmonate-cell death in A549 cells seems to be controlled by cascadeof reaction that involvesproduction of H_2O_2 and higher expression of pro-apoptotic proteins (Kim *et al*., 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals

Testosterone propionate, Methyl jasmonate, Hydrochloric acid, trichloroacetic acid, O-dianisidine, phosphoric acid, aniline hydrochloride, reduced nicotinamide adenine dinucleotide phosphate salt, *p*-aminophenol and sulfanilamide were obtained from Sigma Aldrich, USA.Ethylenediaminetetraacetic acid, reduced glutathione, epinephrine, Thiobarbituric acid andtris powder were also bought from Sigma Aldrich, United State of America. Ellman's reagent and N-(1-naphthyl) ethylenediamine dihydrochloride were procured from British Drug HouseChemical, United Kingdom. The ELISA kit from testosterone was procured from Calbiotech Inc, Califonia while Finasteride was procured at a residentapothecary store in Ibadan, Nigeria and the remaining chemicals used were analytical reagent.

3.2 Animals

Adult male Wistar rats (12 weeks old) were purchased from the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. They were kept in ventilated cages at room temperature (28-30°C) and maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee with the approval number UI-ACUREC/APP/2015/045. The animals were allowed to acclimatize for two weeks before the experiment.

3.3 Experimental BpH

In order to eliminate role of endogenous androgen, the rats were castrated (using ketamineat 25mg/kg as anesthesia)and allowed to recuperate for 28 days prior to the initiation of the study. During castration, the testicles and epidymal fat were removed

through the scrota pouch(Coppenolle *et al*., 2000).The cord supporting the testis together with the blood vessels were then tied up using suturematerial and resected; thecastrated rats were later given tetracycline (0.5g/L) for five days to prevent infection.

3.4 Collection of Blood and Preparation of tissue samples

After 28 days, the rats were fasted overnight and sacrificed 24 hour after the last dose of drugs. Blood was collected via retro-orbital plexus intonon-EDTA bottle and allowed to stand for 1 hour before it was centrifuged for 15 minutesat 3000 rpm. The supernatant (serum) wastransferred into welllabeled tubes and used for biologicalinvestigations. The tissue (prostate) was also cut out, cleansed inKCl(1.15%) liquid and weighed. Oneprostate from each group was put inbuffered formalin(10%) for immunohistochemistry and histologicalinvestigation. Each of the remaing tissue in the groupwas homogenized in 6volumes of phosphate buffer (50 mM: pH 7.4) and centrifugedfor 15 min at 10,000 g so as to getthe supernatant.All procedures were carriedout at 0-4^{o}C.

3.5. Assessment of the Activity of Alkaline Phosphatase (ALP)

This wasmeasured by the techniqueof Englehardt (1970) using Fortress Diagnostics kits.

Principle:

This is based on the formation of para-nitrophenol and phosphoric acid from para-nitrophenyl phosphate by the activity of alkaline phosphatase. Para-nitrophenol gives a yellow colour in the reaction mixture and the intensity of the colour was monitored for 3 minutes at 405 nm using 721S Visible Spectrophotometer (SEARCHTECH).

$$p-\text{nitrophenylphosphate} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} \text{phosphate} + p-\text{nitrophenol}$$

Procedure

One milliliter of the working reagent was added to the serum (20 µL). This was mixed moderately and the initial absorbance was measured while the rate of the reaction was monitored for 3 minutes at 405.

Calculation

$$\text{ALP Activity (U/L)} = \frac{2760 \times \text{Absorbance of sample}}{3}$$

3.6. Determination of Acid Phosphatase (ACP) Activity

This was evaluatedaccording to the technique of Fishman *et al.*(1953) using Fortress Diagnostics kits.

Principle:

The enzyme (acid phosphatase) catalyses the hydrolysis of the phosphate group, in acidic medium, from α-naphthyl phosphate. The α-naphthol formed reacts with a diazonium salt (fast Red TR) forming a chromogen. The activity of the enzyme was determined from the rate of the chromogen formed at 405 nm.

$$\alpha - \text{naphthyl phosphate} + H_2O \xrightarrow{\text{ACP}} \text{Pi} + \alpha - \text{naphthol}$$

$$\alpha - \text{naphthol} + \text{Fast Red TR salt} \rightarrow \text{Azoic Chromogen}$$

Where Fast Red TR salt = 4-chloro-2-methylphenyldiazonium salt

Procedure

One hundred microliter (100µL) of sample was added to one milliliter of reagent solution A (10mL of buffer + 1 vial of substrate (1-naphthyl phosphate and Fast Red TR salt) or B (reagent solution a + substrate (sodium tartarate). The combination was vortexed and incubated for five minutes at 37°C. Initial absorbance was measured against air blank at 405nm while others were read at 60 seconds interval up to 3 minutes.

Calculation

$$\text{Total ACP Activity (U/L)} = \frac{\Delta\text{Abs of solution A}}{\text{minute}} \times 743$$

$$\text{Prostatic ACP } \left(\text{U/L}\right) = \left(\frac{\Delta\text{Abs of solution A}}{\text{minute}} - \frac{\Delta\text{Abs of solution B}}{\text{minute}}\right) \times 743$$

Where

ΔAbs = Change in Absorbance

3.7. Determination of Protein Content

This was evaluatedusing Biuret reagent by the technique of Gornal *et al*. (1949).

Principle

In alkaline condition substances that have more than two peptide bonds produce a purple compound by interacting with copper salts found in Biuret reagent($CuSO_4$, KI and sodium potassium tartarate). The procedure is calibrated with a standard bovine

serum albumin(BSA) curve and the intensity of the colour is proportional to the concentration of protein in the sample.

Procedure

The sample was diluted five times with distilled water. Three milliliters of Biuret reagent was mixed withone milliliter of the diluted sample (serum or prostates post-mitochondria fraction). This was incubated at 25°C for thirty minutes and read at 540 nm using spectrophotometer against distilled waterblank. Theconcentration of protein in the tissues was extrapolated from the BSA curve.

Preparation of Reagents: See Appendix 2

3.8. Determination of the level of Lipid Peroxidation

This was evaluated bythe technique ofRice-Evans *et al*. (1986).

Principle

Theprinciple is based on the relationshipbetweenmalondialdehyde (MDA) and 2-thiobarbituric acid. After heating at acidic pH, the pink cromophore formed is a measure of the quantity of free MDAformed per mg protein.

Procedure

The sample (0.4 mL) was added to Tris-KCl buffer (1.6 mL) followed by TCA (0.5 mL) as well as TBA (0.5 mL) and heated at 80°C in a water bath for forty-five minutes. The mixture was permitted to cool and centrifuged at 3000 rpm for 15 minutes. Then, the resultant pink solution was measured at 532 nm against distilled water blank. A constant of 1.56 x $10^5 M^{-1}Cm^{-1}$ was used to calculate the level of lipid peroxidation.

Calculation $$\text{MDA (units/mg protein)} = \frac{A_S \times V_m}{E \times Vs \times P}$$

Where,

A_s = Absorbance of sample

V_m = Volume of mixture

Extinction coefficient (E) = 1.56 x $10^5 M^{-1}Cm^{-1}$.

V_s = Volume of sample

P = Protein content of the sample

Preparation of Reagents: See Appendix 3

3.9. Estimation of the Concentration of Prostatic Reduced Glutathione

This was assessed by the technique of Beutler *et al*.(1963).

Principle

The majority of sulfhydryl groups (not proteins) in cells are found in the reduced form of glutathione. The principle of this assay is built on the formation of a fairlysteadypigment (yellow) when Ellman's reagent reacts with the sulfhydryl compounds.

Procedure

To the diluted volume(1:10), 3 mL of 4% sulphosalicylic acid was added to remove the proteins. Thiswas spunfor 5 minutes at 3,000rpm. Subsequently, the supernatant (0.5 mL) and the Ellman's Reagent (4.5 mL) were mixed together and read at 412 nm against water blank.The level of the reduced glutathione in the sample wasextrapolated from the standard GSH curve.

Preparation of Reagents: See Appendix 4

3.10. Determination of the activity of Prostatic Catalase

This was evaluatedby the technique ofClaiborne (1985).

Principle

This technique is based on the reduction of absorbance detectedas hydrogen peroxide is split by catalase at 240nm. Althoughhydrogen peroxide does not absorb maximally at 240 nm, its absorbance correlates with concentration and is used for a measureable assay. The extinction coefficient of $0.0436 mM^{-1}cm^{-1}$ was used.

Procedure

To 50 µL of sample, 2.95 mL of hydrogen peroxide was added into 1 cm quartz cuvette mixed carefully and the change in absorbance was recorded for five minutes at 240 nm every 60 seconds.

Calculation

$$\text{Catalase activity} = \frac{\left(\frac{\Delta \text{Abs}}{\text{min}}\right) \times \text{Volume of reaction} \times \text{Df}}{0.0436 \times \text{Volume of sample} \times \text{Protein concentration/mL}}$$

Where

ΔAbs=Change in Absorbance of sample

Df=Dilution factor

Preparation of Reagents: See Appendix 5

3.11. Estimation of the Superoxide DismutaseActivity in the Prostate

This was assessed bythe procedure of Mistra and Fridovich (1972).

Principle

This is based on the inhibition of the autoxidation of epinephrine by superoxide dismutase at highpH (>10). The superoxide radical produced by the reaction catalyzed by xanthine oxidase oxidizes epinephrine to form adrenochrome. The amount of adrenochrome formed by the superoxide increasesas the pH andconcentration of epinephrine increases. The autoxidation of epinephrine pass through two `different pathways,one of which is a cascade of free radical reaction that involves superoxide radical and hence can be inhibited by superoxide dismutase.

Procedure

The diluted (1:10) sample (0.2 mL) was added tothe carbonate buffer (2.5 mL) before adrenaline (0.3 mL) was added to stimulate the reaction. This was mixed gently by inversion. The reference cuvette was prepared in the same way by replacing the sample with distilled water. The change in absorbance was measuredat 480nm every thirty seconds for 2.5 minutes.

Calculation

$$\text{Change in absorbance per minute} = \frac{A_{f-}A_I}{2.5}$$

Where A_I = initial absorbance

A_f = final absorbance

$$\% \text{ Inhibition} = \left(\frac{\text{Increase in absorbance of sample}}{\text{Increase in absorbance of blank}}\right) \times 100$$

A unit of superoxide dismutase activity is the amount of superoxide dismutaserequired to inhibit the oxidation of adrenalineby at least 50%.

Preparation of Reagents: See Appendix 6

3.12. Determination of the Glutathione-S-Transferase (GST) Activity in the Prostate

The activity of GST was assayed as described byHabig *et al.*(1974).

Principle:

The principle is based on the fact that glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4, - dinitrobenzene (CDNB) as the second substrate.When this substance is conjugated with reduced glutathione, its absorption maximum shifts to a longer wavelength. The absorption increase at the new wavelength of 340nm provides a direct measurement of the enzymatic reaction.

Procedure

Reduced glutathione (30 µL) was added to 150 µL of CDNB in the cuvette. 2.80 mL of the buffer was added to the solution after which 30 µL of sample was added.The absorbance was read at 340 nm and the rate of the reaction was monitoed for 3 minutes at 60 seconds interval. The blank was prepared as the above but 30 µL of distilled water was used to replace the sample. The extinction coefficient of $9.6mm^{-1}cm^{-}1$ was used to compute the enzyme activity.

Calculation

$$\text{Glutathione} - \text{S} - \text{transferase activity} = \left(\frac{\text{Change in Absorbance/minute}}{9.6}\right) \times \left(\frac{1}{\text{mg Protein}}\right)$$

=µmole/min/mg protein.

Preparation of Reagents: See Appendix 7

3.13. Determination ofGlutathione Peroxidase Activity in the Prostate

This was done by the technique ofRotruck *et al*. (1973).

Principle:

Glutathione peroxidase catalyses the oxidation of reduced glutathione to oxidized glutathione using hydrogen peroxides. The formation of 5-thio-2-nitrobenzoic acid from the interaction of 5',5' – dthiobis-2-nitrobenzoic acid (DTNB) and reduced glutathione (GSH) is therefore measured at 412 nm using spectrophotometer.

Procedure

The assay was performed by adding phosphate buffer (250 µL), 100 µL of reduced glutathione, NaN_3 (50 µL), 50 µL of hydrogen peroxide, sample (250 µL) and 300 µL of water (distilled). The entire mixture was incubated at 37°C for 180 seconds and 250 µL of TCA was then added and centrifuged at 3000 rpm for five minutes. 1000 µL of

K_2HPO_4 and 500 µL DNTB were mixed with the supernatant (500 µL) and the absorbance was read against reagent blank at 412 nm.

Calculation

The concentration of the reduced glutathione left was extrapolated from the reduced GSH standard curve and the activity of glutathione peroxide was derived by calculation.

$$\text{GSH consumed} = 245.84 - \text{GSH remaining}$$

$$\text{Glutathione peroxidase activity} = \frac{\text{GSH consumed}}{\text{1 mL protein}}$$

Preparation of Reagents: See Appendix 8

3.14. Determination of the Percentage of Fragmented Deoxyribonucleic acid (DNA)

This amount of fragmented DNA was assessed by the technique of Wu *et al*. (2005).

Principle

The method assessed the endonuclease cleavage of the end product of apoptosis. DNA was extracted from the homogenate and treated with diphenylamine (DPA). Theabsorbance of the chromophore formed was measured in the spectrophotometer at 620 nm.

Procedure

The prostates were homogenized in 10 volume of TE. Homogenates were centrifugedfor 20 minutes at 27,000 gso as to detached the intact chromatin (pellet named K) from broken ones (supernatant named M). The pellet (K) was suspended in 2 mL of TE'. 0.5 mL of the suspension (K) and 0.5 mL of supernatant(M) were placed in different test tubes and 1.5 mL of diphenylamine solution(made fresh) was added to each tube. This was incubated at 37°C for twenty hours. The radiation capacityof the resulting solution wasrecorded at 620 nm.

Calculation $$\% \text{ Fragmented DNA} = \left(\frac{A_M}{A_K + A_M}\right) \times \left(\frac{100}{1}\right)$$

Where,

A_M = Absorbance of supernatant

A_K = Absorbance of Pellet

Preparation of Reagents: See Appendix 9

3.15. Determination of Prostatic Zinc and Selenium Levels

The quantity of selenium and zinc and were assessedby the technique of Ammerman *et al*.(1974).

Procedure

The levels of zinc and selenium in the prostate were assessed using atomic absorption spectroscopy(AAS) as described by the manufacturers guide. Prostate post mitochondrial fraction (1 mL) was deproteinised with 9 mL of 10% TCA in 0.1% Lanthanum liquid and centrifugedfor ten minutes at 3000 g. The supernatant was diluted with 0.1% Lanthanum for Selenium and aspirated to the AAS, the supernatant was diluted in ratio 1:4 with water for Zinc and aspirated to the AAS while thestandard and blank were prepared by diluting with 5% glycerin.

Calculation $\mathrm{A\,(mg/L)} = \mathrm{R} \times \mathrm{D}$

Where:

A= Zn/Se, R= AAS reading, D=Dilution factor

Preparation of Reagents: See Appendix 10

3.16. Determination of Serum Total Cholesterol Level

The level of serum cholesterol was assessed as described by Richmond(1973) using Randox kit.

Principle

The principle is centered on the enzymatic hydrolysis and oxidation of cholesterol which resultsin the production of H_2O_2. Once the H_2O_2 formed reacts with phenol and 4-aminophenazon, a red color is formed. The color intensity correlates with the level of cholesterol.

Procedure

The sample (10 µL) was mixed with 1.0 mL of cholesterol reagent (mixture of 4-aminopyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and pipes) and incubated for five minutes at 37°C. The blank and the standard were treated the same way by replacing the sample with distilled water or standard respectively. The absorbance was read at 500 nm against reagent blank.

Calculation $$\text{Cholesterol content} = \left(\frac{A_{sample}}{A_{standard}}\right) \times \left(\frac{195\ mg/dL}{1}\right)$$

A_{sample} = Absorbance of sample

$A_{standard}$ = Absorbance of standard

Preparation of Reagents: See Appendix 11

3.17. Determination ofSerum High Density Lipoprotein Cholesterol (HDL-C) Content

This was assessed as described by Friedewald *et al*. (1972).

Principle

This is based on the precipitation of Low density lipoprotein and chylomicrons fractionsby the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined at 500 nm.

Procedure

The sample (200 µL) was added to 500 µL ofprecipitant (phosphotungstic acid and magnesium chloride) mixed and allowed to settle for 10 minutes at 25°C. The mixture was then centrifuged at 4000 rpm for ten minutes. 1.0 mL of reagent was mixed with 100 µL of the supernatant in a test tube,mixed and incubated for five minutes at 37°C. The blank and the standard were treated similarly. The absorbance was measured against reagent blank at 500 nm.

Calculation

$$\text{Cholesterol level in sample} = \left(\frac{A_{sample}}{A_{standard}}\right) \times \left(\frac{195\ mg/dL}{1}\right)$$

Where

A_{sample} = Absorbance of sample

$A_{standard}$ = Absorbance of standard

Preparation of Reagents: See Appendix 12

3.18. Determination of Serum Triglyceride Level

The levels of serum triglyceride were measured by thetechniques of Jacobs *et al*. (1960).

Principle

In the presence of lipase, triglycerides are hydrolyzed to fatty acids and glycerol. Glycerol kinase actson glycerol formed to yield glycerol-3-phoshate and adenosine diphosphate. The glycerol-3-phoshate thenundergoes oxidization to produce

dihydroxyacetone phosphate and H_2O_2catalysed by glycerol phosphate oxidase. The H_2O_2 produced then reacts with 4-aminophenazone and 4-chlorophenol to give quinoneimine.

Procedure

The sample (10 µL) was mixed with 1.0 mL of triglyceride reagent [buffer (4-chloro-phenol and magnesium ions R1a and enzyme reagent (4-aminophenazone, ATP, Lipases, Glycerol-kinase, Glycerol-3-phosphate oxidase and peroxidase- R1b)] and incubated for five minutes at 37°C. In a similar way, the blank and the standard were made by exchanging the sample with distilled water and standard reagent respectively. The absorbance was measured against reagent blank at 500 nm.

Calculation: $\text{Cholesterol content} = \left(\frac{A_{sample}}{A_{standard}}\right) \times \left(\frac{196\ mg/dL}{1}\right)$

3.19. Estimation of the levels of Nitric Oxide

This was determinedby the technique ofPalmer*et al*. (1987).

Principle

In aqeous solution, nitric oxide rapidly degrades to nitrate and nitrite. Using Griess reagent, the concentration of nitrite in the sample was measured as an indicator of nitric oxide production.

Procedure

The content of nitrite in supernatants or in serum were measured by incubating 0.5 mL ofsample and0.5 mL of Griess reagent at 25°C for twenty minutes and the absorbance recorded at 550 nm. The amount of nitrite was extrapolated from the calibration curve. Calibration curve was prepared over a linear range of nitrite between 10 and 100 µmol/L.

Preparation of Reagents: See Appendix 13

3.20. Determination of Myeloperoxidase Activity (MPO)

This was measuredas described by Trush *et al.*(1994).

Principle

The principle is based on the ability of MPO, in the presence of H_2O_2 as an oxidizing agent, to catalyse the oxidation of o-dianisidine yielding a brown coloured product with a minimum absorbance at 470 nm, according to the following overall reaction:

$$2H_2O_2 \rightarrow O - \text{dianixidine (Oxidixed)} + 4H_2O$$

Procedure

An aliquot of 400 µL of O-dianisidine, 100 µL of H_2O_2 (diluted) and 14 µL of sample was mixed in a cuvette and the absorbance of the brown colored product was recorded at 60 seconds interval for 3 minutes at 470 nm.

Calculation

$$\text{MPO activity (U/mg Protein)} = \frac{\Delta\text{Abs}\,(t_2 - t_1/\text{min} \times \text{volume of mixture} \times \text{dilution factor}}{(11.3 \times 10^{-3}) \times \text{volume of sample} \times \text{mg protein}}$$

Preparation of Reagents: See Appendix 14

3.21. Determination of Aniline Hydroxylase (AnH) Activity

This was evaluatedby the techniques of Ko *et al*.(1987).

Principle:

This is based on the quantity of *p*-aminophenol formed during the hydroxylation of aniline hydrochloride. The *p*-aminophenol formed reacts with phenol to form an indophenol dye (blue),in an alkaline medium, whose absorbance can be measured spectrophotometrically at 630nm.

Procedure

The solution of NADPH (100 µL), aniline hydrochloride (500 µL) and post-mitochondrial fraction of the prostate (500 µL) were mixed in a test tube and incubated for thirty minutes at 37^0C. The reacting mixture was stopped by adding 20% TCA solution (1.5 mL)and then centrifuged at 2000 g for five minutes to remove the precipitated protein. The supernatant (1.0 mL) was mixed with 1.0 mL of NaOH/Phenol and 1.0 mL of sodium carbonate (1.0M) at 37°C for thirty minutes. The blue colored conjugate of phenol and*p*-aminophenol formed was measuredat 630 nm. The standard and blank wereprepared by adding p-aminophenol (500 µL) and aniline hydrochloride (500 µL), respectively instead of the prostate before the addition of TCA.

Calculation:

$$\text{The activity of AnH} = (A_{\text{test}} \times 7 \times 60 \times 2)/A_{\text{standard}} \times 30 \times \text{mg Protein}$$

Where

A_{test} = Absorbance of test sample

$A_{standard}$ = Absorbance of standard

Preparation of Reagents: See Appendix 15

3.22 Determination of Aminopyrine-*N*-Demethylase (AmD) Activity

This was assessed as described by Tu and Yang (1983).

Principle:

The aminopyrine-*N*-demethylase activity was assesed based on the *N*-demethylation of aminopyrine (4-dimethyl aminoantipyrine) to 4-aminoantipyrine with a step-wise formation of formaldehyde. The amount of formaldehyde formed was taken as a function of AmD activity.

Procedure

TheNADPH solution (1.0 mL), *p*-aminopyrine (500 µL), semicardazide (200 µL) and sample (500 µL) were mixed in a test tube and incubated for ten minutes at 37°C. Addition of 20% $ZnSO_4$ (1.5 mL) stopped the reaction. To prepare the standard and blank,500 µLofformaldehydeand 500 µLof *p*-aminopyrine was added to the mixture instead of sample, respectively. Addition of two millilitre of saturated barium hydroxide and 1.0 mL of $B_4Na_2O_7$ solutions precipitated the proteins and the mixture was centrifuged at 2000 g for five minutes. The amount of formaldehyde in the supernatant was evaluated by incubating 1.5 mL of the supernatant and 1.5 mL of the double-strength Nash reagent at 60°C for forty minutes. The absorbance was measured at 412 nm against the blank.

Calculation

The AmD activity was calculated as follows:

$$\text{Specific Activity} = (A_{\text{sample}} \times 0.6 \times 60 \times 2)/(A_{\text{standard}} \times 10 \times \text{mg protein/mL})$$

Where

Asample = Absorbance of sample

$A_{standard}$ = Absorbance of standard

Preparation of Reagents: See Appendix 16

3.23. Histopathological Studies of Prostate Tissue

A section of the prostate tissue fixed in 10% formalin was dehydrated in 95% ethanol and cleared in xylene before embedded in paraffin. Micro sections (3-4 µm) were prepared and stained with haematoxylin and eosin (H&E) dye, and were examined under a light microscope.

3.23. Immunohistochemical Assessment of Proteins in Prostate Tissue

The immunochemical examination of prostatic tissue for the expressions of some proteins wasbyChakravarthi *et al*. (2010).

Procedure

The deparaffinized prostateslices were subjected to the peroxidase labeled streptavidin-biotin technique, using the monoclonal antibodies against PSA and Ki67 antigens. All the chemicals were manufactured by Novocastra (LEICA). The dilution factor for the antibody used was 1:100 for all the antigen markers. Prostate tissue was split at 2 microns on the rotary microtome and fixed on slides. The slides were placed on the hot plate at 70°C for at least1 hour. The fragments were passed into water, after being passed through two changes of xylene three changes of descending grades of alcohol. Antigen was recovered by boiling the segmentsin citric acid (pH 6.0) for 15 minutes at 100°C. The slices were evenly balancedprogressively with cold water in order to remove the hot citrate for about 5 minutes to allow cooling of the slices.

The blocking of the peroxidase was carried out by covering the sections with 3% $H_2O$for a quarter of an hour. The prostate sliceswere rinsed with PBS and the protein blockingwas done using avidin for 15 minutes.The prostate sections were rinsed again with PBS and the endogenous biotin in the prostate was blocked by biotin for 15 minutes. The slices were then incubated with 5µg/mL each of the proteins antibodies for 60 minutes. The unbound antibodies were rinsed off by PBS (pH 7.4) and a secondary antibody (LINK) was applied on the slices for 15 minutes. The slices were rinsed and Horseradish peroxidase (HRP) label was applied on the slices for 15 minutes and then rinsed with PBS for 5 minutes to remove HRP. Visualization of the reaction products was done by immersing the slice in Karnovsky solution (0.01% diaminobenzidine (DAB) in Tris-buffer containing 0.05% H_2O_2 and 0.01% NaN_3). Excess precipitate and diaminobenzidine solution were rinsed off using water. Haematoxylin solution was used to counterstain the tissue slices for 120 seconds. Lastly, the slides were dried using alcohol, cleaned in xylene, mounted with DPX mountant and then observed under microscope (Magnification X 400).

3.25. Determination of Serum Reproductive Hormones by ELISA Method

The serum testosterone was assayed using ELISA kit following manufacturer's (Abcam) guide.

Principle:

This is based on the affinity and specificity of monoclonal antibody targeted towards a definite antigenic determinant on the whole hormone molecule. The levels of the hormone are directly proportionate to the intensity of the color formed from the sample.

Procedure:

The microplate wells for each sample, calibrator, blank and control serum were set up to carry out the assays in replica. To each calibrator, samples and control serum (25 µL) were pipetted into suitable wells afteradding theconjugate solution (100 µL) to each well except blank. The microplate was whirledslightly between twenty–thirty seconds and then covered. The mixture was then incubated at 25°Cfor sixty minutes. 300 µLof washing solution was added and then decanted. Addition of washing solution and decantation were done five times.TMB-substrate (100 µL) was added into all wells at scheduledtime (shaking of the plate was avoided after adding TMB-substrate). The plate was incubated again at 25°C in the dark for twenty–thirty minutes followed by the addition of stopping reagent (150 µL) to all wellsas before. The incubation mixtures were slightly mixed for five-ten seconds and the absorbance takenat 450nm aftertwenty minutes using microplate reader.

3.26. Statistical Analysis.

All valuesare presentedasmean ± standard deviation of seven rats per group (n=7). The data in this study were evaluatedby the use of one-way analysis of variance and were taken to be statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1. Experiment 1: Ameliorative effects of methyl jasmonateon testosteronepropionate-induced benign prostatic hyperplasiarats

Introduction

Benign prostatic hyperplasia (BpH) isregarded as the increase in the prostate mass and this usually goes with lower urinary tract symptoms, however, the mechanism involved in its pathogenesis is unclear (Park *et al*., 2004). Current drugs used to manage BpH have limited efficacy and caused adverse effects, necessitating the continuous search for better option. Methyl jasmonate (MeJa) is a known anticancer agent via several mechanisms. However, information on its protective rolein benign prostatic hyperplasia development is very rare. Hence, thestudy is aimed at the evaluation of the protective effect of methyl jasmonate in BpH rats.

Procedure

Forty-nine (49) Wistar rats were divided into seven groups of 7 rats per group. Seven of the rats were not castrated and were referred to as control1 (received corn oil) while the rest(forty two)werecastrated and grouped as follows: Control 2 (Corn oil only), rats administered with testosterone propionate (BpH), rats administered with testosterone propionate (Tp)and MeJa (BpH + MeJa), rats administered with Tp and finasteride (BpH +finasteride), rats administered with MeJaonly (CMeJa) and rats administered with finasteride (FN) alone (CFN). The drugs, dissolved in corn oil,were prepared and stored in the refrigerator overnight before use. All drugs, Tp (3 mg/kg*i.p.*), MeJa (50 mg/kg*i.p.*) and FN (10 mg/kg*p.o*)were given daily to the rats for 28 days. The rats were fed with rat pellets and water *ad libitum.* The preparation of serum and tissue for

biochemical assays was as described in page39 and the assay procedures were as stated in the materials and method (pages 38-53).

Results

The result in Table 4.1 depicts that castration decreasedsignificantly the prostateweight as well as the relative weight of control 1(non-castrated rats) relativeto control 2 (castrated rats) by 54% and 50% respectively. Administration of testosterone propionate (Tp) dailyto castrated rats for 28 days(i.e. BpH) increased the prostatic wet weight and its relative weight ratio significantly compared to control 2. The prostatic wet weight and its relative weight ratio in castrated rats that received Tphad 228% and 171% increase, respectively. Administration of finasteride to Tp-treated rats for 28 daysdecreased the prostatic weight by just 11% whileMeJa administeration to BpH ratshad no significant effect.

In Table 4.2, castration did not significantly ($p>0.05$) change the phosphatases (prostatic, total and non-prostatic) activity relative to non-castrated rats (control 1). On the other hand, administration of Tpto castrated rats raised the prostatic, total and non-prostatic acid phosphatase activities by 265%, 455% and 766%, respectively, relative to castrated rats (control 2). Remarkably, MeJa administration reduced the activity of non-prostatic phosphatase in benign prostatic hyperplasia rats significantly ($p<0.05$) by 34% while treatment with FN reduced the non-prostatic acid phosphatase activity by 83% relative to BpH rats.The activity of prostatic alkaline phosphatase (ALP) was significantly ($p<0.05$) reduced by castration by 66% when compared with the those that are not castrated whereas administration Tp for 28 days to castrated rats improved the ALP activity by 522% when matched with the castrated rats. However, only a decrease of 18% was noticed in ALP activity when BpH rats were treated with MeJa. In addition, administration of Tp injection for 28 days to castrated rat brought aboutanupsurge in the amounts of zinc in the prostate homogenate by 20% whereasthe selenium levels was reduced by 11% relative to control 1(castrated control rats (Figure4.1 and Figure 4.2). Intraperitoneal injection of MeJa to BpH ratsreduced the zinc level significantly by 33% relative to castrated rats that received Tp but insignificant change in the selenium level in castrated rats that received Tp and methyl jasmonate was observed. Nevertheless, the concentration of prostatic protein in all groupswas reduced significantly when compared to control 1 (Figure4.3).

Moreover, castration improvedthe levels of malondialdehyde (lipid peroxidation index-MDA) by 89% relative to the non-castrated controls while injection of Tp for 4 weeks

increased the MDA levels by 39% as compared to control 2. Remarkably, injection of MeJa or FN to BpH rats for 28 days reduced the levels of MDA by 70% or 62%, respectively when compared with BpH rats (Figure 4.4). Furthermore, in rats with BpH, the activities of SOD and CAT (Figure 4.5), GSH levels (Figure 4.6) and the activity of GPx (Figure 4.8) were decreased ($p<0.05$) significantly by 33%, 62%, 36% and 29%, respectively relative to Control 2. In the BpH+ MeJa rats, the level of GSH was improved by 22%, the activities of CAT and SOD were improvedconsiderablyby 22% and 35%, respectively while insignificant difference was noticed in GPx activity relative to BpH rats. Also, prostatic GSTactivity in BpH rats was significantly elevatedas compared with Control 2 (Figure 4.7),conversely, the activity of the enzyme was reduced by 41% or 23%, respectively in the BpH + MeJa and BpH + FN rats relative to the BpH rats. The study also observed aninsignificant difference in the amount of fragmented DNA in BpH rat relatice to control 2 while the injection of MeJa to BpH rats decrease the amount of fragmented DNA by 22% when compared to BpH rats (Figure 4.6).

Also, in BpHrats (Figure 4.9), the structural features of the prostatic cells were deformed with hyperplasia of luminal epithelial region. Specifically, the nuclear-cytoplasmic ratio of the prostatic epithelial cells of BpH rats was elevated relative tocontrol 2. Remarkably, in the BpH + MeJagroup, the observeddisparities in the histological featuresof the tissue was restored close to normal (Figure 4.9) whereas, in the BpH + FN rats the aberrations caused by Tp was not reversed (Figure 4.9). In addition, the immunohistological examination of prostate tissue of BpH ratsexhibited higher expression ofPSA(Figure 4.10 and 4.11) and Ki67 (Figure 4.12 and 4.13)when compared with castrated control and treatment with MeJa or FN decreased the expression of the proteins. The expression of PSA and Ki67 castrated rats administered with MeJa only and FN only were similar to castrated control.

Table 4.1 Changes in body weight and relative weight of prostate in each group

Treatments	Body Weight			Prostate	Relative
	Initial	Final	Weight Gain	Weight	Weight
	(g)		(g)	(g)	(% body weight)
Control 1	175.00±13.63	264.29±26.24	100.00±3.54	0.39±0.04	0.14±0.02
Control 2	196.43±15.65	246.43±22.92	60.00±13.64[a]	0.18±0.06[a]	0.07±0.02[a]
BpH	235.71±24.54	317.86±40.28	87.50±14.43[b]	0.59±0.08[b]	0.19±0.03[b]
BpH + MeJa	210.71±19.82	278.57±27.51	80.00±11.43	0.54±0.05	0.19±0.01
BpH + FN	206.43±17.07	282.14±34.55	83.33±14.43	0.40±0.06[c]	0.15±0.02
CMeJa	176.43±19.68	228.57±31.43	56.67±12.52	0.13±0.03	0.06±0.01
CFN	175.71±17.38	239.29±30.89	75.00±0.00	0.12±0.04[b]	0.05±0.01[b]

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]$p<5\%$ was considered significant relative to Control 1, [b]$p<5\%$ was considered significant relative to Control 2, [c]$p<5\%$ was considered significant relative to BpH.

Table 4.2 Effects of MeJa on the activities of acid (total, prostatic and non-prostatic) and alkaline phosphatases

Treatments	PHOSPHATASES (U/L)			
	Acid			
	Total	**Non-Prostatic**	**Prostatic**	**Alkaline**
Control 1	1.73±0.35	0.83±0.29	0.90±0.06	248.40 ± 14.60
Control 2	1.36±0.18	0.50±0.35	0.86±0.17	82.80 ± 11.04[a]
BpH	7.55±0.83[b]	4.33±0.88[b]	3.22±0.05[b]	515.20±52.17[b]
BpH + MeJa	6.93±0.89	2.85±0.88[c]	4.08±0.01	423.20± 47.60[b]
BpH + FN	8.42±0.70	0.74±0.00[c]	7.68±0.70	507.84 ± 72.18
CMeJa	0.87±0.18	0.37±0.18	0.50±0.00	147.20 ± 13.89
CFN	2.11±1.23	1.11±0.53	1.00±0.30	198.72± 16.56[b]

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a] $p<5\%$ was considered significant when compared with Control 1, [b] $p<5\%$ was considered significant relative to Control 2, [c] $p<5\%$ was considered significant relative to BpH.

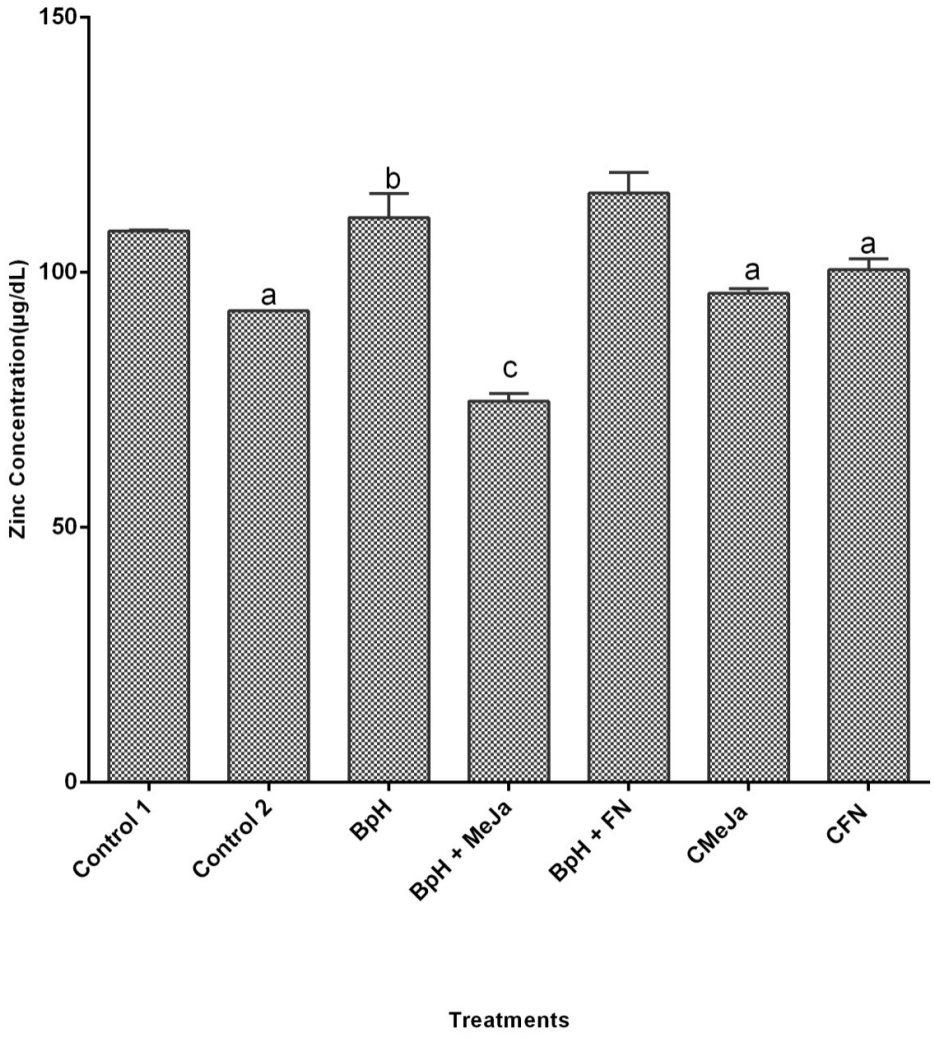


Figure4.1 Effects of MeJa on the concentration of zinc in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only.[a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c]p<5% was considered significant relative to BpH.

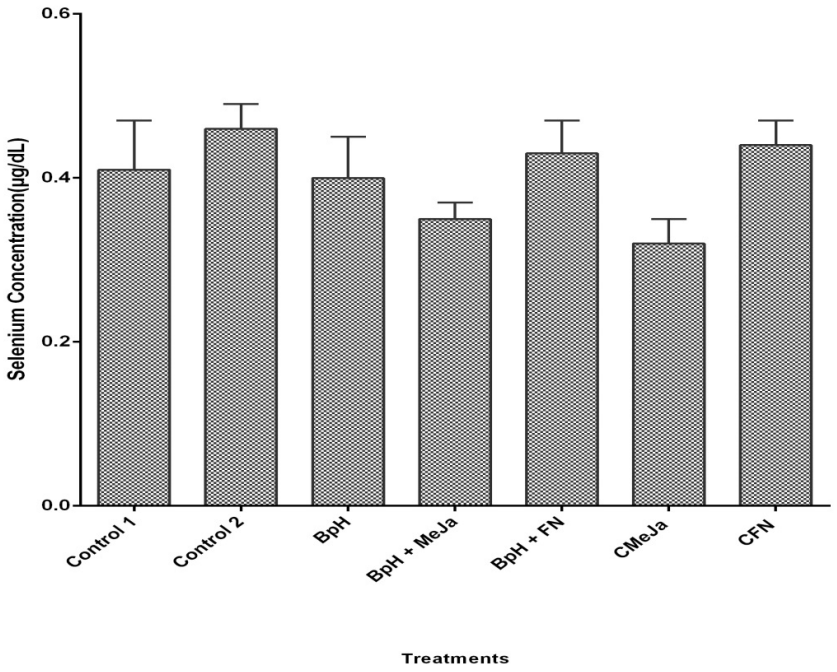


Figure4.2 Effects of MeJa on the concentration of selenium in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only.

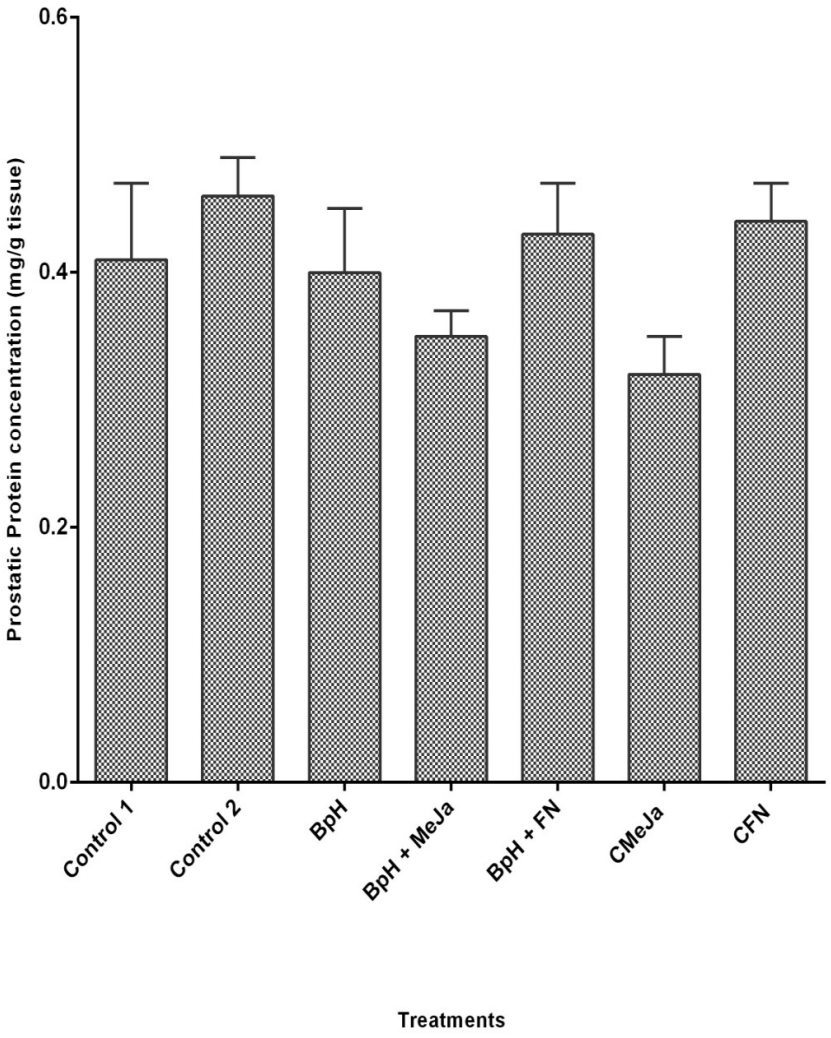


Fig 4.3 Effects of MeJa on protein concentration in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only.

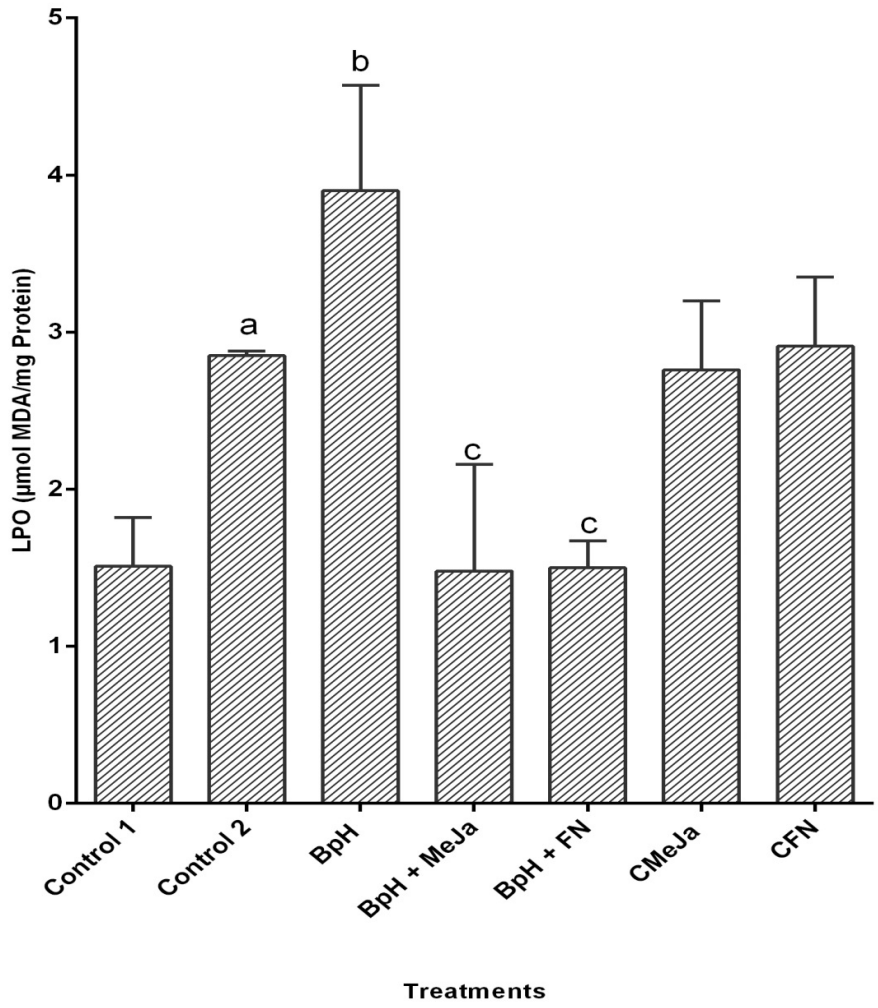


Figure4.4 Effects of MeJa on lipid leroxidation (LPO) level in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{a}p<5\%$ was considered significant relative to Control 1, $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

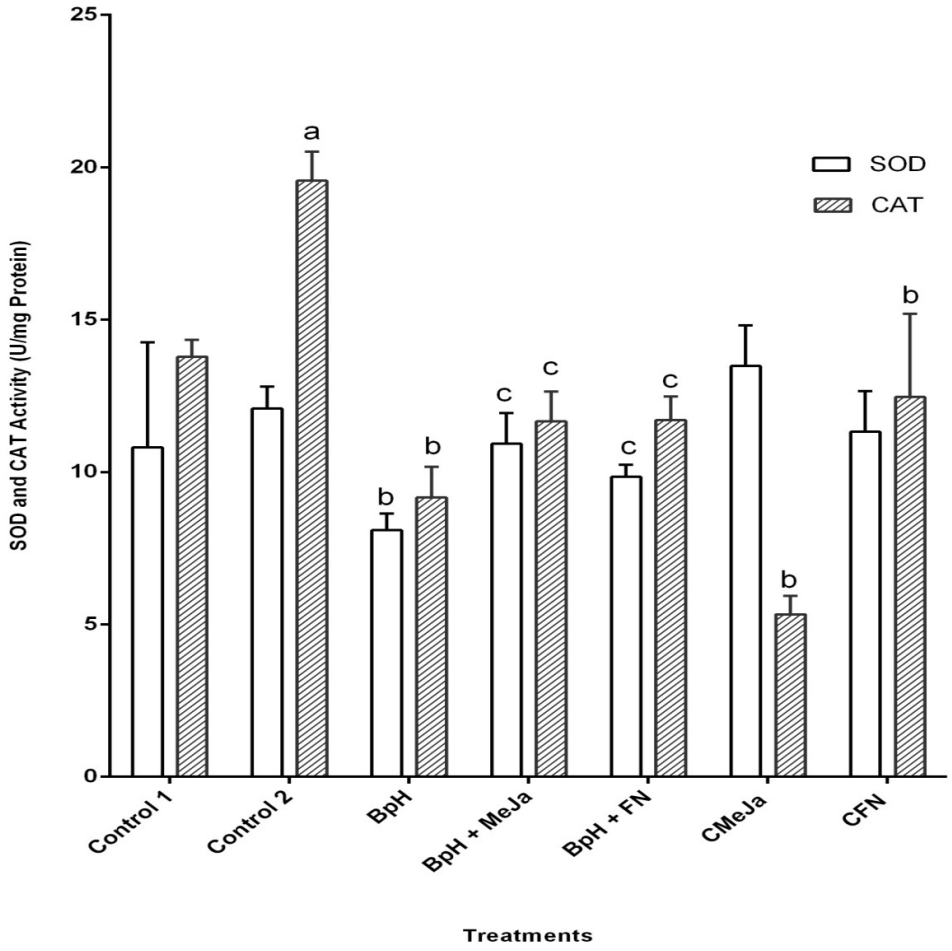


Figure4.5 Effects of MeJa on catalase and superoxide dismutase activities in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{a}p<5\%$ was considered significant relative to Control 1, $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

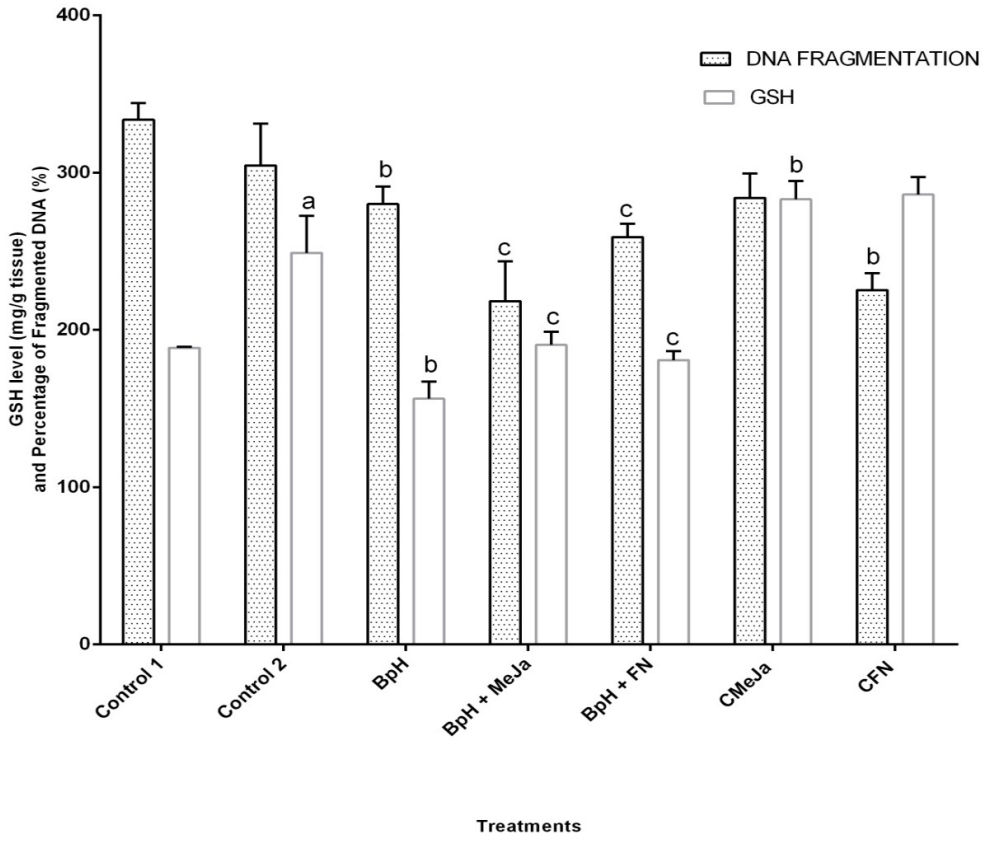


Figure 4.6 Effects of MeJa on reduced glutathione (GSH) levels and the percentage of fragmented DNA in the prostate

All values are presented as mean ± standard deviation of 7 rats in each group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c] p<5% was considered significant relative to BpH.

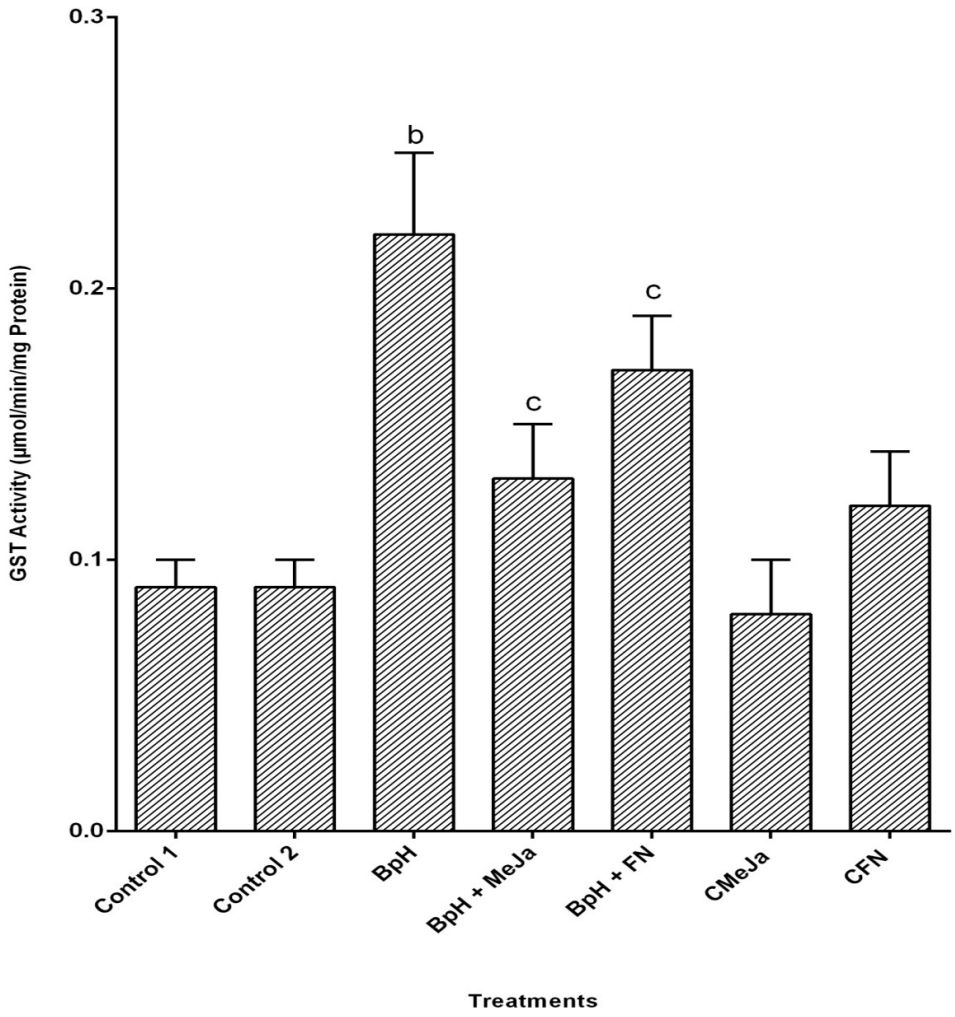


Figure4.7 Effects of MeJa on prostatic glutathione-S-transferase activity (GST)

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

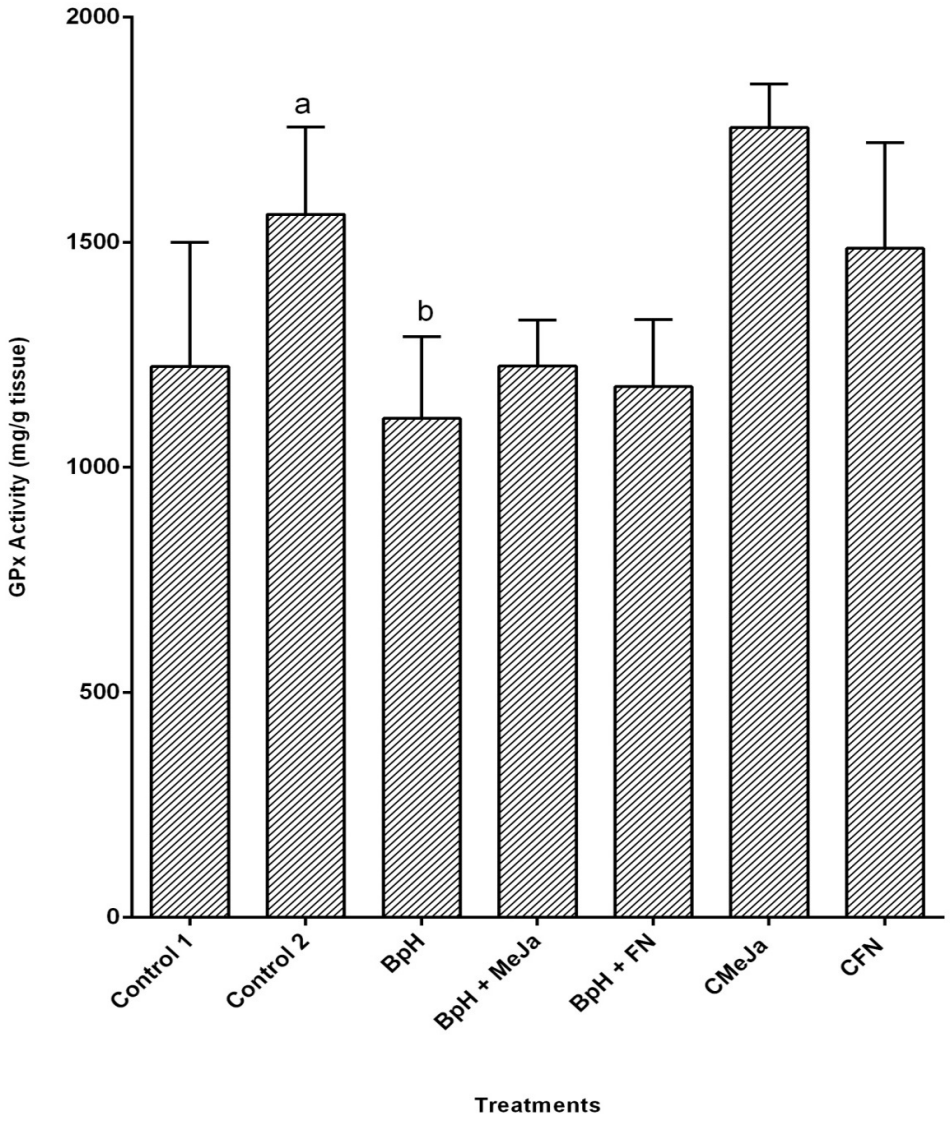
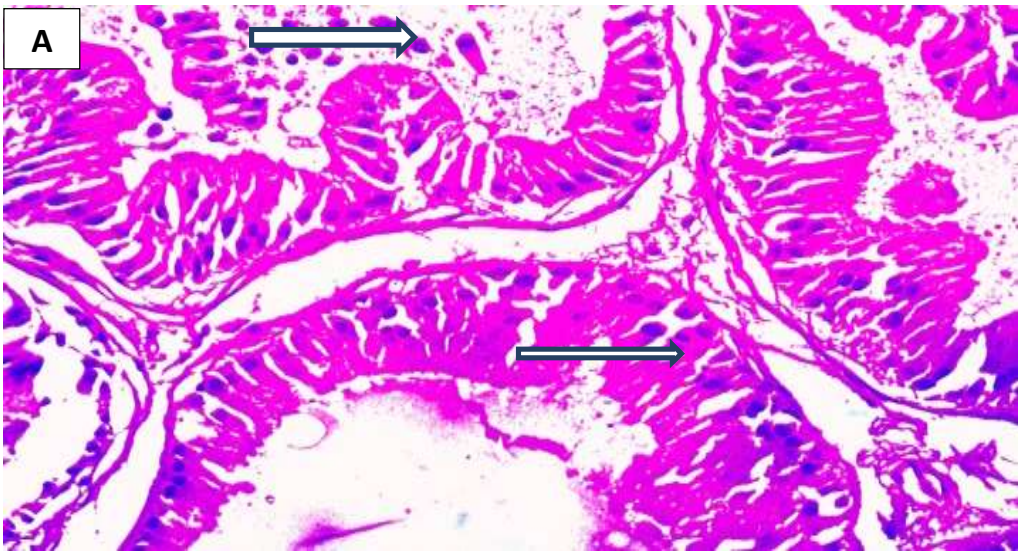
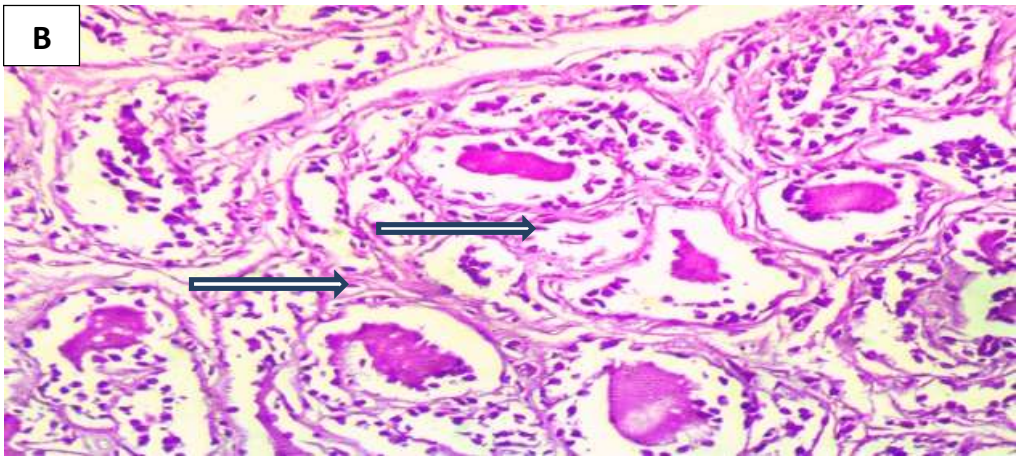


Figure4.8 Effects of MeJa on prostatic glutathione peroxidase (GPx) activity

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2.



Control 1 (Rats that were not castrated rats) –Gland shows columnar epithelium with normal luminar secretion (white arrow) and mild defoliation of columnar epithelial cells into the lumen (M X 400).



Control 2 (Castrated rats) –The epithelial cells have very high nuclear-cytoplasmic ratio (white arrow), irregular outline and severe defoliation of epithelial cells of the gland (M X 400).

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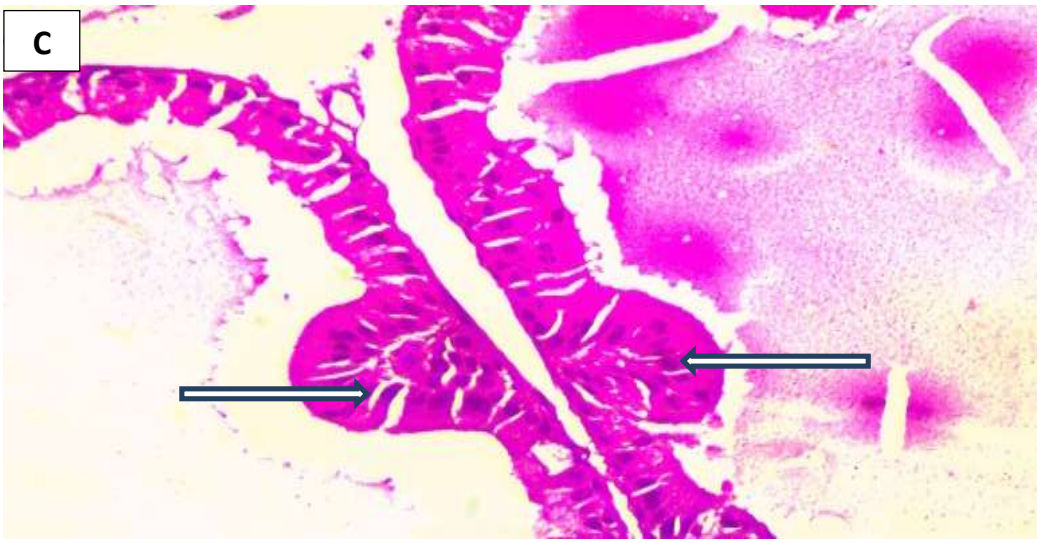
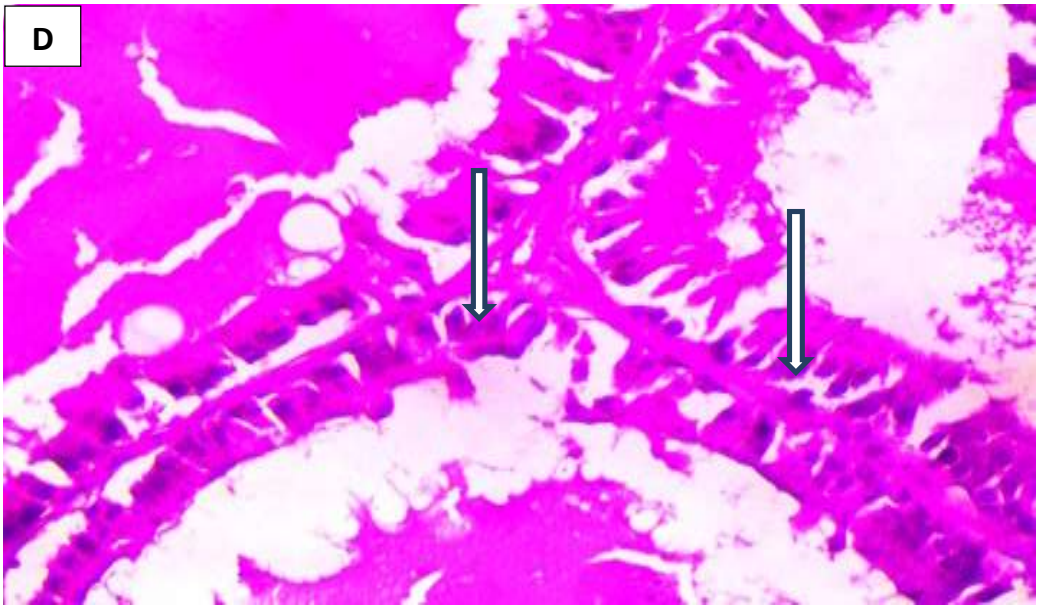
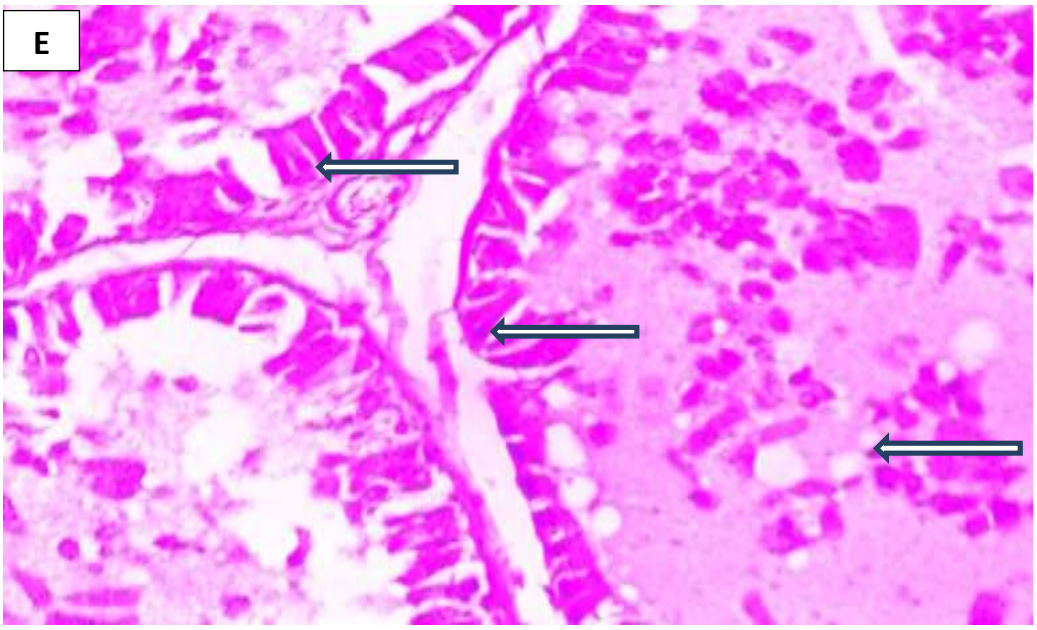


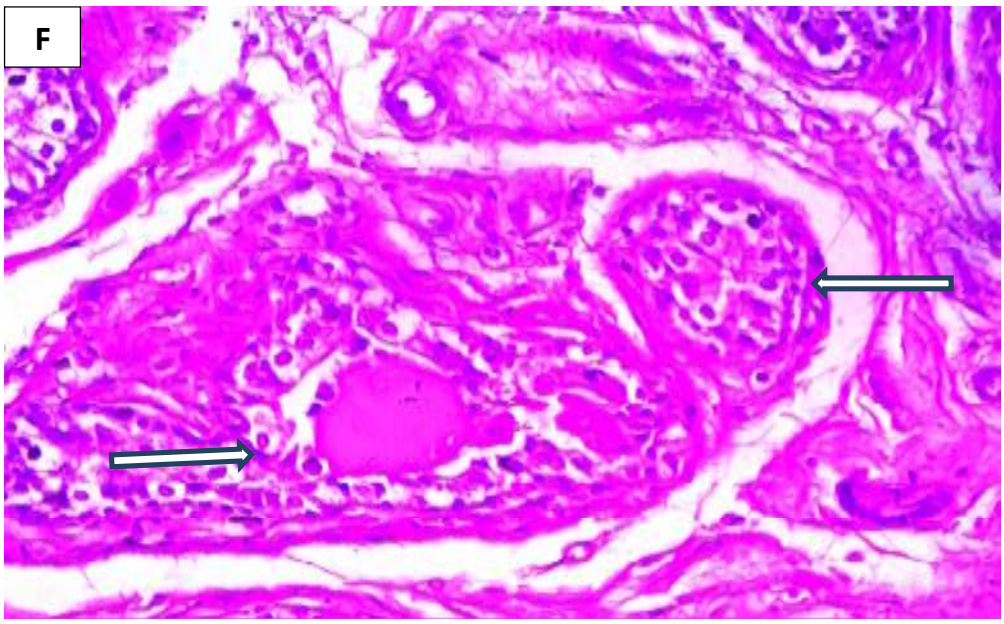
BpH (Castrated rats that received testosterone propionate only) – Glands shows numerous large glands with simple cuboidal to columnar epithelium (Hyperplasia). These glands are distended with copious amounts of luminal secretions(M X 400).



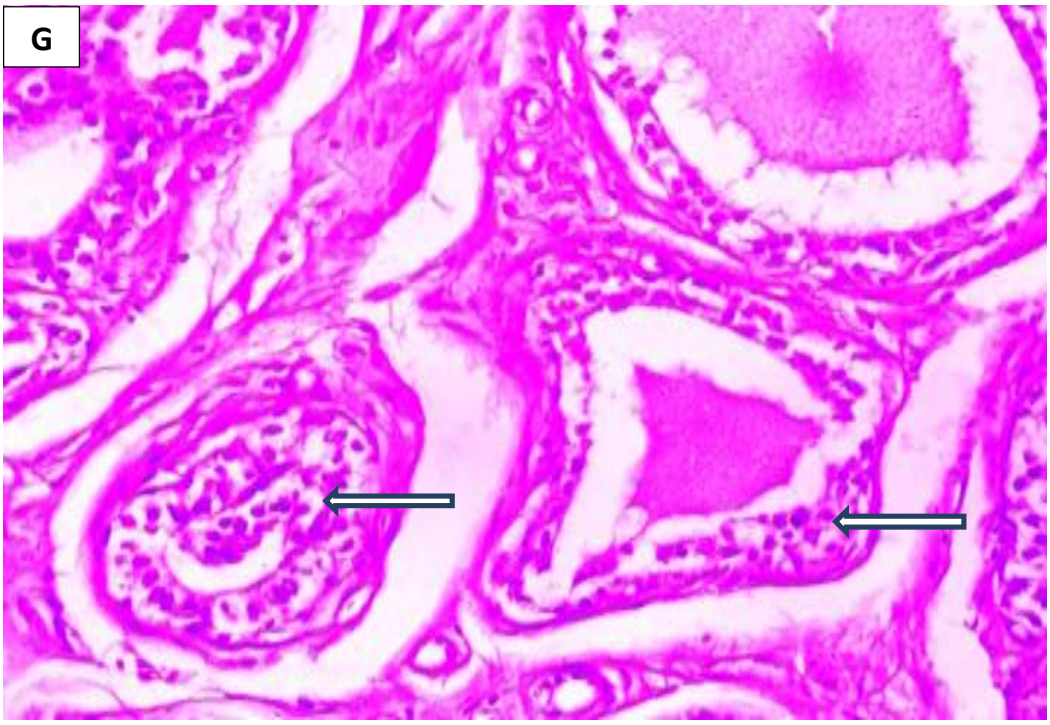
BpH + MeJa (Castrated rats that receivedtestosterone propionate and methyl jasmonate) – Gland is distended with abundant (white arrows) luminal secretions (M X 400).



BpH + FN (Castrated rats that receivedTpand Finasteride (FN).Glands are irregular and small with a disorganized epithelial lining (white arrows). There is increase in the amount of fibrous connective tissue surrounding the glands (M X 400).

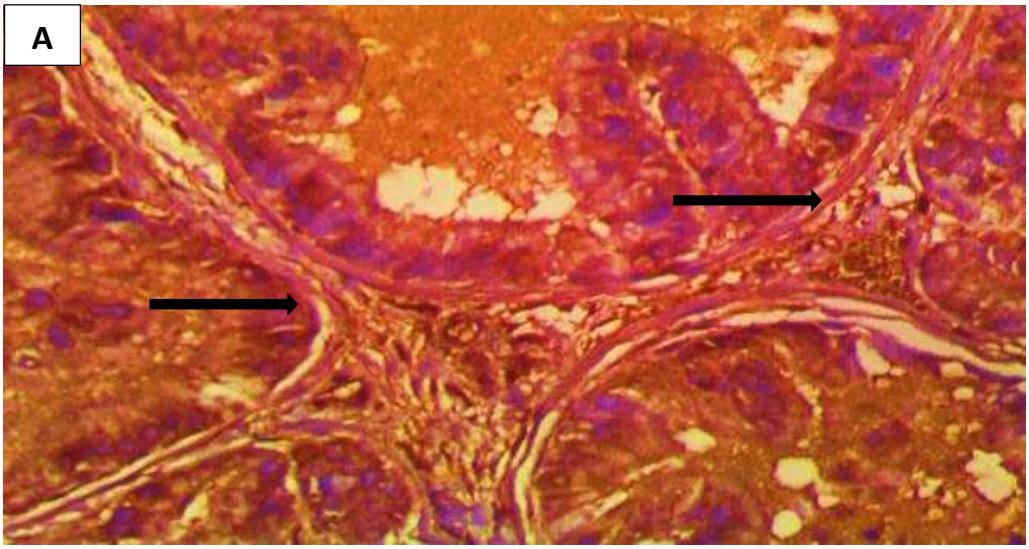


CMeJa (Castrated rats that receivedMeJa only) -- There are numerous large glands with simple columnar epithelium andmild defoliation of the epithelial cells (white arrow)into the luminal secretion (M X 400).

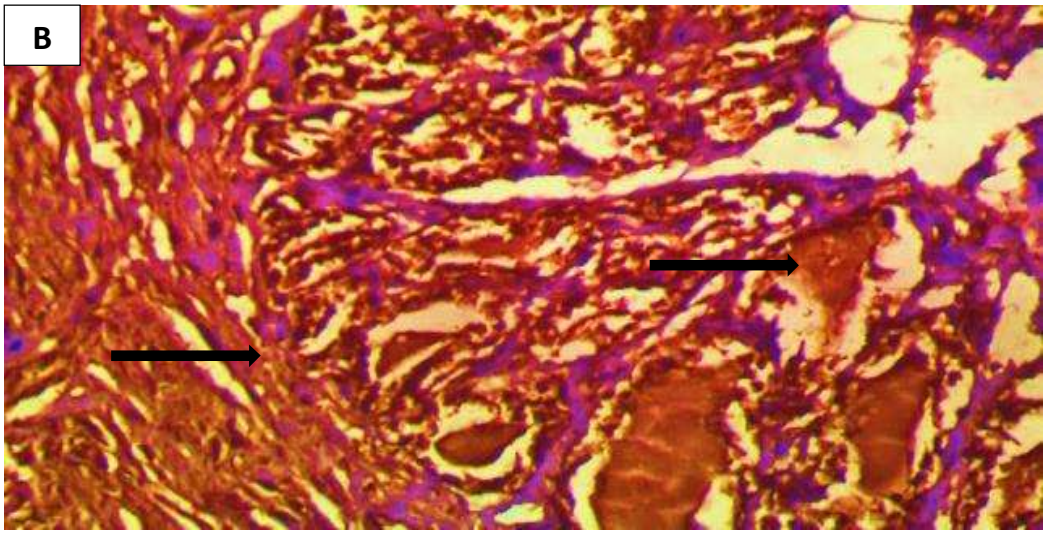


CFN (Castrated rats that received FN only) -- The glands are irregular and variably-sized, there arescant luminal secretions (white arrow) and mild disorganized epithelial lining (M X 400).

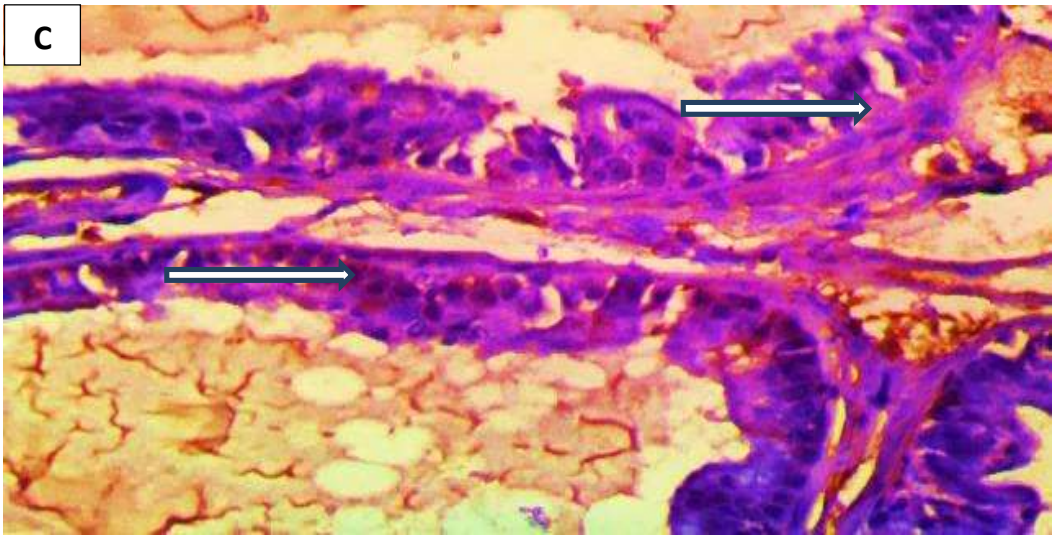
Figure 4.9(A-G) Photomicrograph of cross section of prostate tissue of BpH rats treated with MeJa for 28 days (M X 400)



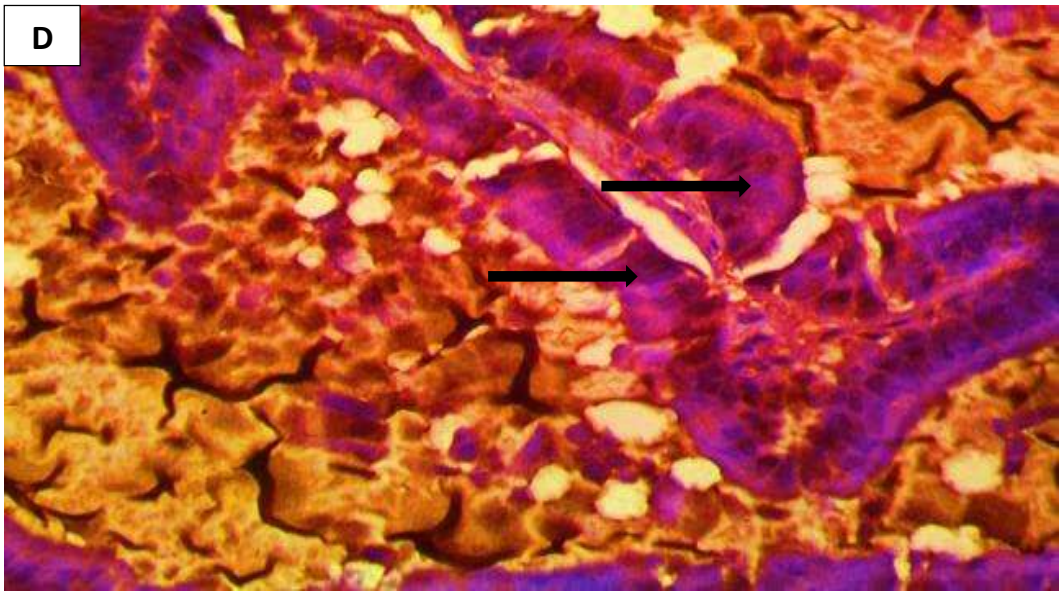
Control 1 (Rats that were not castrated rats) – Mild expression of prostate specific antigen (black arrows) indicated with black arrows (M X 400).



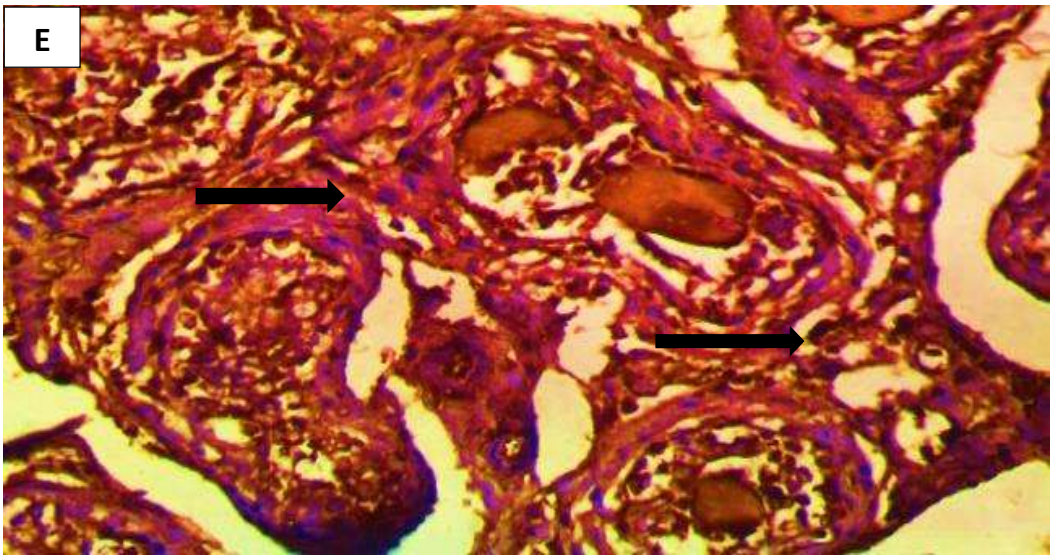
Control 2(Castrated) – Mild expression of PSAwith numerous connective tissue stroma indicated with black arrows (M X 400).



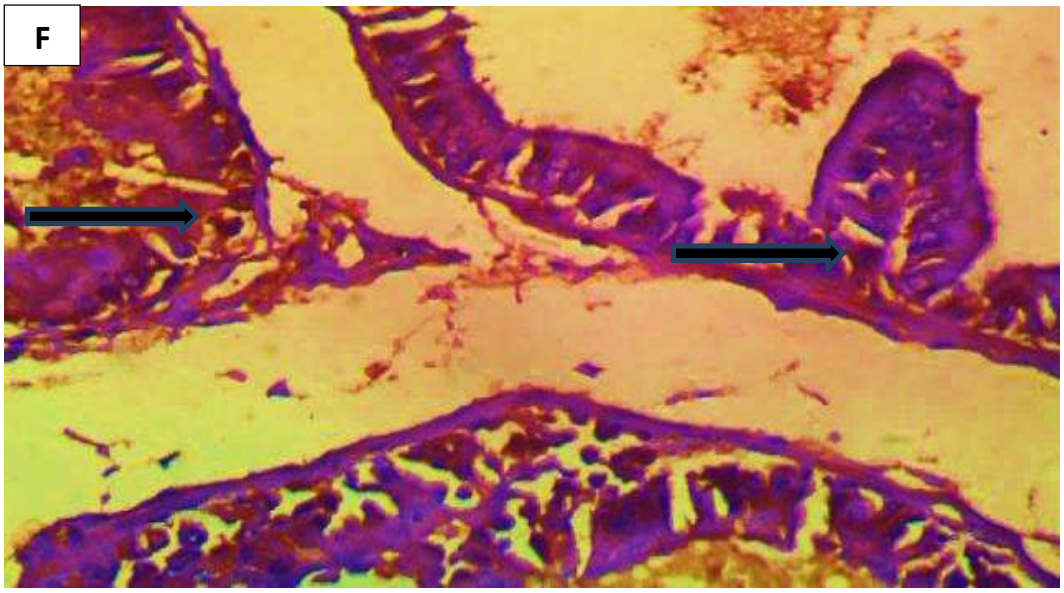
BpH (Castrated rats that received testosterone propionate (Tp) only) -- Strong expression of PSA indicated with white arrows (M X 400).



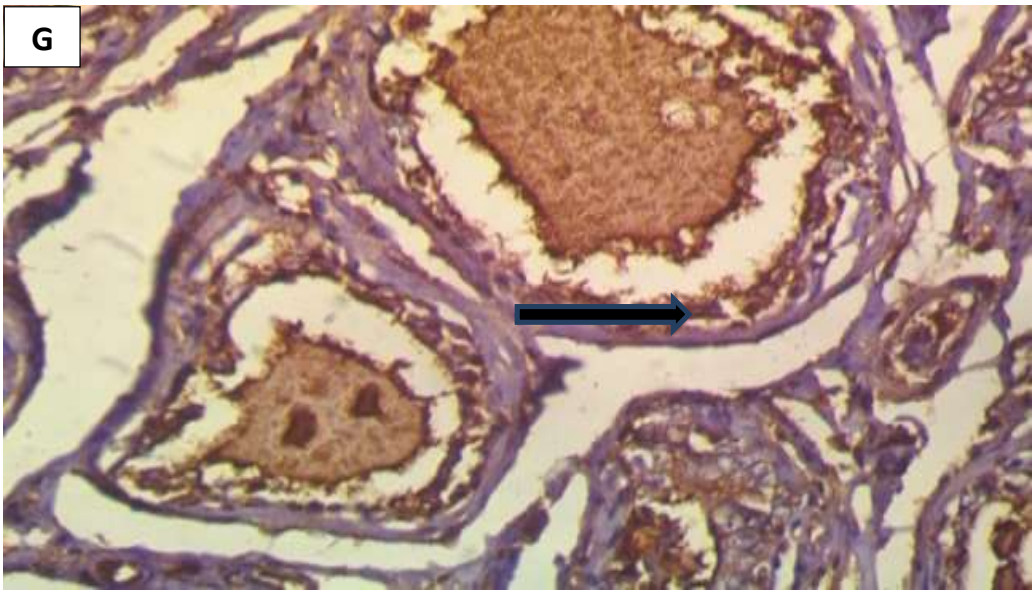
BpH + MeJa (Castrated rats that receivedTpand methyl jasmonate) – Moderate expression of PSA indicated withblack arrows(M X 400).



BpH + FN(Castrated rats that receivedTpand Finasteride) – Moderate expression of PSA by the gland indicated with black arrows(M X 400).



CMeJa (Castrated rats that received methyl jasmonate only) --Mild expression of PSA by the gland indicated with black arrows (M X 400).



CFN (Castrated rats that received finasterideonly) – Mild expression of PSA indicated with black arrow (M X 400).

Figure4.10(A-G): Photomicrograph of cross section of prostate showing the effects of MeJa on the expression of PSA via immunohistochemiscal staining of prostate tissue (M X 400)

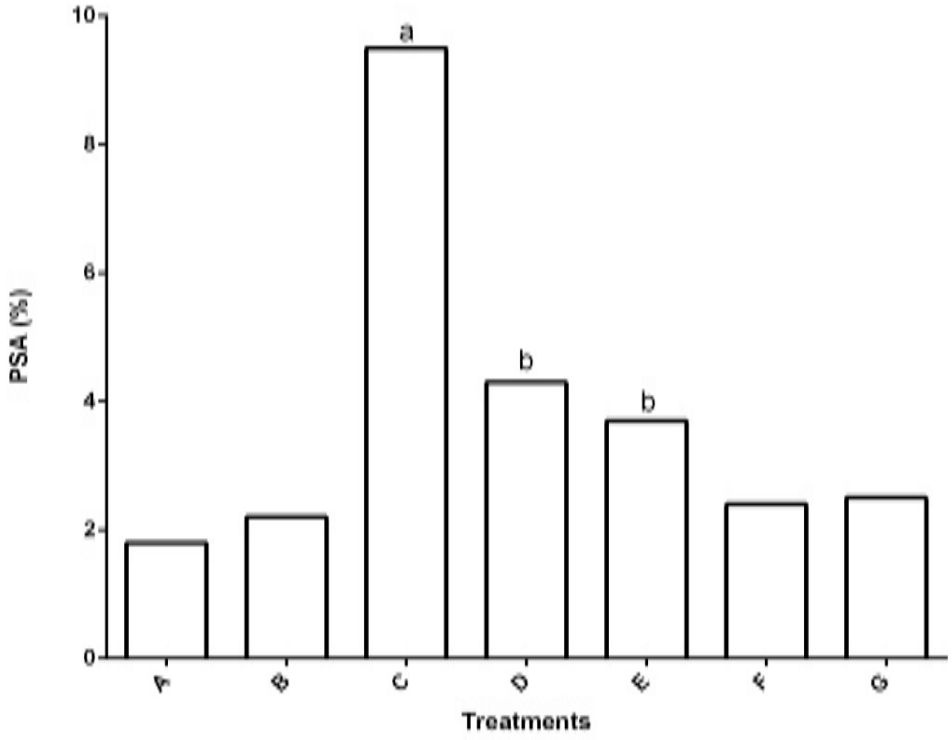
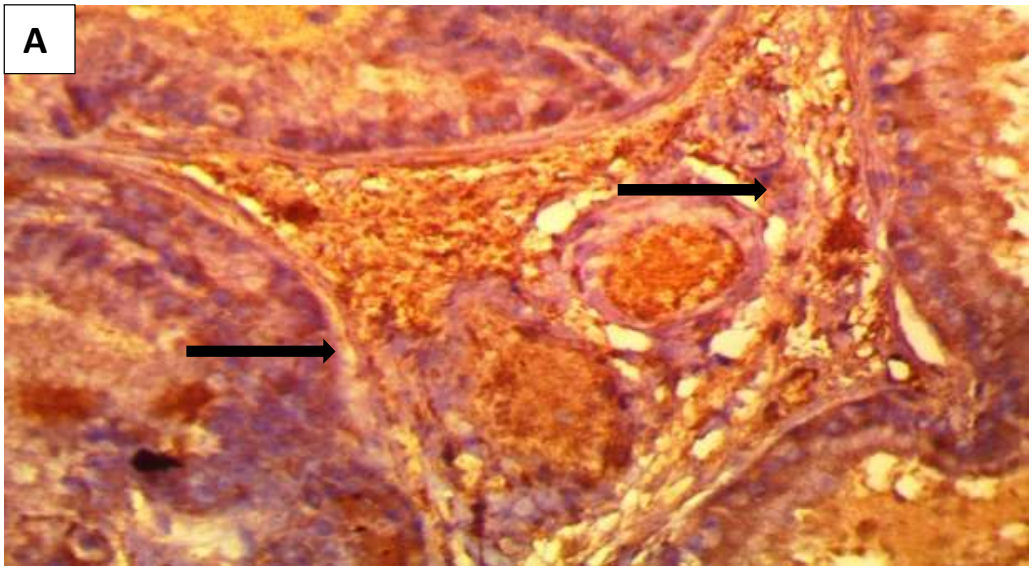
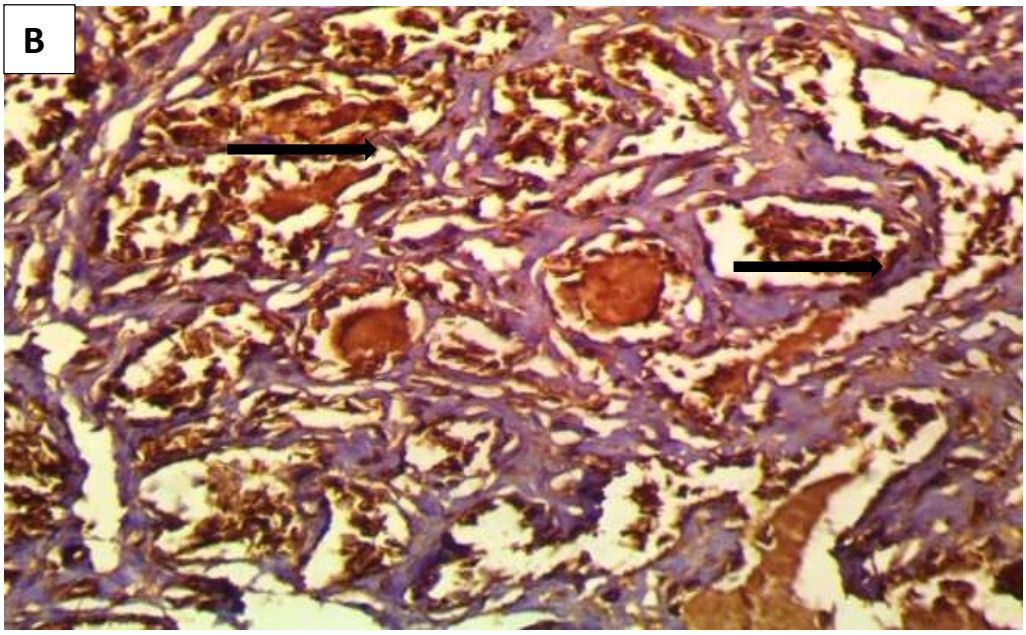


Figure4.11 Effects of MeJa on PSA expression via immunohistochemiscal staining of prostate tissue (MX400)

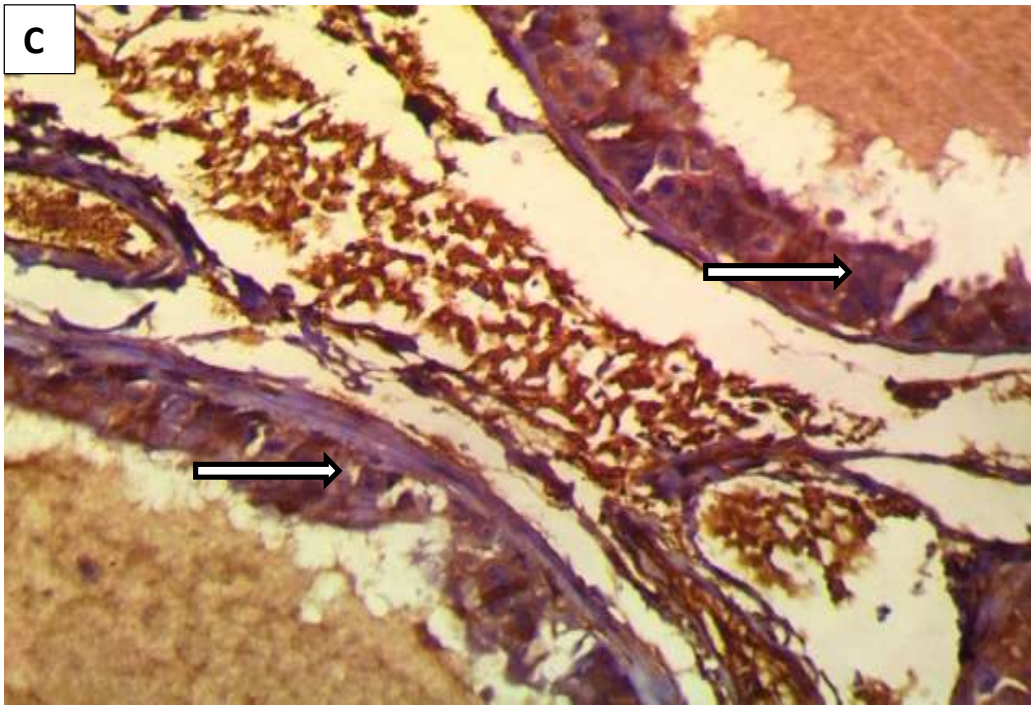
All values are presented as the mean ± standard deviation of 7 rats per group, A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. catrated rats that were given testosterone propionate, D=BpH + MeJa i.e. BpH rats treated with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats treated with finasteride (FN), F=CMeJA i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only.
[a]$p<5\%$ was considered significant relative to A and B, [b] $p<5\%$ was considered significant relative to C.



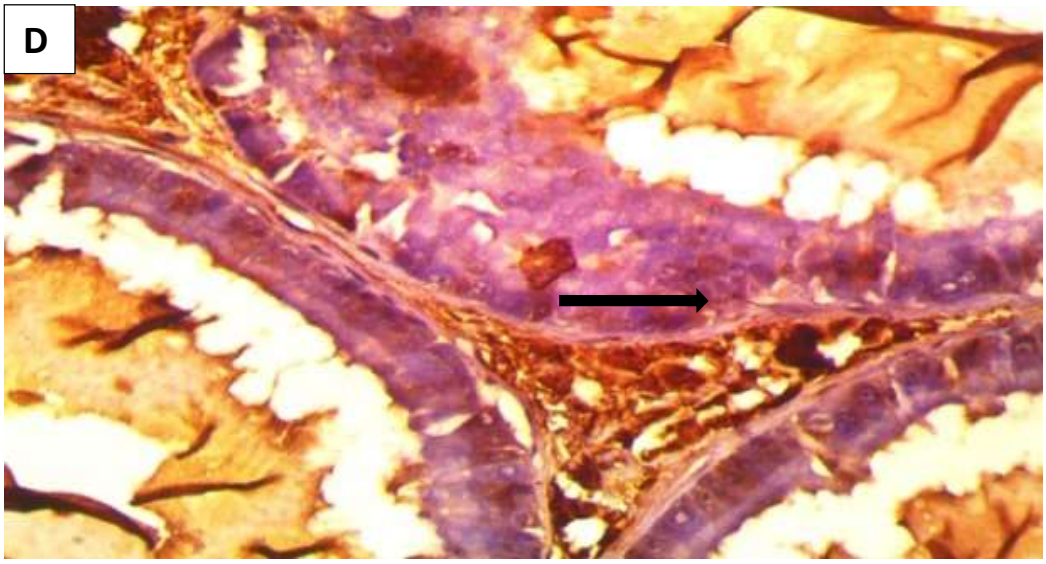
Control 1(Rats that were not castrated rats) – Mild expression of Ki67 indicated with black arrows (M X 400).



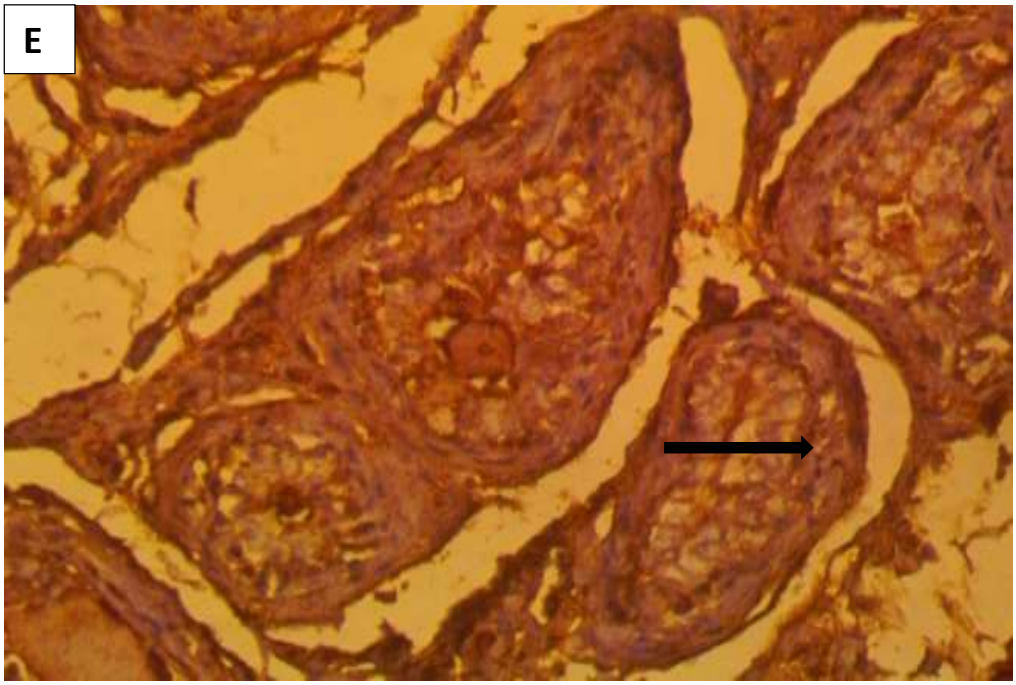
Control 2 (Castrated rats) -- Mild expression of Ki67 indicated with black arrows (M X 400).



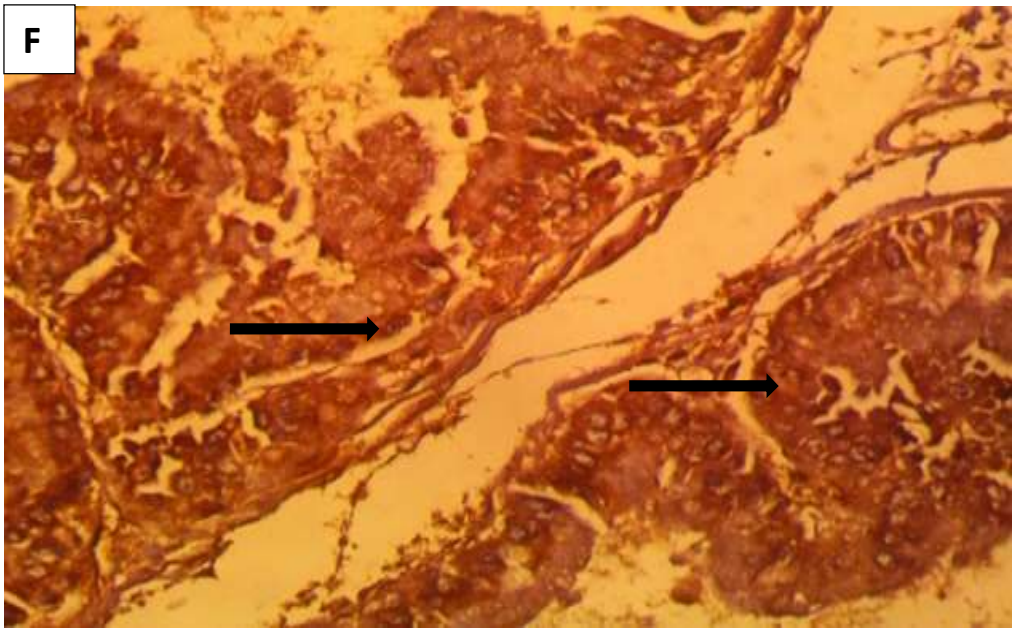
BpH (Castrated rats that received testosterone propionate (Tp) only) – Strong expression of Ki67 indicated with white arrows (M X 400).



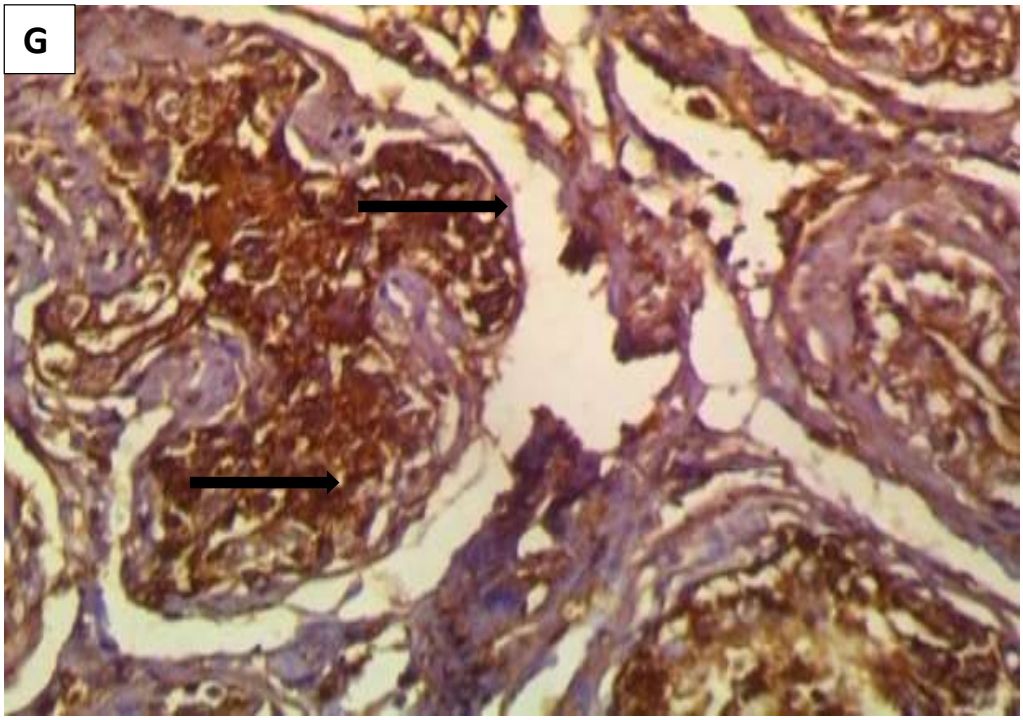
BpH + MeJa (Castrated rats that receivedTpand methyl jasmonate) – Moderate expression of Ki67 indicated with black arrow(M X 400).



BpH + FN (Castrated rats that receivedTpand Finasteride) – Weak expression of Ki67 indicated with black arrow (M X 400).



CMeJa (Castrated rats that receivedmethyl jasmonate only) – Weakexpression of Ki67 indicated with black arrows(M X 400).



CFN (Castrated rats that receivedFinasteride only) -- Weakexpression of Ki67 indicated with black arrows(M X 400).

Figure4.12(A-G): Photomicrograph of cross section of prostate showing the effects of methyl jasmonate on Ki67 expression via immunohistochemical staining of prostate tissue (M X 400)

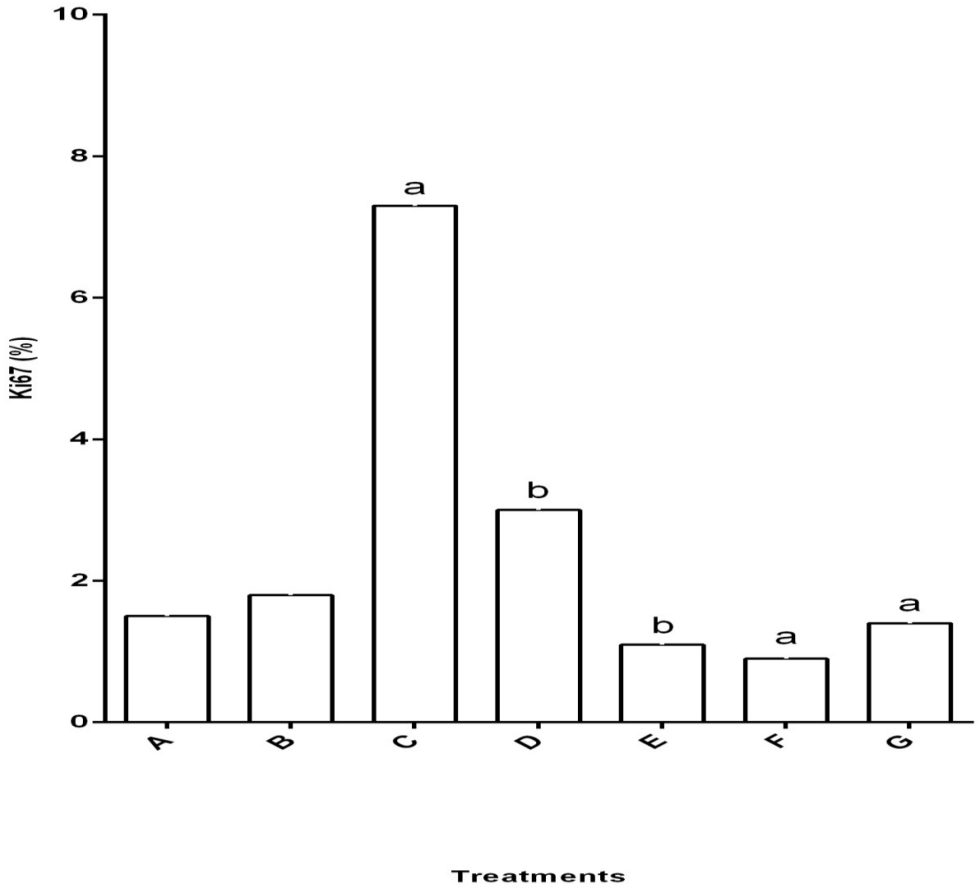


Figure4.13 Effects of methyl jasmonate on Ki67 expression via immunohistochemical staining of prostate tissue

All values are presented as mean ± standard deviation of 7 rats per group. A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. catrated rats that were given testosterone propionate, D=BpH + MeJa i.e. BpH rats treated with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats treated with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only. [a] $p<5\%$ was considered significant relative to Control A and B, [b]$p<5\%$ was considered significant relative to C.

4.2. Experiment 2:Protective role of methyl jasmonate on inflammatory and apoptotic processes in benign prostatic hyperplasia rats.

Introduction

Benign prostatic hyperplasia (BpH) isidentified asincrease in prostatemass which contributes to arange of voiding problems in elderlymales (Roehrborn, 2011). It is regarded as the fourth most widespreadailment in men above fifty years old. Pathologic BpH involves the investigation of the histologyof non-neoplastic prostatic growth in elderly males. The causal factors suggested to be implicated in the BpH development are androgen and estrogen function, embryonic reawakening, aging,inflammation and oxido-reductase, though the particular molecular modelnecessary for the initiation and maintenance of clinical sequelae resulting from BpH are partially understood (Bostwick, 2005).

Managementroutes ofbenign prostatic hyperplasia are; radiation, radical prostatectomy, hormonal therapy, 5α-reductase inhibitors and α1-adrenoceptor antagonists. Nevertheless, there are several side effects related with the treatments. The high-cost oftreatment supported the search for less-expensive and safe chemopreventive agents of plant origin for the management of BpH. In the first experiment, it has been shown that methyl jasmonatehas the ability to improve BpH by decreasingthe prostatic weight, prostatic ACPactivityand expressions of Ki67and PSA. Nevertheless, the protective role of methyl jasmonate on inflammation and apoptosis in BpH is still unclear. This second experiment was therefore aimed at the determination of the exact mechanism employed by MeJaand the synergistic effects of finasteride and MeJain BpH.

PROCEDURE

Forty eight (48) Wistar rats allotted into 8 groupsof 6 rats each were used in the study. Six of the rats were not castrated(Control 1) and were fed with the usual rat feed together with corn oil while the rest(forty two) had their testis removed i.e castrated and divided as follows: Control 2 (rats that were given corn oil), rats that were givenTp (BpH), rats that were givenTp and MeJa (BpH + MeJa),rats that were givenTp and FN (BpH + FN), rats that were givenMeJa alone(CMeJa), rats that were given FNalone (CFN) and rats the were given Tp, MeJa and FN (BpH + MeJA + FN) . The vehicle used to dissolve the drugs is corn oil. These drugs were prepared at least 12 hours and preserved in the refrigerator prior to use. Methyl jasmonate (50 mg/kg) and testosterone propionate (3 mg/kg) were given via intraperitoneal route whilefinasteride(10 mg/kg) were given by oral gavage, respectively,28 days. The preparation of serum and tissues

for biochemical assays was as described on page 38 and the procedures for the experimentswere as described in pages 38-53.Rats were fed with rats feed and water *ad libitum.*

RESULTS

The study observed no noticeable change in the weight of BpH rat (Table 4.3) relative to control 2. However, the weight gain of BpH rats that were given FN and MeJa (singly or when combined) significantly ($p<0.05$) increased by 126%, 116% and 211%, respectively relative to BpH rats. Prostate weight and relative weight are generally used to estimate the progression of BpH. In Table 4.3, the weight of the prostate inBpHrats improved by 580% relative toControl 2, specifying that BpH was successfully established by Tpin the control 2. However, co-administration of FN and MeJa toBpH rats lowered the observed increase by 44%, although treatment with MeJaand FN(singly) also decreased the prostatic wet weight by 32% and 44%, respectively. A 400% increase was observed in the prostatic relative weight of castrated Rats that were administered with Tp relative to those that did not receive Tp while managementwith MeJa and FN(individuallyor when combined) reduced the weight by 20%,35% and 40%, respectively (Table 4.3).

Castration had no significant influence on the level of serum total cholesterol, triglyceride (Figure 4.14 and 4.15) and HDL-c (Figure 4.16) relative tocontrol 1. The levels of serum triglyceride and total cholesterol was raised by 43% and 53% while that of HDL-c was reduced by 12%, respectively in BpH rats compared tocontrol 2. However,treatment of BpH rats withMeJa and FN (when combined)reduced the volume of total cholesterol and triglyceride while HDL-c levels increased significantly relative to BpH rats. Also, administration of MeJa alone to BpH rats also decreased total cholesterol and triglyceride by 25% and 45%, (Figure 4.14and 4.13) respectively and increased HDL-c by 12% (Figure 4.16) with effects similar with BpH + FN- treated group.

Furthermore, Tp administration significantly raised the production of nitric oxide (NO)by 29% and 23% in the serum and prostate of BpH rats, respectively when compared with Control 2 (Figure 4.17 and 4.18) whereas when MeJa and FNwere co-administered to BpH rats, an insignificant decrease was observed. However, sole administration of MeJa and sole administrationof finasteride lowered the levels of serum NOby 21% and 13%, respectively relative to BpH rats (Figure 4.17). Also,administration of MeJa and finasteride (singly) to Control 2(castrated rats)

decreased the levels of prostatic NOby 21% and 19%, respectively (Figure 4.18). In Figure 4.19, an insignificant increase was detected in the activity of prostatic myeloperoxidase (MPO) in control 2 relative to Control 1. Tp injection also producedan insignificant effect in the activity of prostatic myeloperoxidase relative to control 1. Administration of FN and MeJa (when combined) to BpH rats significantly reduced the MPOactivity by 30% (Figure 4.19). Also, sole administration of MeJa or finasteride to BpH rats decreased the MPO activity significantly by 85% and 70%, respectively (Figure 4.19).

Additionally,the level of serum testosterone was increased significantly in BpH rats by 152% relative tothe Control 2 while combined administration of MeJa and finasteridedecreased the elevated level of serum testosterone by 64% relative to BpH rats (Figure 4.20). However, sole administration of finasteride to BpH rats produced a significant reductionin the testosterone level by 49% while MeJa insignificantly decreased the testosterone levels. Furthermore, in BpH rats, an increase of 28% was discovered in the activity of aniline hydroxylase (AnH) relative to Control 2(Figure 4.21). Administration of MeJa and FN (combination) decreased the AnH activity by 36% similar to treatment of BpH with MeJa or finasteride (Figure 4.21). In contrast, inconsequential changeswere noticed in the activity of amylopyrine-*N*-demethylase in all the groups (Figure 4.22).

Figure 4.23shows the histological changes in prostate tissue. Daily injection of Tp to castrated rat for 28 days led to increased hyperplasia of the epithelia of the prostatic tissue while in the castrated rats, the inflammatory cells that permeate the prostatic stroma were mildly congested (Figure 4.23). Co-administration of MeJa and finasteride to BpH rats showed normal prostatic glands with normal secretions and corpora amylacea lined with normal epithelia cells (Figure 4.23). However, treatment of BpH rats with MeJa showed prostatic glands with undefined with some glands showing epithelia hyperplasia and infiltrating inflammatory cells (Figure 4.23). Bcl-2 was highly expressed in the prostategland of BpH and castrated rats treated with finasteride only while its expression in the group treated with combination of MeJa and finasteride was similar to that of non-castrated control (Figure 4.24 and 4.26).

Also, the expression of Bax (Figure 4.26 and 4.27) and p53 (Figure 4.28 and 4.29) in BpH rats were downregulated while co-administration of MeJa and finasteride increased the expression of Bax and p53 moderately in the nuclei of the epithelium of the prostate glands. Cyclooxygenase-2 (COX-2) was highly expressed in BpH rats

while weak expression was noticed in the prostatic tissue of the group treated with combination of MeJa and finasteride (Figure 4.30 and 4.31). However, treatment with finasterideor MeJarevealed weak expression. Also, iNOS was expressed strongly in BpH rats while very low expression was discovered in (BpH + MeJa + FN) group(Figure 4.32 and 4.33).

Table 4.3 Changes in the body weight and relative weight of prostate in each group

Treatments	Body Weight		Weight Difference	Prostate Weight	Relative Weight
	Initial	Final			
	(g)		(g)	(g)	(% body weight)
Control 1	249.17±15.92	262.50±20.92	13.33±5.00	0.41±0.06	0.15±0.01
Control 2	248.00±12.93	260.00±13.69	12.00±0.77	0.10±0.02[a]	0.04±0.00 [a]
BpH	288.33±23.40	304.17±18.82	15.83±4.58	0.68±0.13 [a, b]	0.20±0.04 [a, b]
BpH + MeJa	254.17±20.46	290.00±13.69	35.83±6.67 [c]	0.46±0.04[c]	0.16±0.01 [c]
BpH + FN	260.83±10.18	295.00±34.16	34.17±10.74[c]	0.38±0.07 [c]	0.13±0.01 [c]
CMeJa	250.00±9.12	269.00±10.84	19.00±1.72	0.18±0.03 [a]	0.07±0.01 [a]
CFN	243.33±7.28	266.00±10.84	22.67±3.56	0.10±0.02 [a]	0.06±0.01 [a]
BpH + MeJa + FN	284.17±23.52	333.33±12.91	49.17±10.61[c]	0.38±0.07 [b, c]	0.12±0.01 [b, c]

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= catrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a] $p<5\%$ was considered significant relative to Control 1, [b] $p<5\%$ was considered significant relative to Control 2, [c] $p<5\%$ was considered significant when compared with BpH.

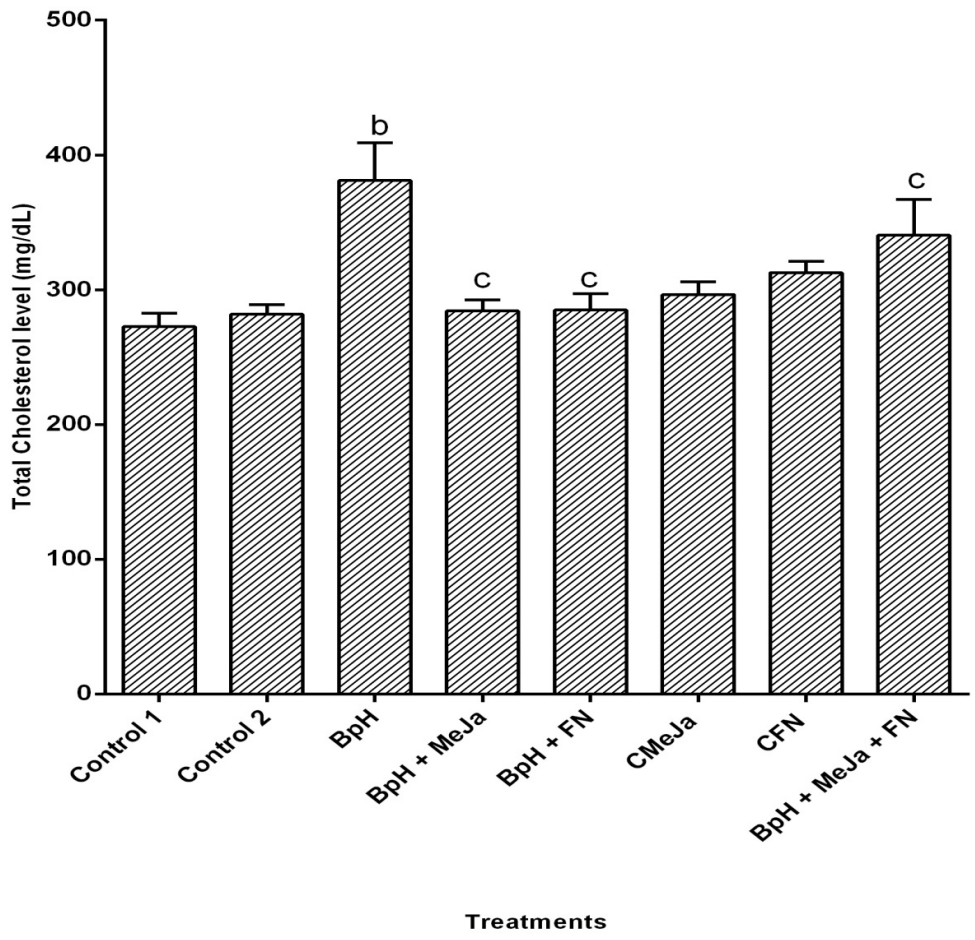


Figure4.14 Effects of MeJa on total cholesterol level in the serum

All values were presented by way of the mean ± standard deviation of 6 rats pergroup Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= catrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only.$^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

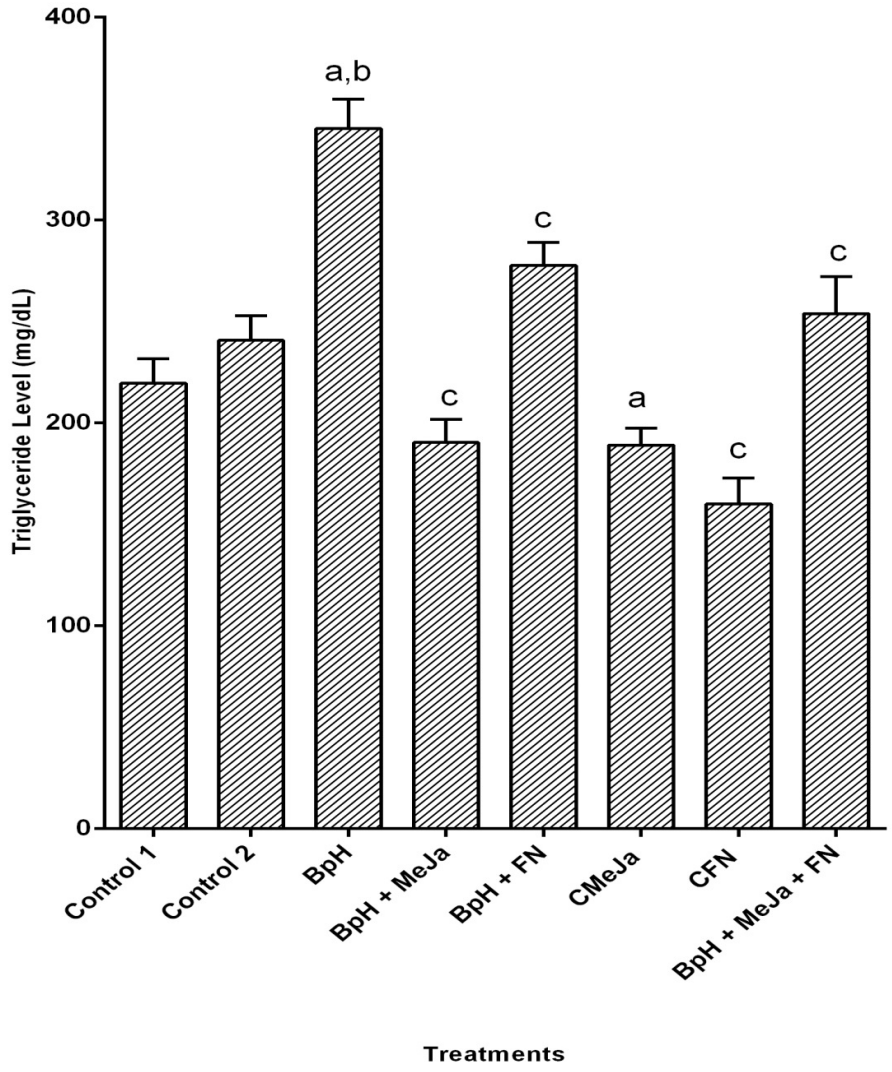


Figure4.15 Effects of MeJa on triglyceride level in the serum

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{a}p<5\%$ was considered significant relative to Control 1, $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

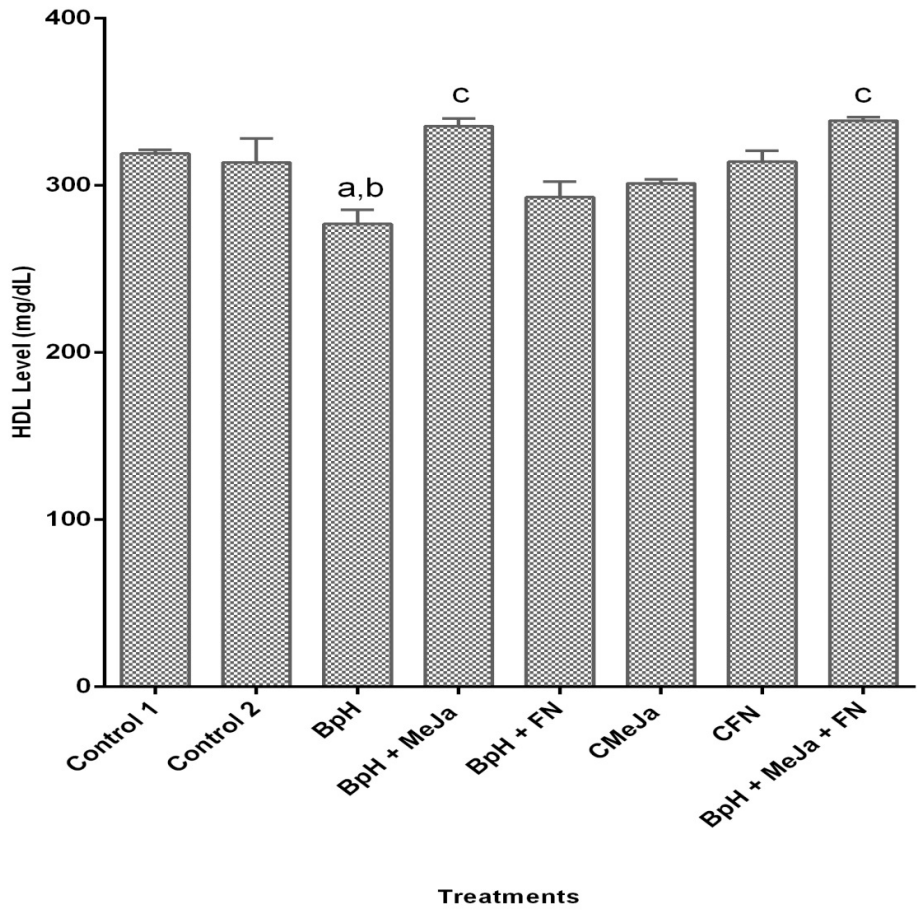


Figure4.16 Effects of MeJa on high density lipoprotein (HDL-c) in the serum

All values are presented by as mean ± standard deviation of 6 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c] p<5% was considered significant relative to BpH.

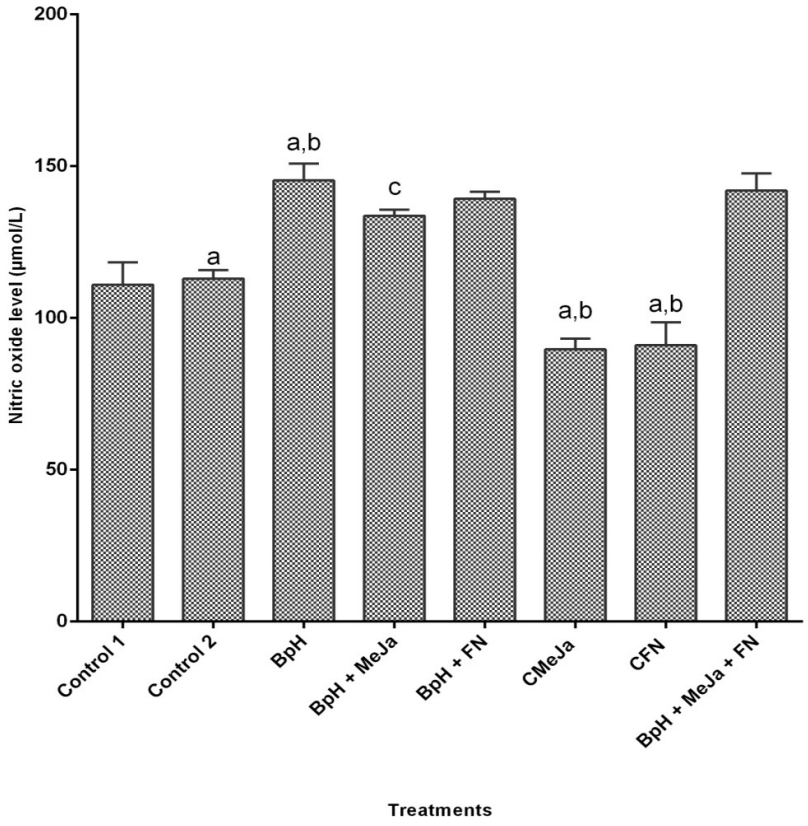


Figure 4.17 Effects of MeJa on nitric oxide levels in the prostate

All values are presented as mean ± standard deviation of 6 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c] p<5% was considered significant relative to BpH.

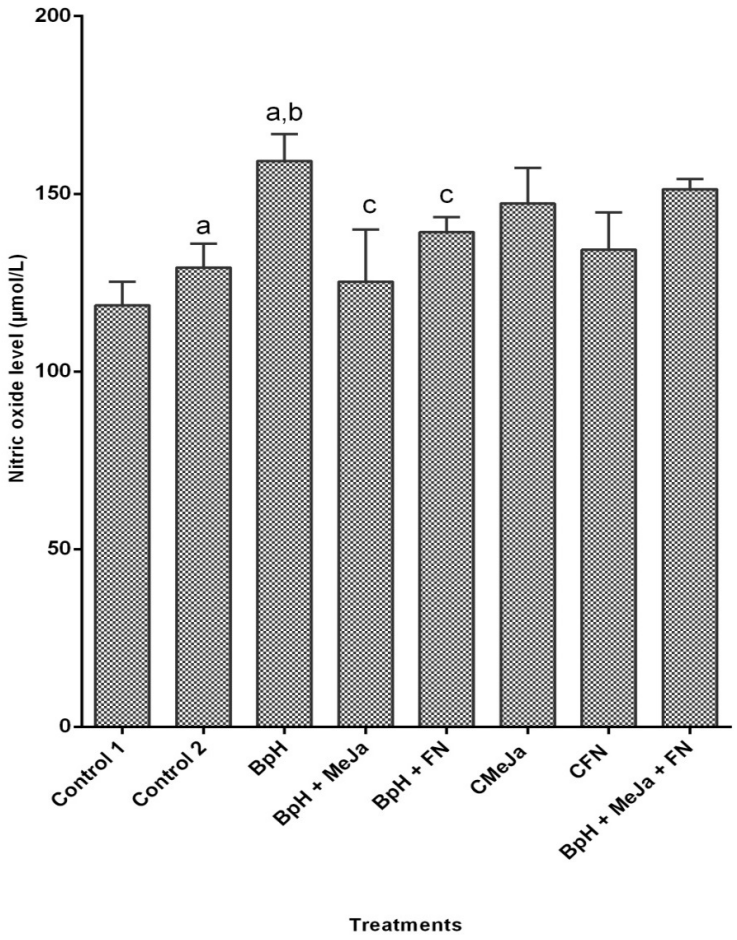


Figure4.18 Effects of MeJa on the nitric oxide levels in the serum

All values were presented by way of the mean ± standard deviation of 6 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c] p<5% was considered significant relative to BpH.

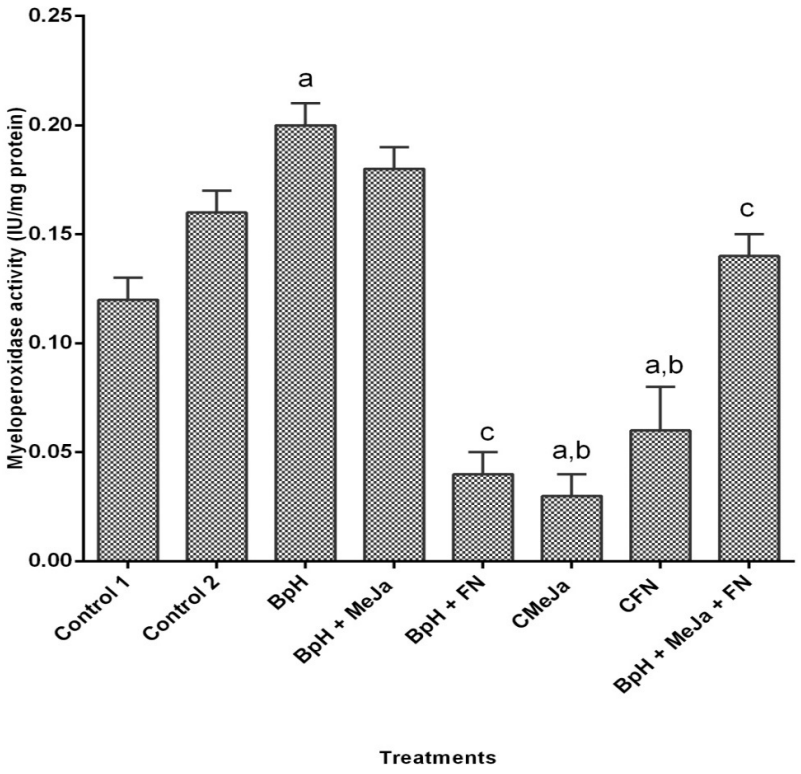


Figure4.19 Effects of MeJa on myeloperoxidase activity in the prostate

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{a}p<5\%$ was considered significant relative to Control 1, $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

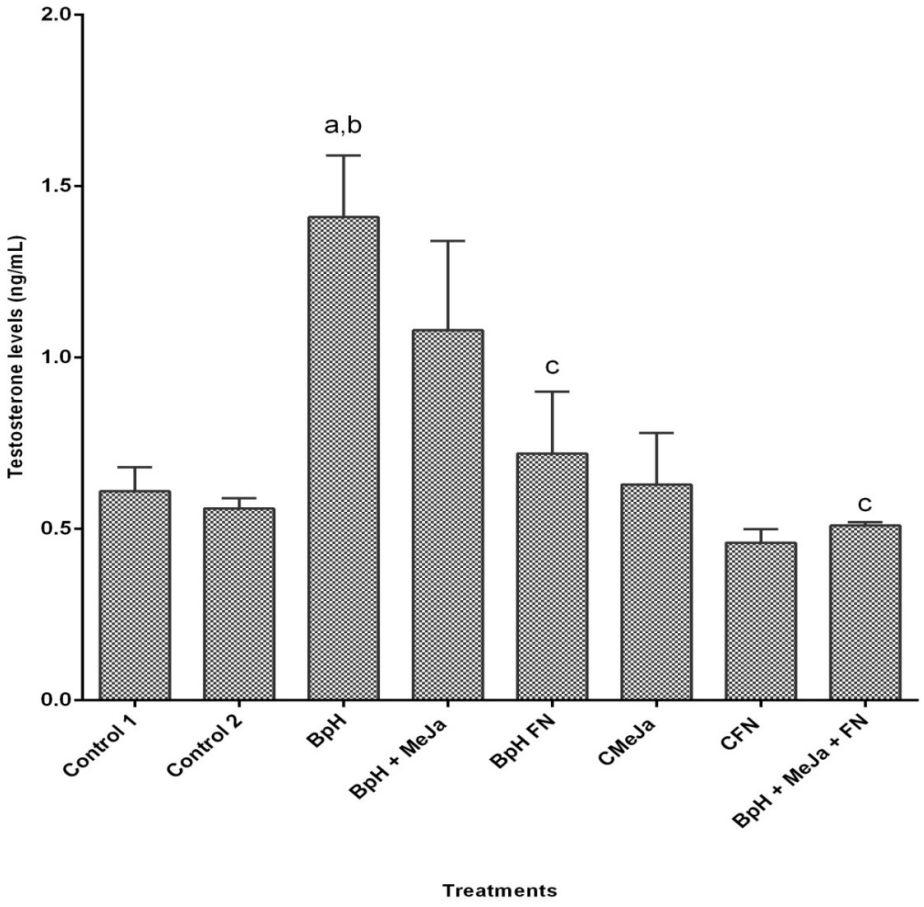


Figure 4.20 Effects of MeJa on testosterone production in the serum

All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. [a]p<5% was considered significant relative to Control 1, [b]p<5% was considered significant relative to Control 2, [c]p<5% was considered significant relative to BpH.

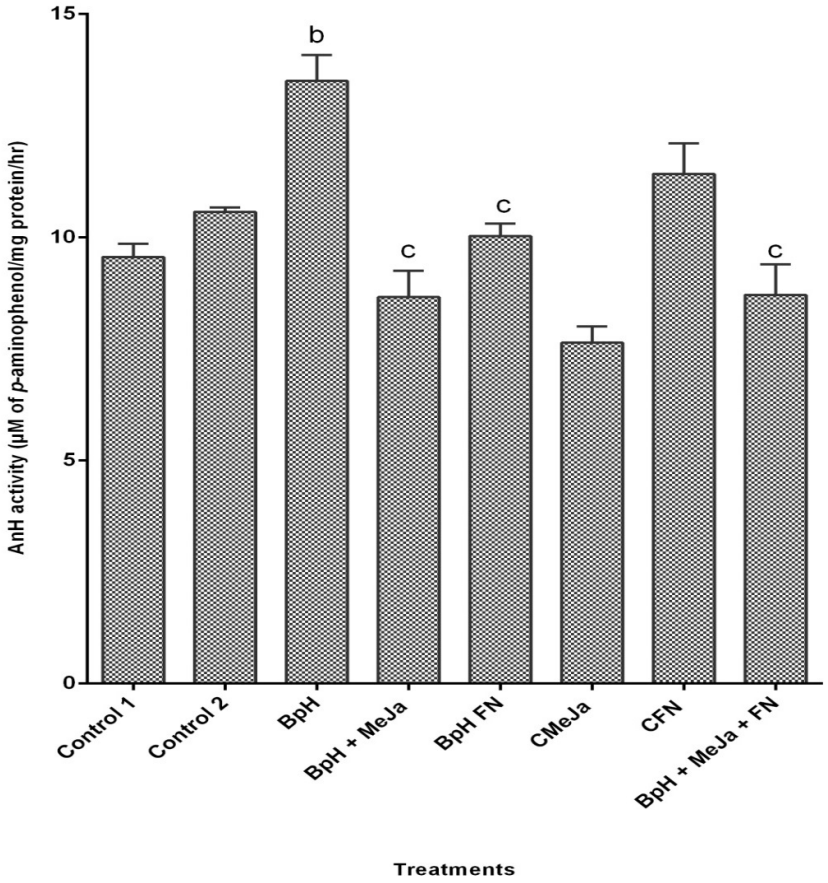


Figure4.21 Effects of MeJa on aniline hydroxylase (AnH) activity in the prostate

All values are presented as mean ± standard deviation of 6 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only. $^{b}p<5\%$ was considered significant relative to Control 2, $^{c}p<5\%$ was considered significant relative to BpH.

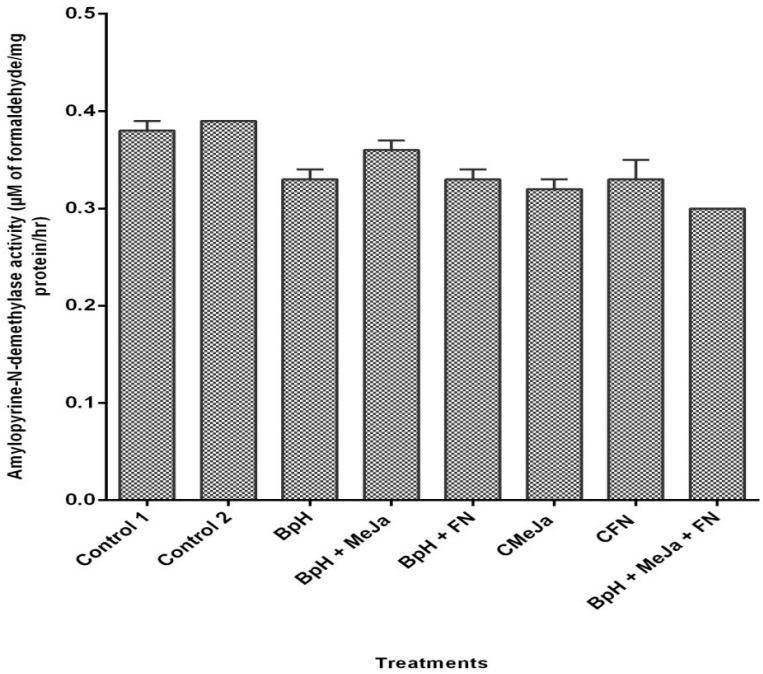
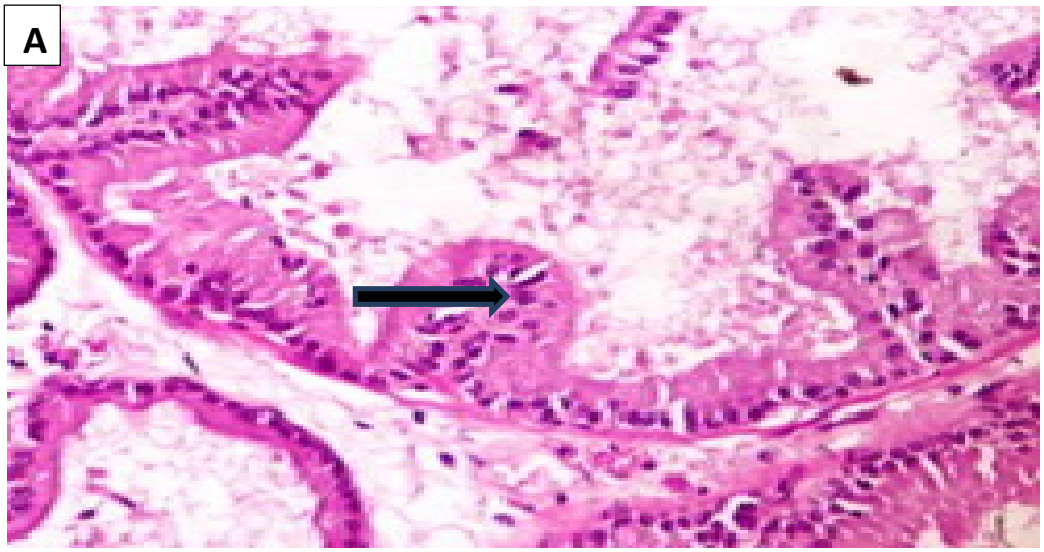
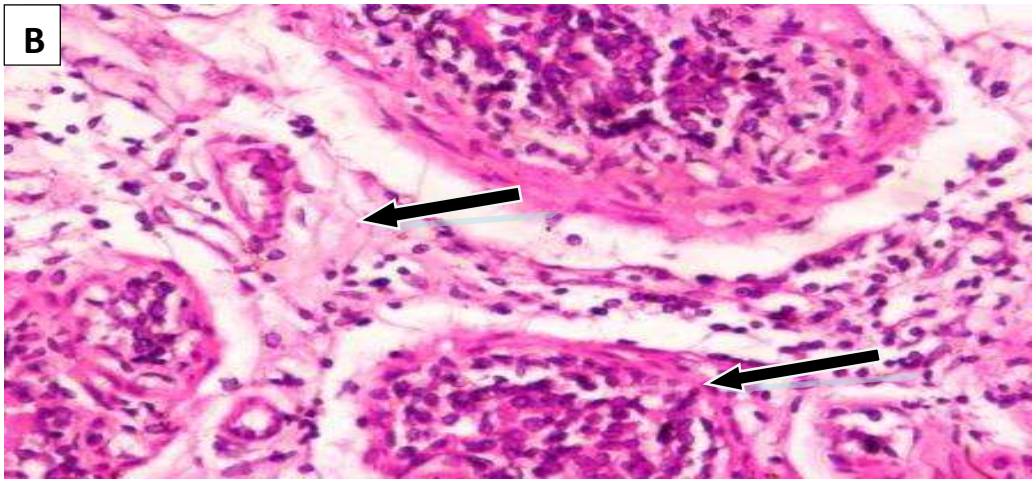


Figure4.22 Effects of MeJa on amylopyrine-*N*-demethylase activity in the prostate

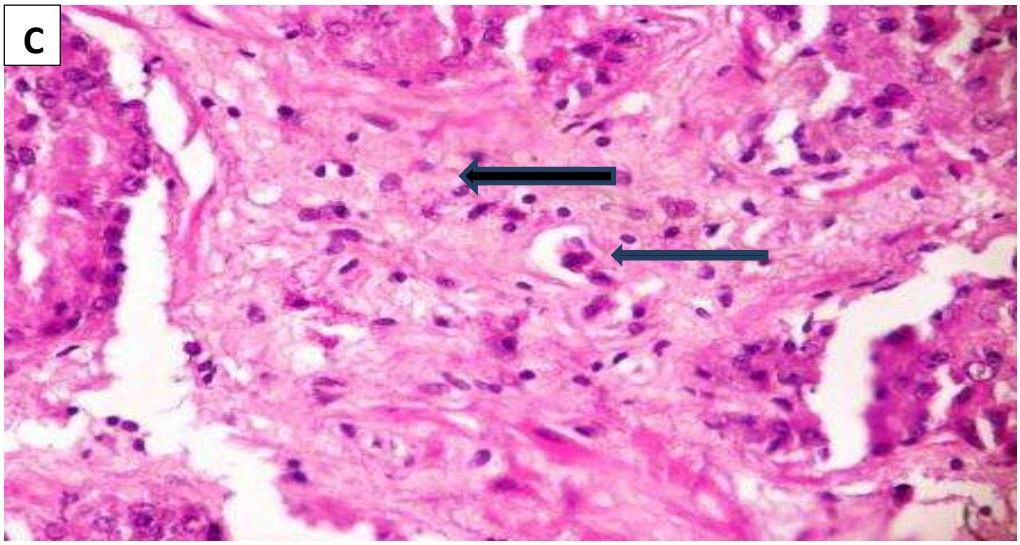
All values are presented as mean ± standard deviation of 7 rats per group, Control 1=Rats that are not castrated, Control 2= Rats that are castrated, BpH= castrated rats that were given testosterone propionate, CMeJa= Castrated rats that were given methyl jasmonate (MeJa) only, CFN= Castrated rats that were given finasteride (FN) only.



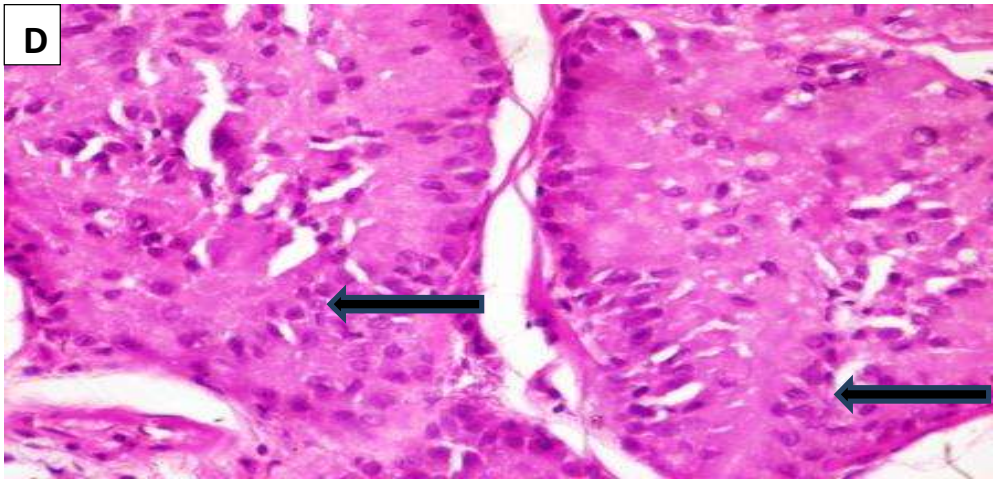
A = Control 1 (Rats that were not castrated) -- Gland shows normal secretions and corpora amylacea lined by normal columnal epithelial cells (black arrow). The prostatic stroma also appears normal (M X 400).



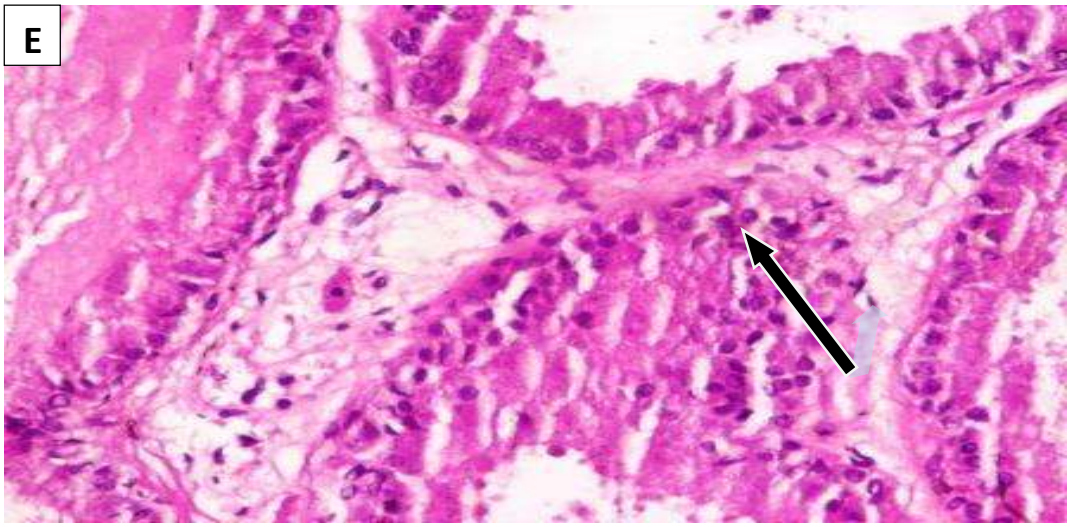
B = Control 2 (Castrated rats) –Gland shows mild congestion (black arrows) and severe to chronic fibrosis involving the prostatic stroma and the inflammatory cells infiltrate the stroma and the glands(M X 400).



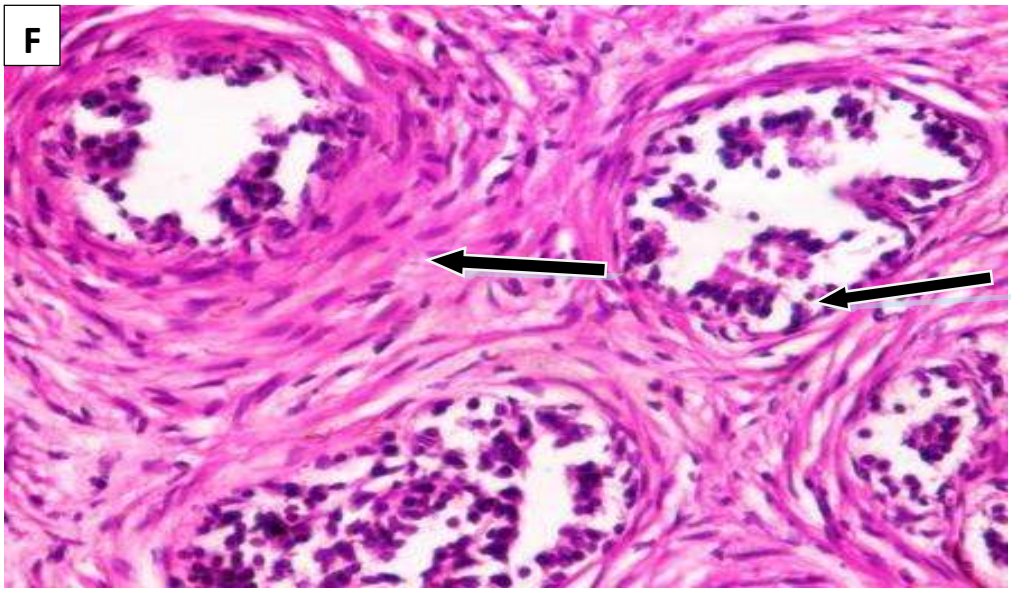
C = BpH (Castrated rats that received testosterone propionate only) -- Gland shows hyprplasia and severe atrophy (black arrows), chronic infiltration of inflammatory cells involving the glands and stroma and severe fibrosis (M X 400).



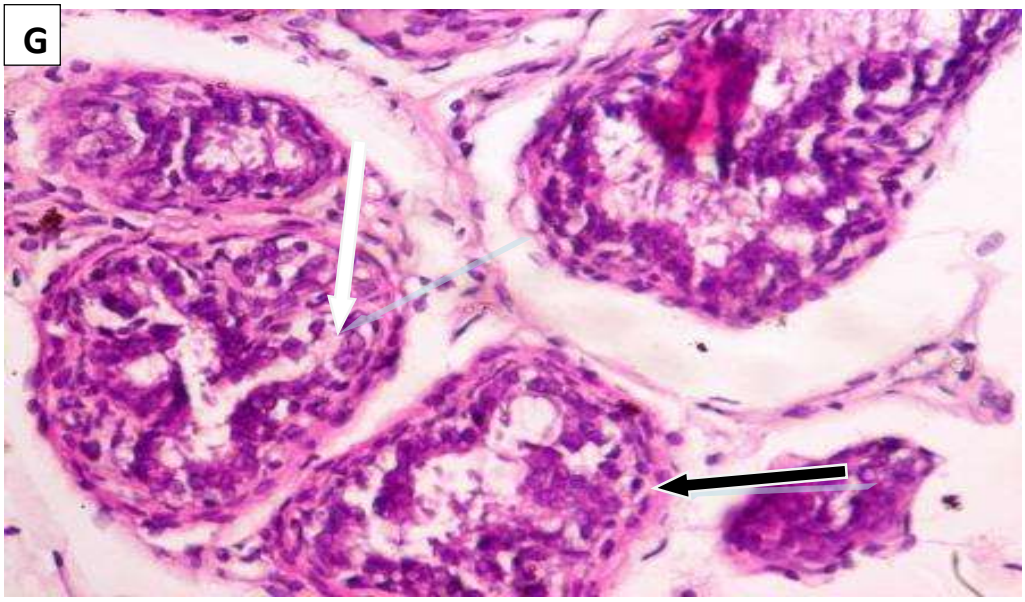
D = BpH + MeJa (Castrated rats that receivedTpand methyl jasmonate) -- Glands shows undefined margins and moderate epithelial hyperplasia (black arrows) with infiltrating inflammatory cells. The tissue also appears moderately fibrotic (M X 400).



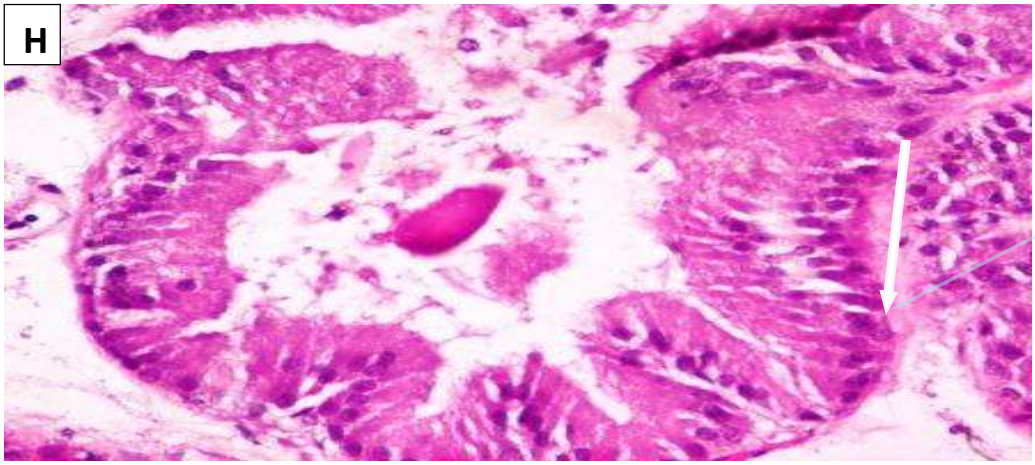
E = BpH + FN (Castrated rats that receivedTpand Finasteride) – Gland shows mild infiltration of inflammatory cells (black arrow) within the prostatic stroma and no fibrosis was noted (M X 400).



F = CMeJa (Castrated rats that receivedmethyl jasmonateonly) -- Gland shows severe deposition of connective tissues (black arrows), moderate hyperplasia of the epithelia cells and cellular degeneration (M X 400).

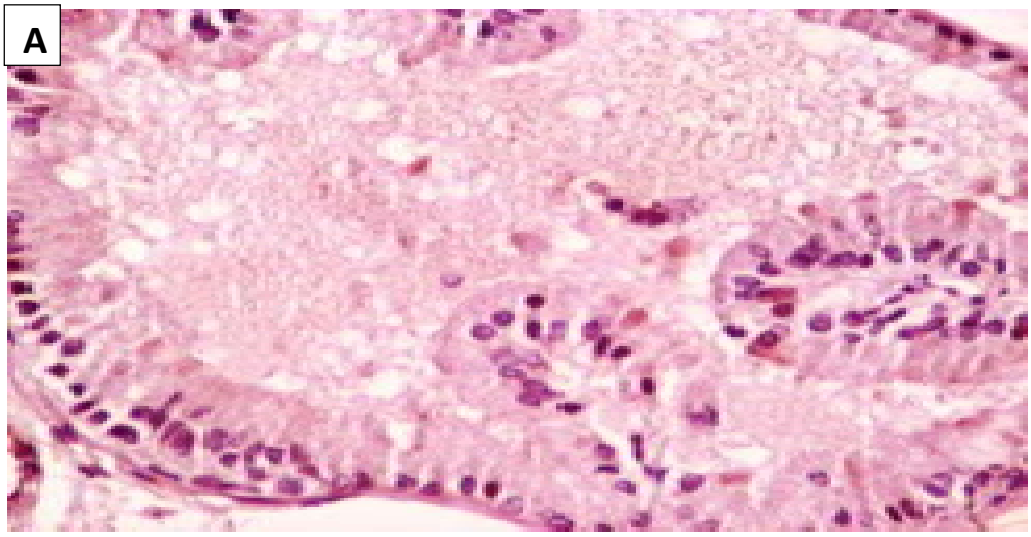


G = CFN (Castrated rats that receivedFinasteride only) -- Gland shows moderatelynormal epithelial arrangement without proliferation (black arrow), however, some of the prostatic glands are mildly infiltrated by inflammatory cells (white arrow) (M X 400).

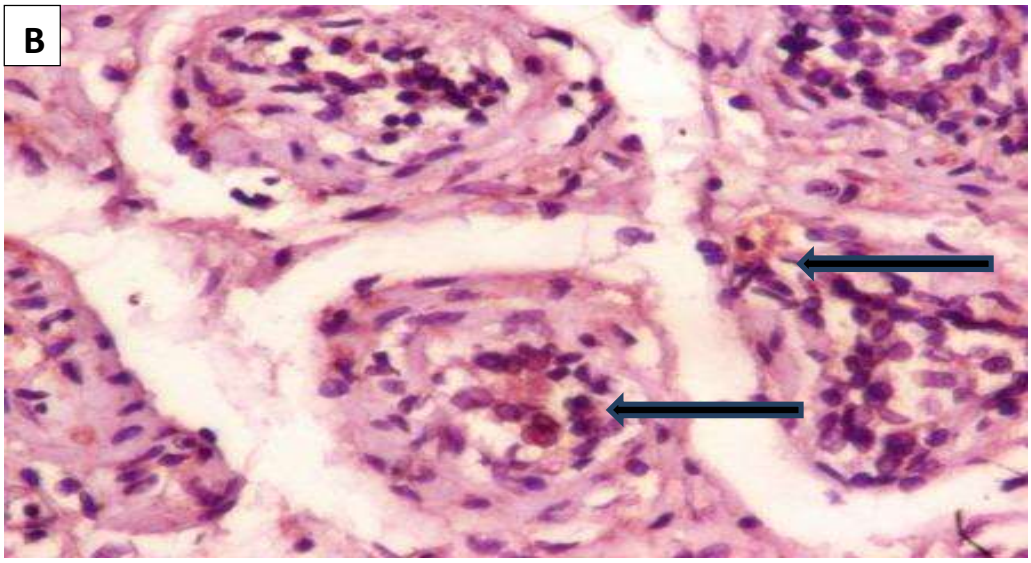


H = BpH + MeJa + FN (Castrated rats that received Tp, methyl jasmonate and Finasteride – Gland shows normal secretions and corpora amylacea (white arrow), normal epithelial cells and normal stroma with few infiltration of inflammatory cells (M X 400).

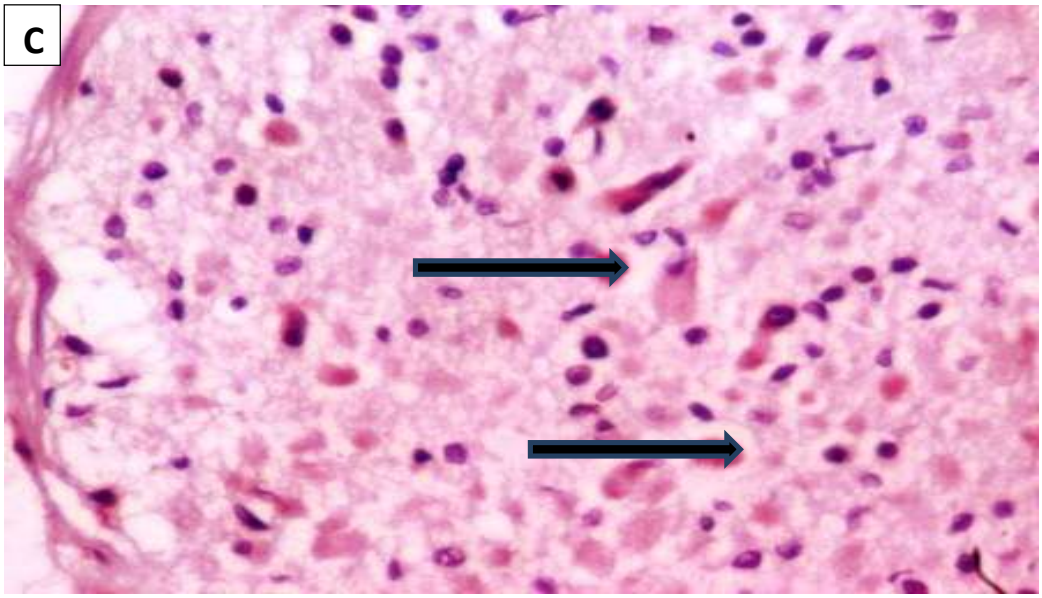
Figure4.23(A-H): Photomicrograph of cross section of prostate tissue BpH rats treated with MeJa for 28 days (M X 400)



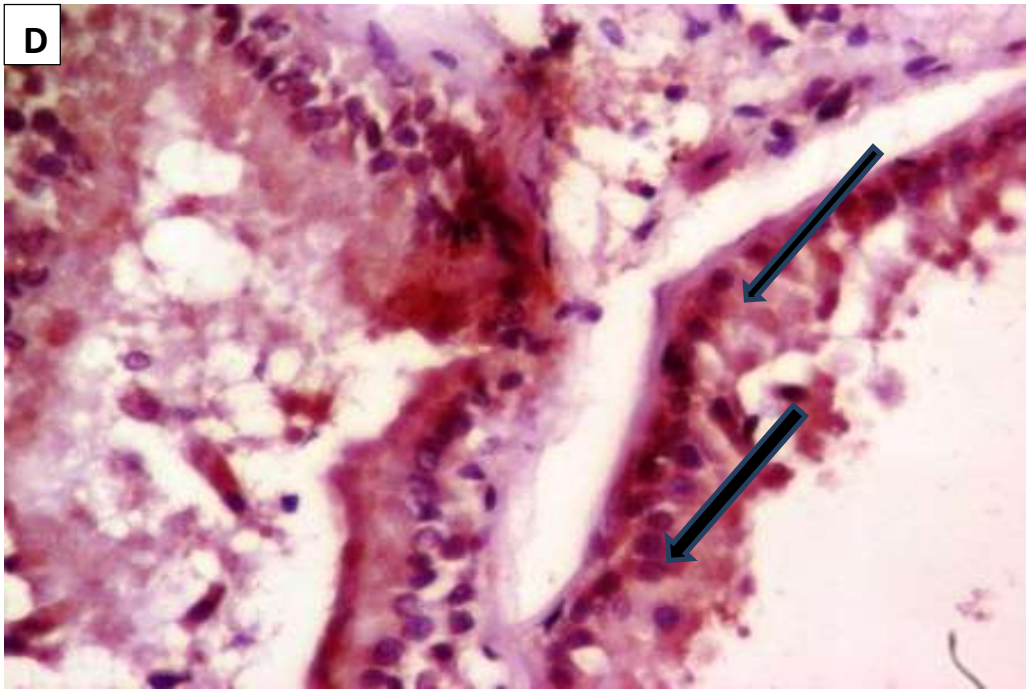
A = Control 1 (Rats that were not castrated) – Gland shows weak expression of Bcl-2 (M X 400).



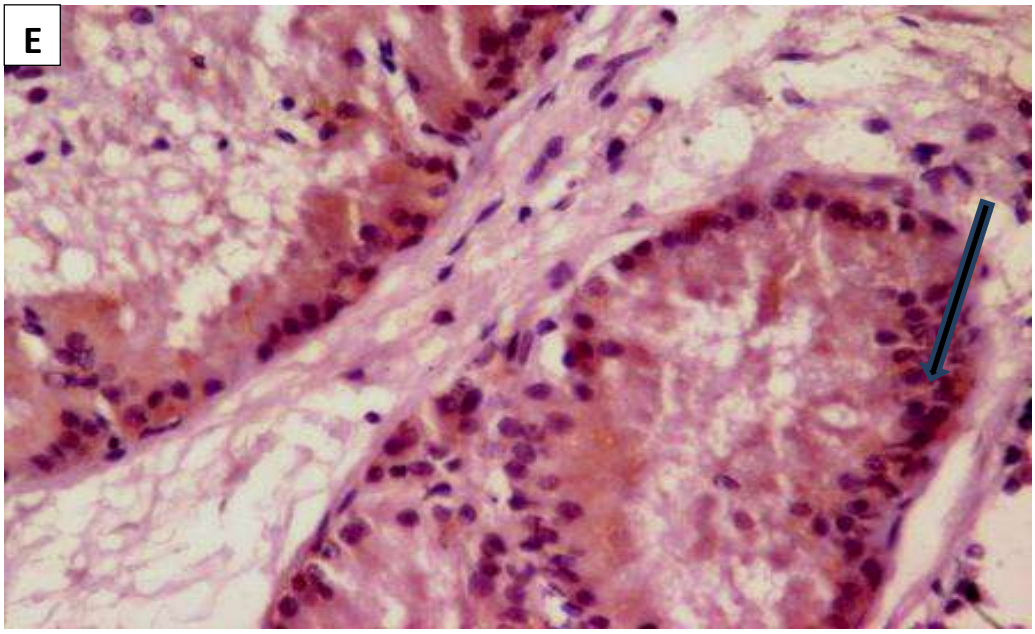
B = Control 2 (Rats that were castrated) – Gland shows weak expression (black arrows) of bcl-2 on the glandular epithelium (M x 400).



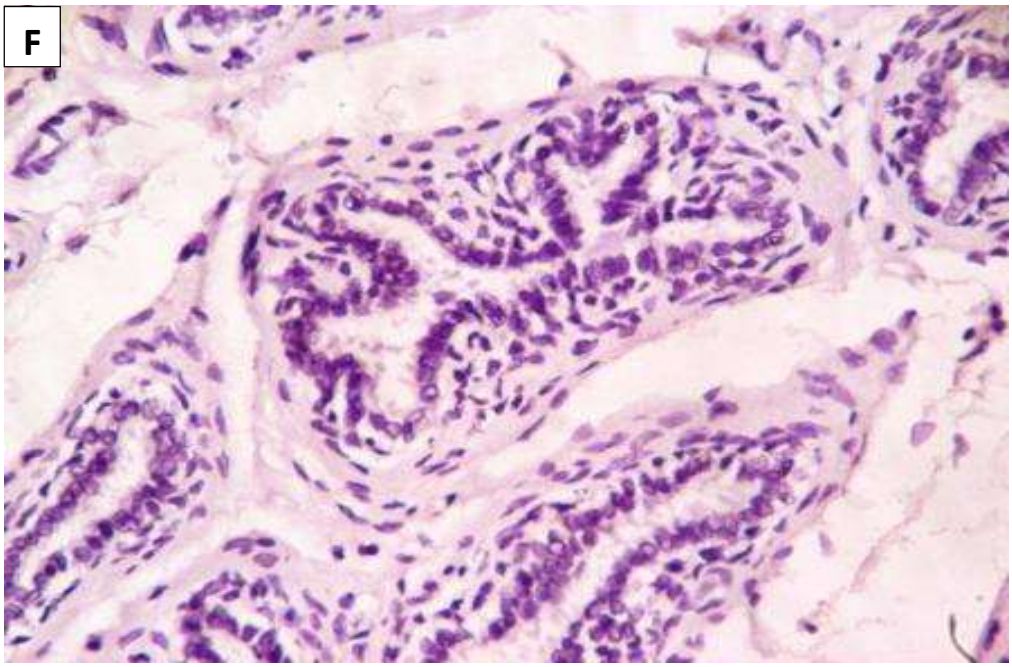
C = BpH (Castrated rats that received testosterone propionate (Tp) only) -- Gland shows strong extression of Bcl-2 indicated with black arrows (M X 400).



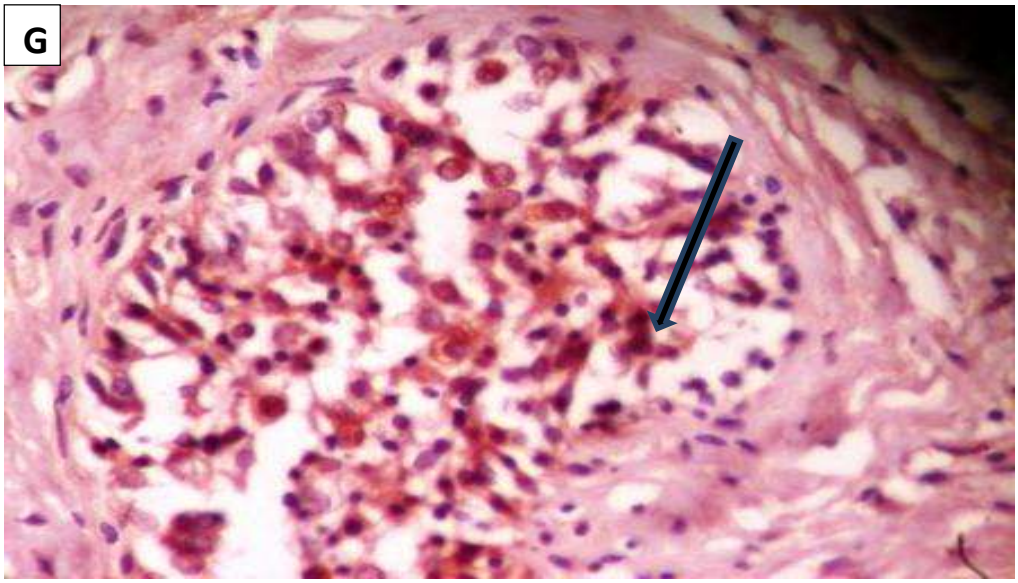
D = BpH + MeJa (Castrated rats that received Tp and methyl jasmonate) --Gland shows moderate expression of Bcl-2 (black arrows) in epithelia cytoplasm (M X 400).



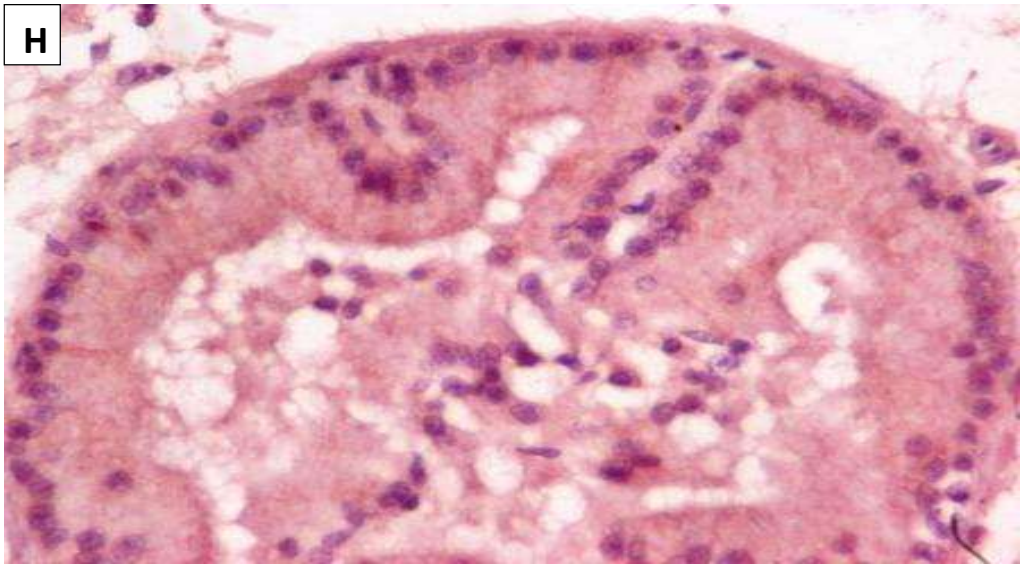
E = BpH + FN (Castrated rats that received Tp and Finasteride) – Gland shows moderately weak expression of Bcl-2(black arrow)on the epithelia cytoplasm (M X 400).



F = CMeJa (Castrated rats that receivedmethyl jasmonate only) – Gland shows weak expression of Bcl-2 (M X 400).



G = CFN (Castrated rats that received Finasteride only) – Gland shows moderate expression of Bcl-2 (M X 400).



H = BpH + MeJa + FN (Castrated rats that received Tp, methyl jasmonate and Finasteride) – Gland showsweak expression of Bcl-2 (M X 400).

Figure4.24(A-H): Photomicrograph of cross section of prostate tissue showing the effects of MeJa on Bcl-2 expression via immunohistochemistry staining

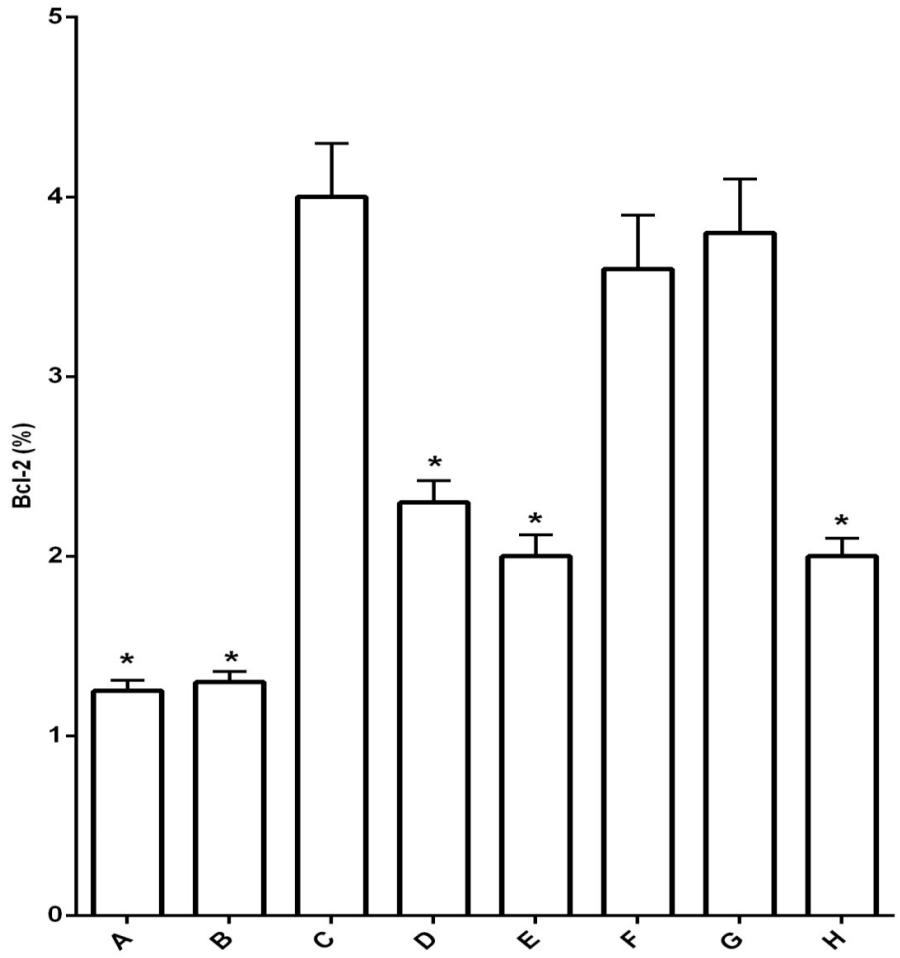
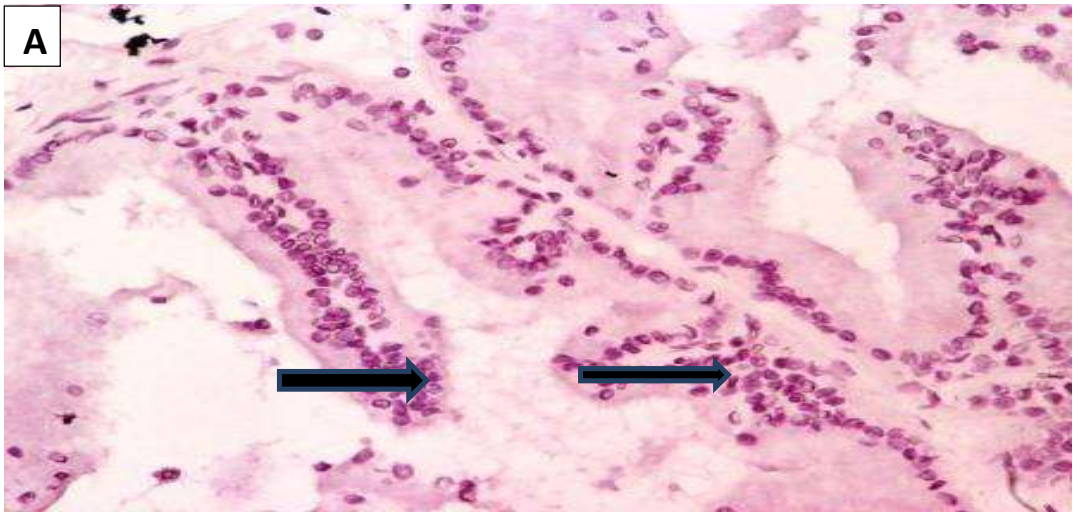
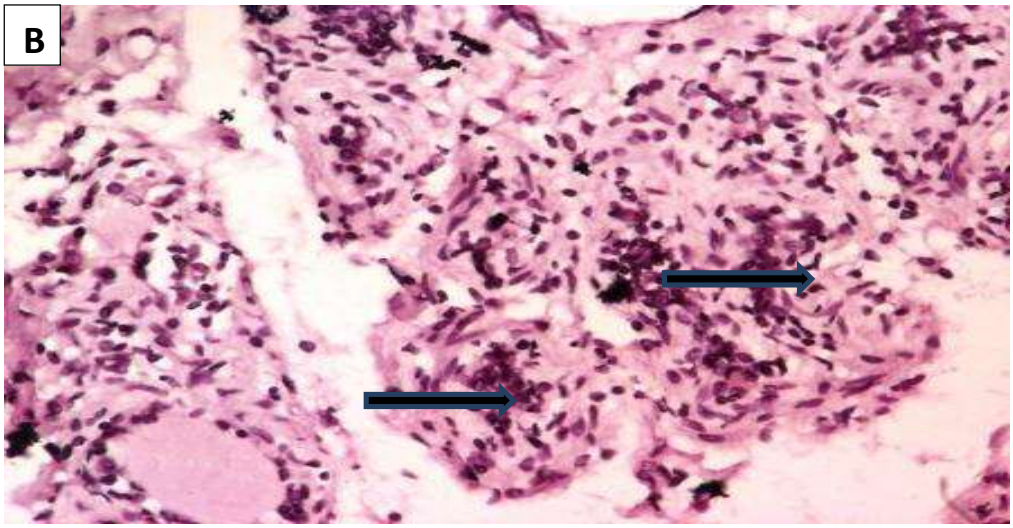


Figure4.25 Effects of MeJa on Bcl-2 expression via immunohistochemistry staining in the prostate tissue

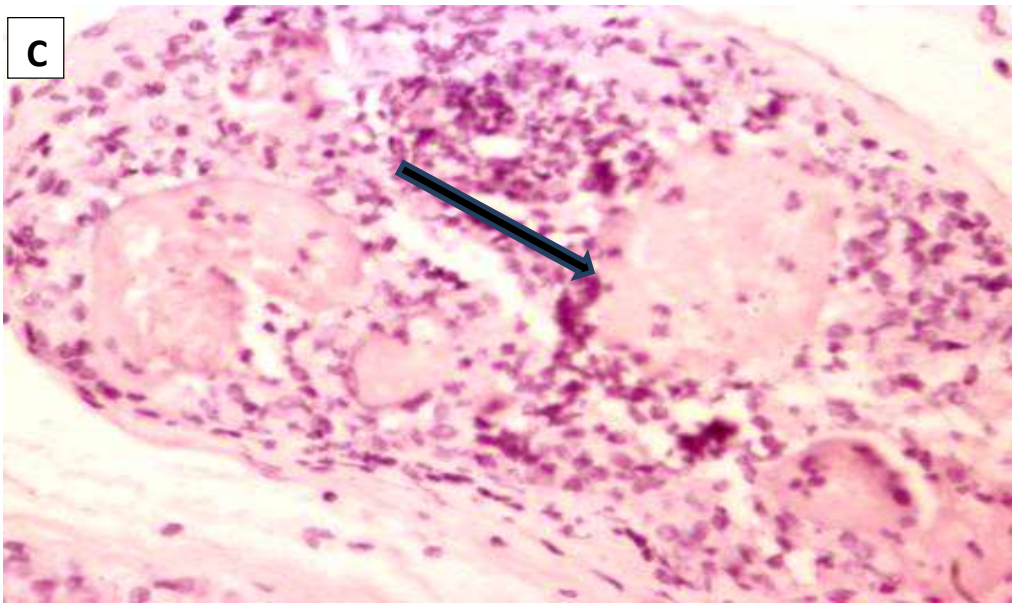
A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. castrated rats that were given TP, D=BpH + MeJa i.e. BpH rats administered with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats administered with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only. *Significantly different from C ($p<0.05$).



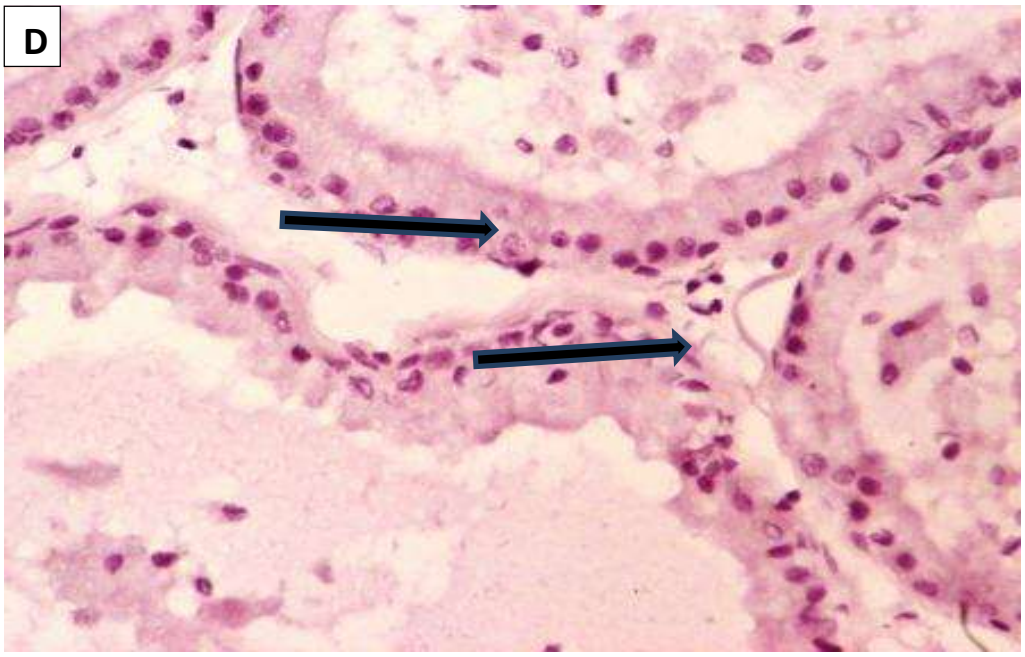
A = Control 1 (Rats that were not castrated) – Gland shows moderate expression of Bax indicated with black arrows (M X 400).



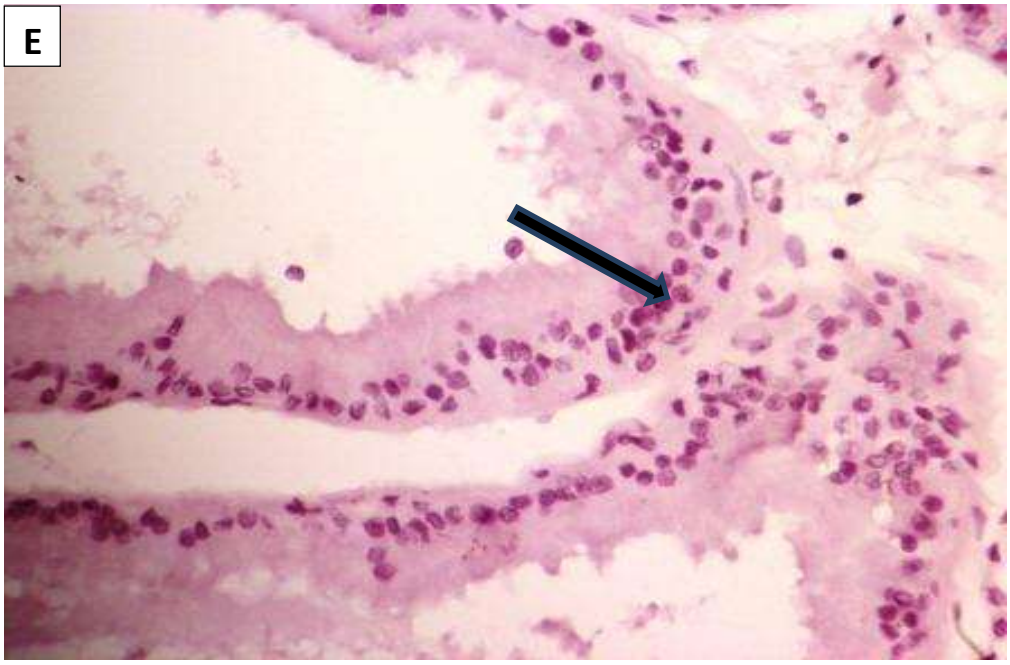
B = Control 2 (Rats that were castrated) – Gland showsstrong expression of Bax(black arrows) in the infiltrating inflammatory cells (M X 400).



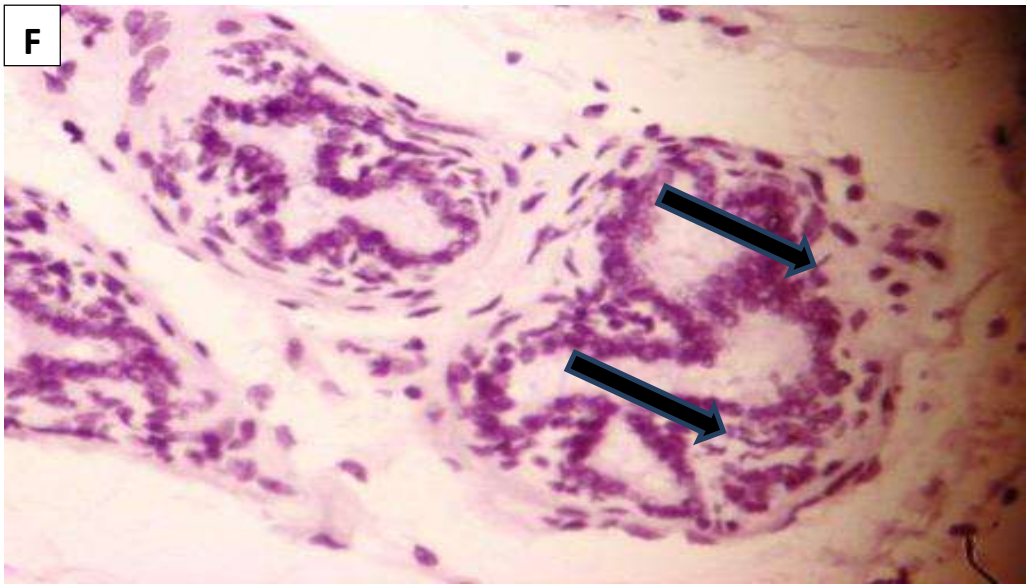
C = BpH (Castrated rats that received testosterone propionate (Tp) only) –Glands show weak expression of Bax (black arrows) in the epithelium (M X 400).



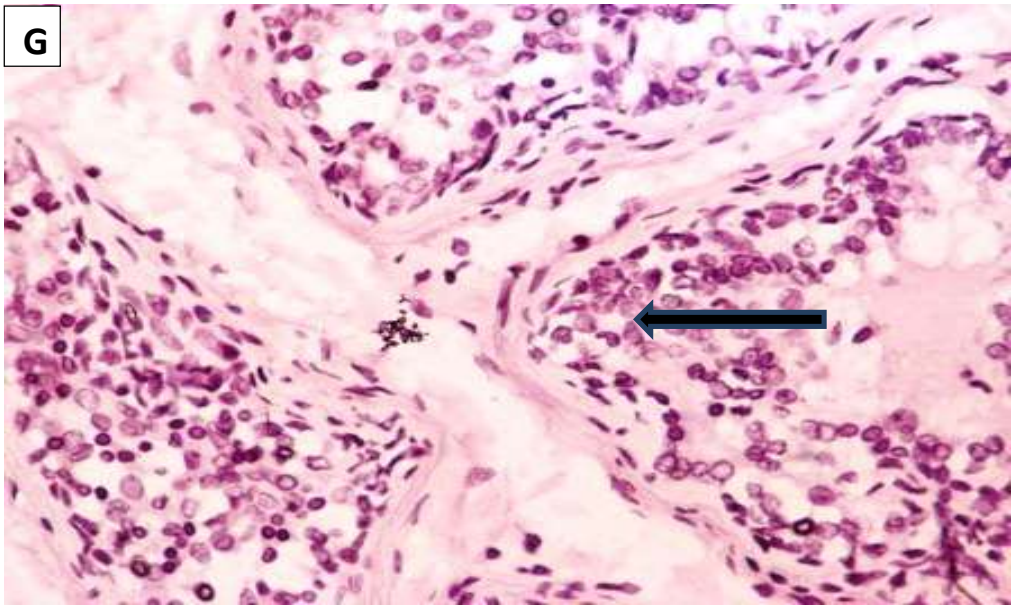
D = BpH + MeJa (Castrated rats thatreceived Tpand methyl jasmonate) – Gland show moderate expression(black arrows) of Bax(M X 400).



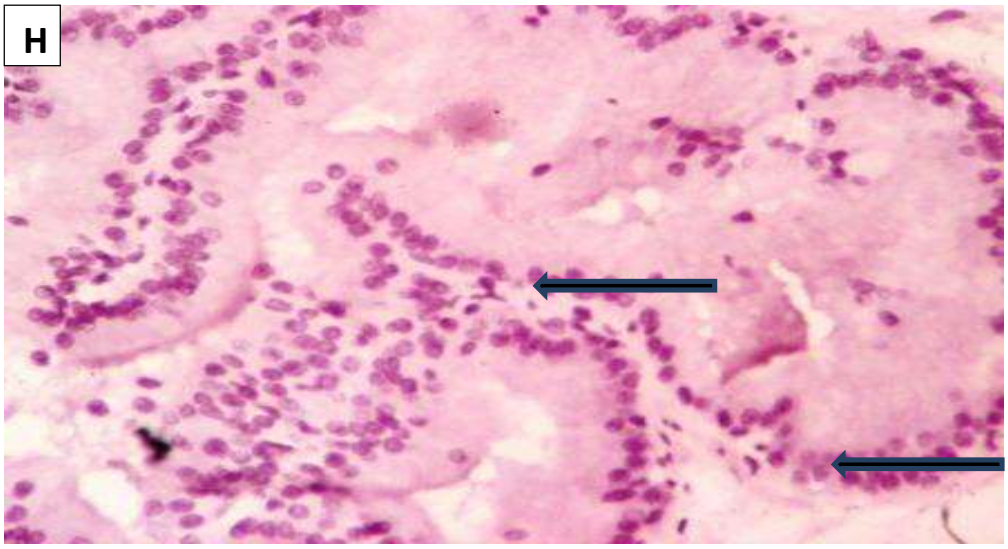
E = BpH + FN (Castrated rats that receivedTpand Finasteride) --Gland shows moderate expression (black arrow) of Bax (M X 400).



F = CMeJa (Castrated rats that received mthyl jasmonate only) --Gland shows moderate expression (black arrows) of Bax (M X 400).



G = CFN (Castrated rats that received Finasteride only) –Gland shows weak expression of Bax (M X 400).



H = BpH + MeJa + FN (Castrated rats that received Tp, methyl jasmonate and Finasteride) – Gland shows strong expression (black arrows) of Bax (M X 400).

Figure4.26(A-H): Photomicrogrph of cross section of prostate tissue showing the effects of MeJa on the expression of Bax via immunohistochemical staining (M X 400)

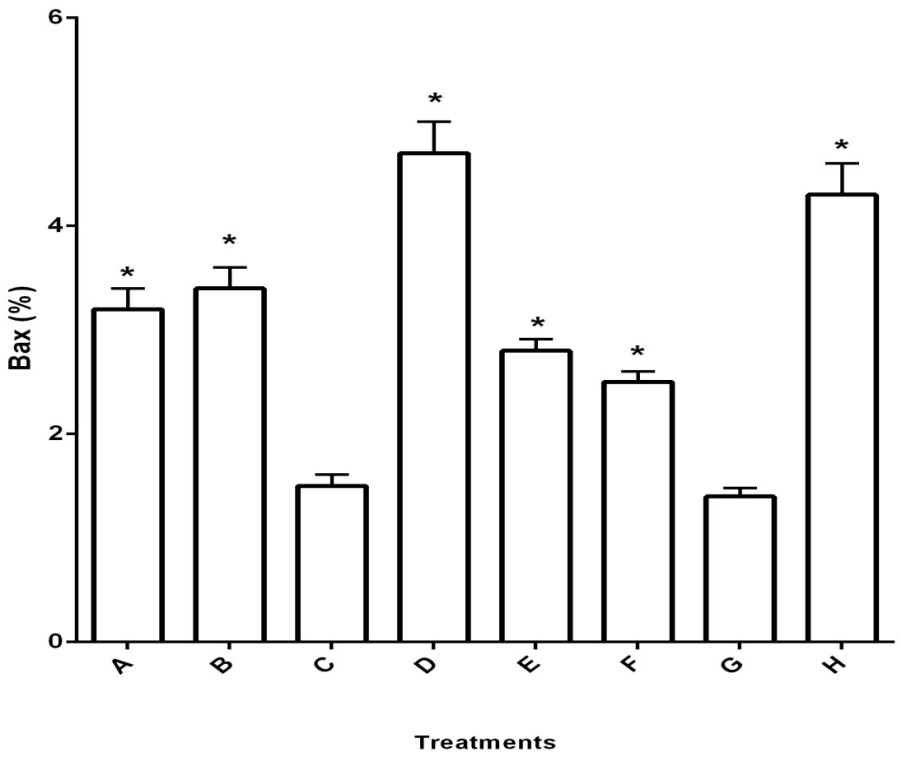
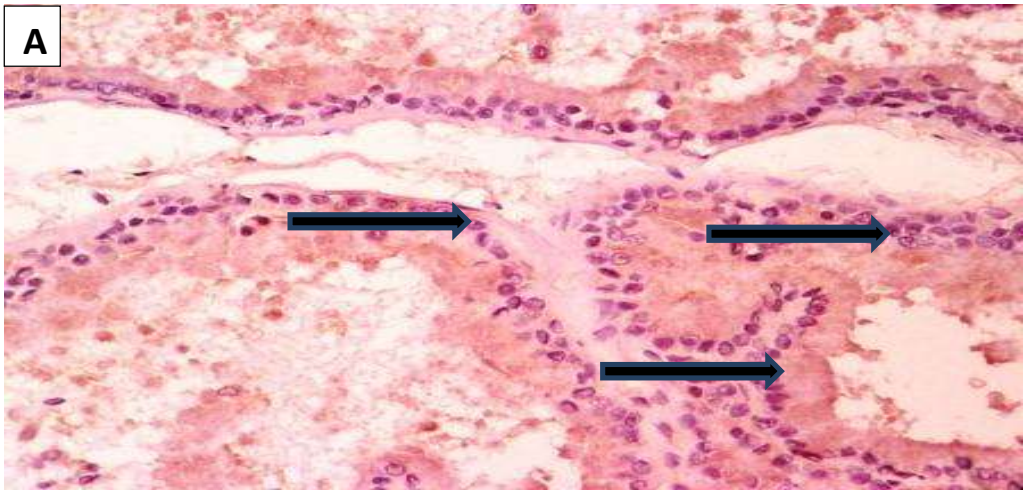
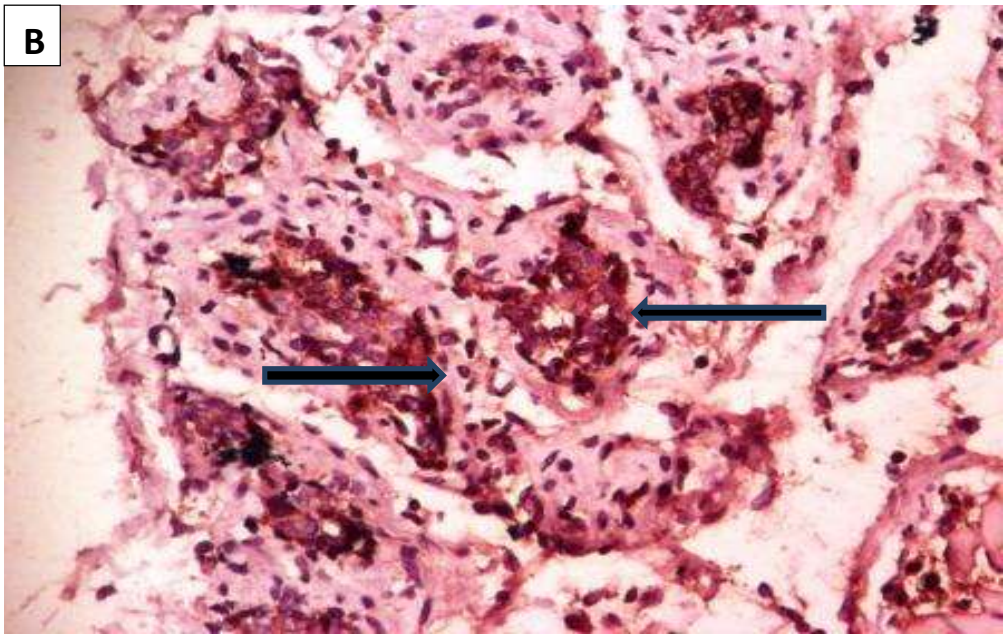


Figure4.27 Effects of MeJa on the expression of Bax via immunohistochemical staining in the prostate tissue

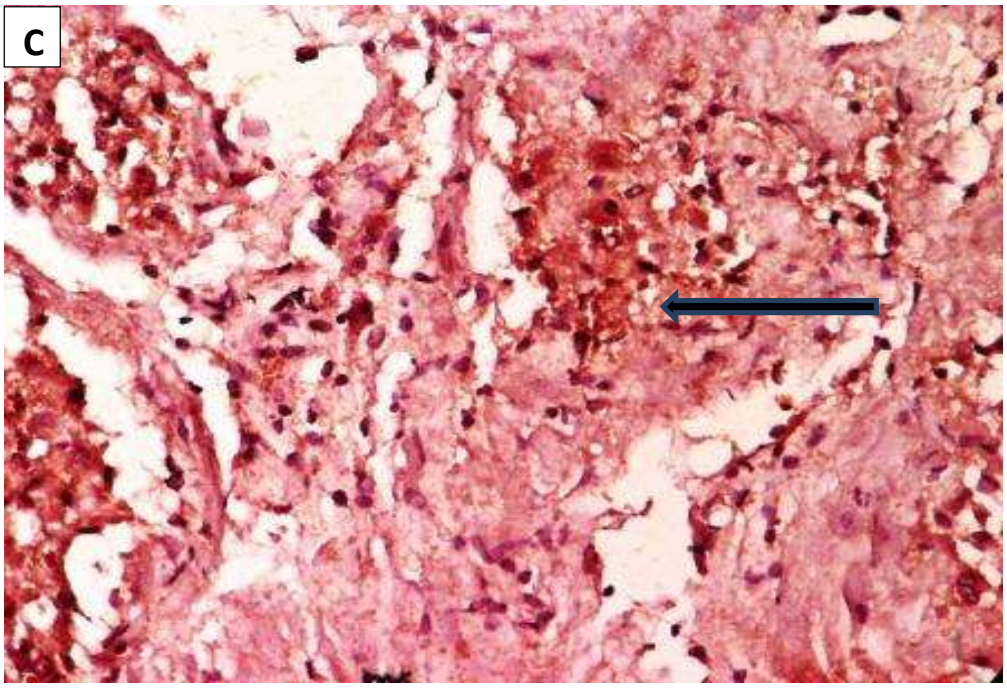
A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. castrated rats that were givenTP, D=BpH + MeJa i.e. BpH rats administered with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats administered with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only. *Significantly different from C ($p<0.05$).



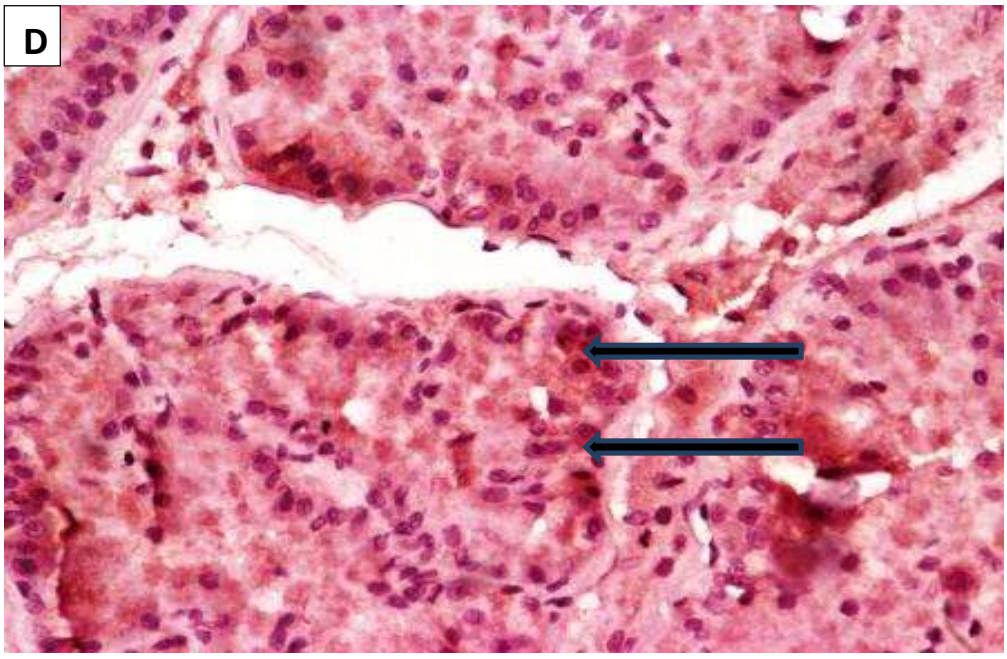
A = Contro 1 (Rats that were not castrated) – Gland shows strong expression (black arrows) of p53 (M X 400).



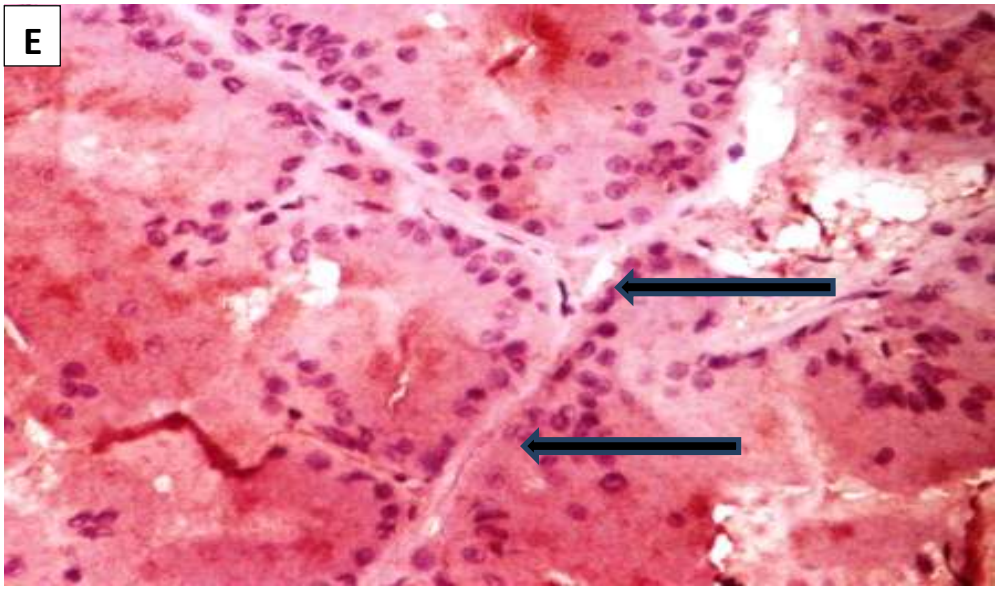
B = Contro 2 (Rats that were castrated) --Gland shows strong expression (black arrows) of p53 (M X 400).



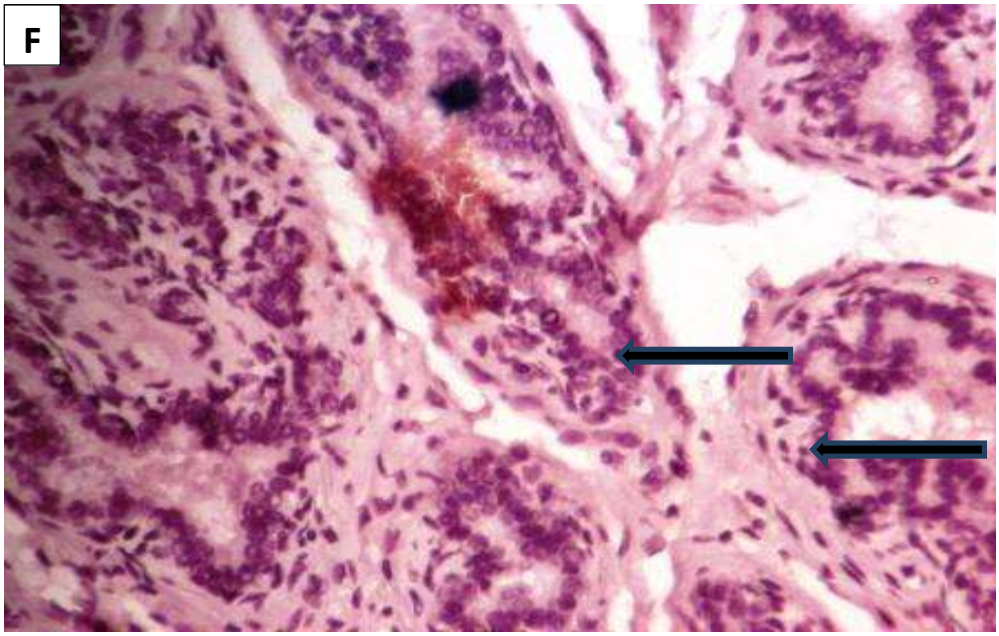
C = BpH (castrated rats that received testosterone propionate only) – Gland shows weak expression of p53 (black arrow) in the epithelium (M X 400).



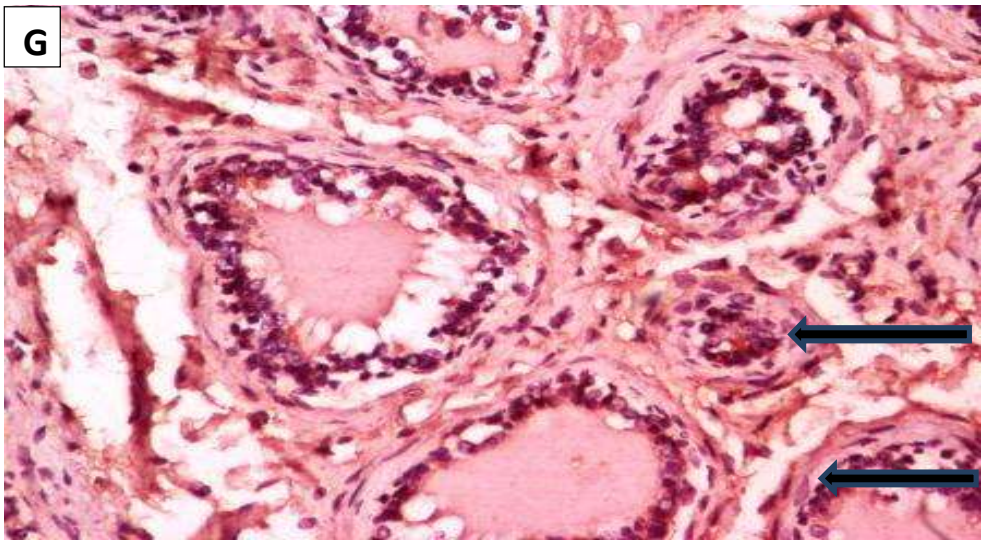
D = BpH + MeJa = (castrated rats that received testosterone propionate and methyl jasmonate) – Gland shows strong expression of p53 (black arrow) in the epithelium (M X 400).



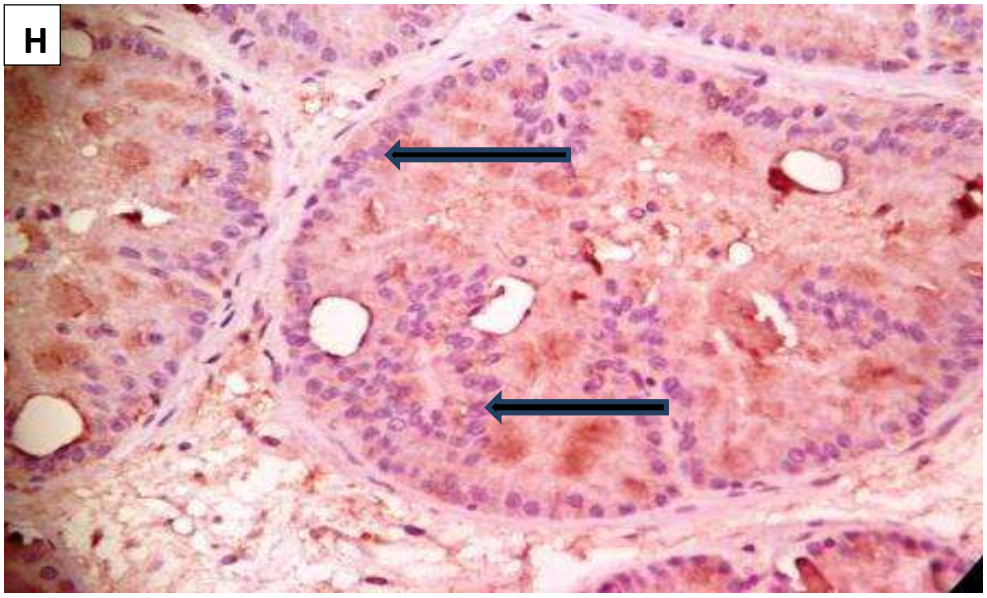
E = BpH + FN(castrated rats that received testosterone propionate and Finasteride) – Gland shows moderate expression of p53 (black arrow) in the epithelium (M X 400).



F = CMeJa(Castrated rats that received methyl jasmonate only) – Gland shows moderate expression of p53 (black arrows) in the epithelium (M X 400).



G = CFN(Castrated rats that received finasteride only) – Gland shows moderate expression of p53 (black arrows) in the epithelium (M X 400).



H = BpH + MeJa + FN (Castrated rats that received Tp, methyl jasmonate and finasteride) – Gland shows moderate expression of p53 (black arrows) in the epithelium (M X 400).

Figure 4.28(A-H): Photomicrograph of cross section of prostate on the effects of MeJa on the expression of p53 via immunohistochemical staining of the prostate tissue

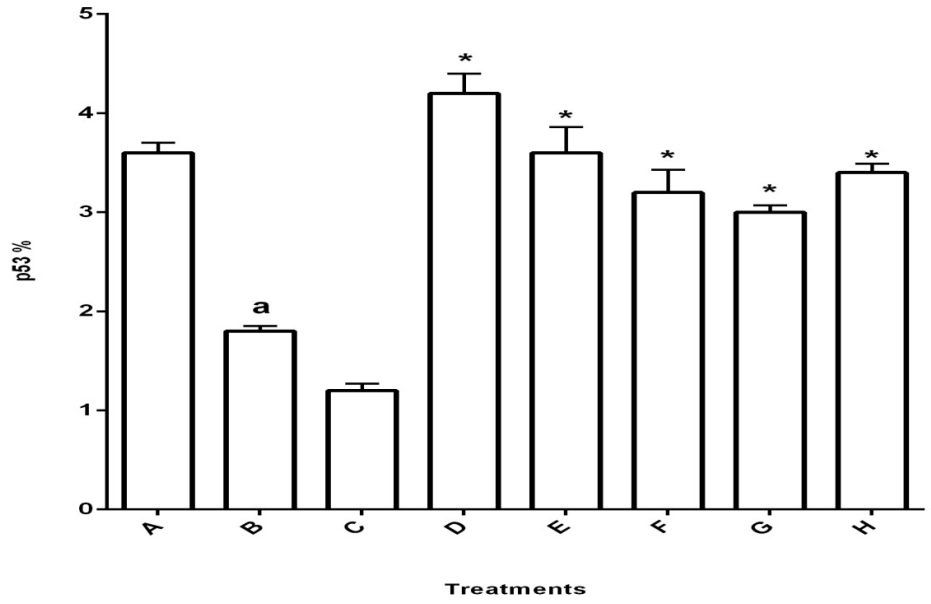
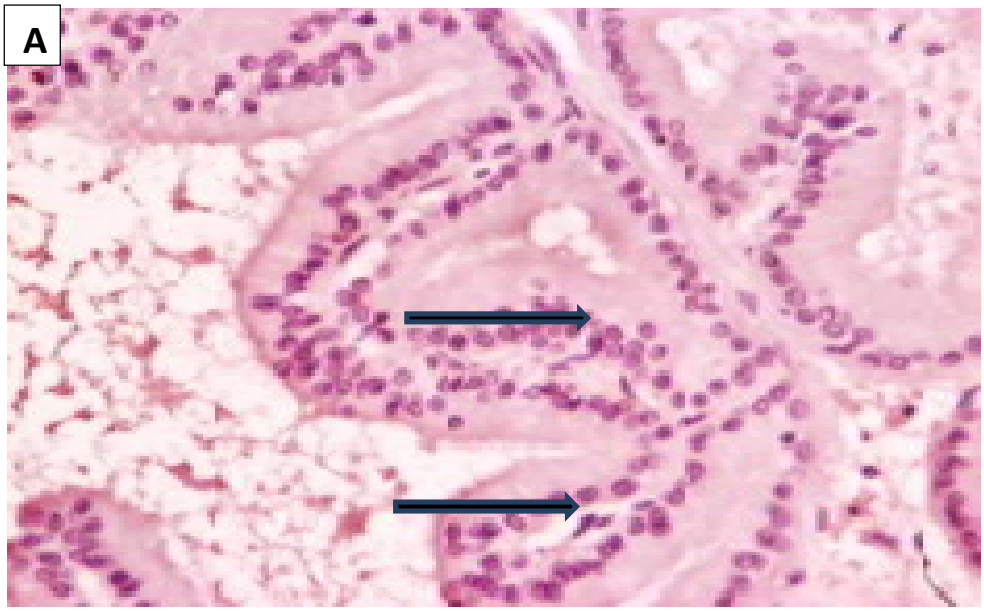
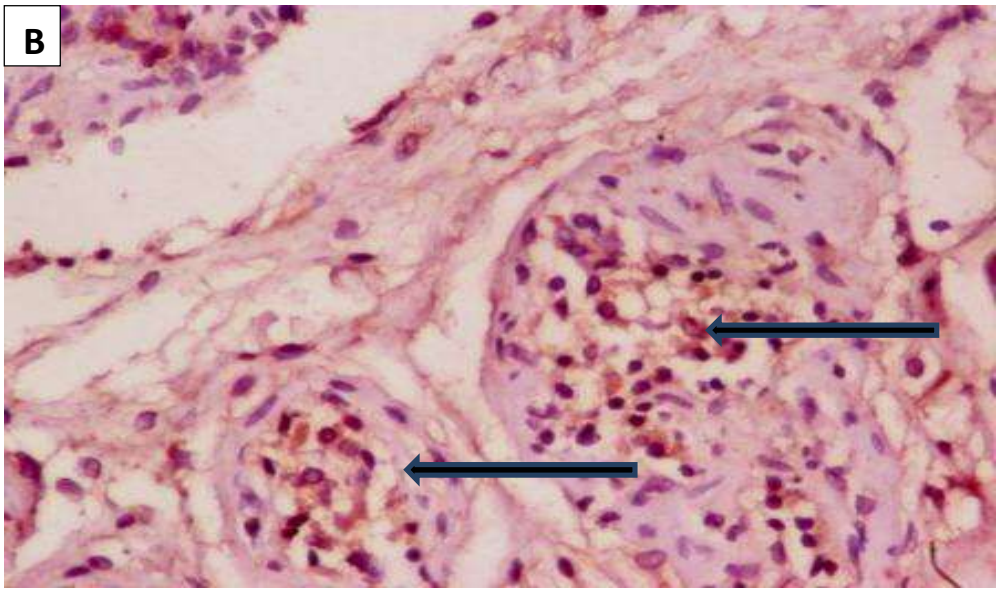


Figure 4.29 Effects of MeJa on the expression of p53 via immunohistochemical staining of the prostate tissue

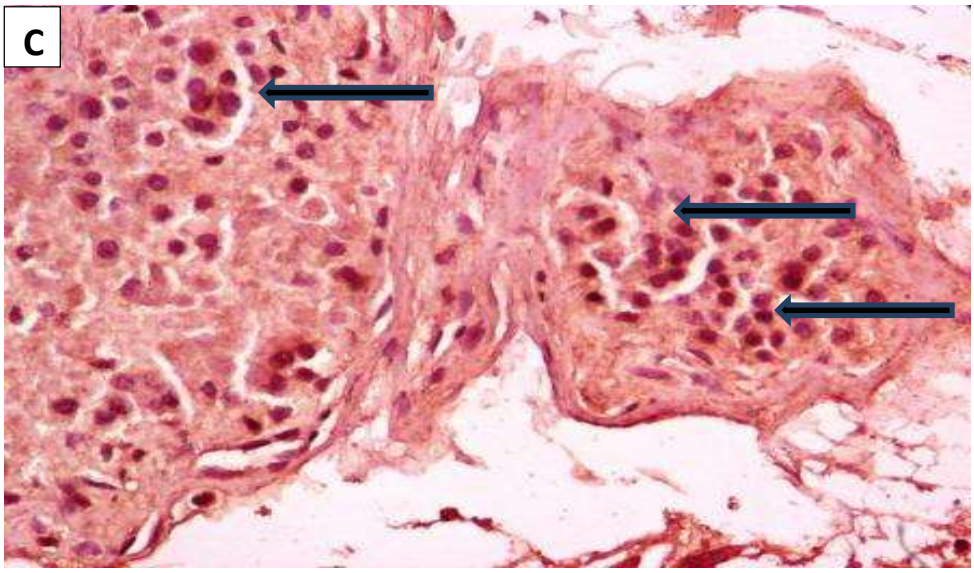
A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. castrated rats that were givenTP, D=BpH + MeJa i.e. BpH rats administered with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats administered with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only. *Significantly different from C ($p<0.05$), [a]Significantly different from C ($p<0.05$).



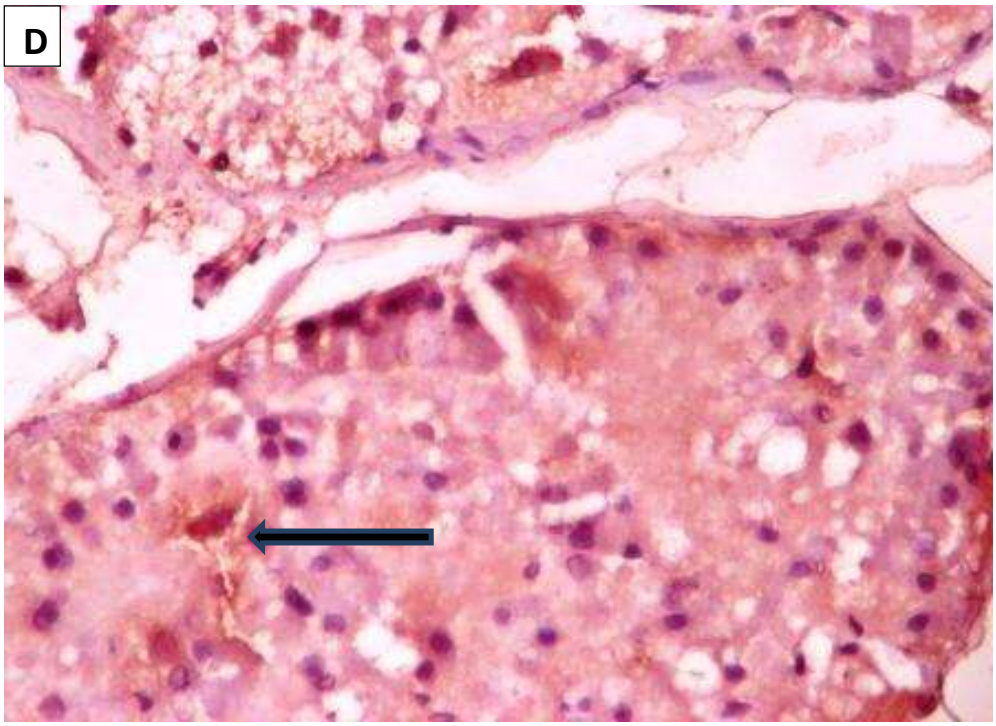
A = Control 1 (Rats that were not castrated) – Gland shows weak expression (black arrows) of COX-2 in the epithelium (M X 400).



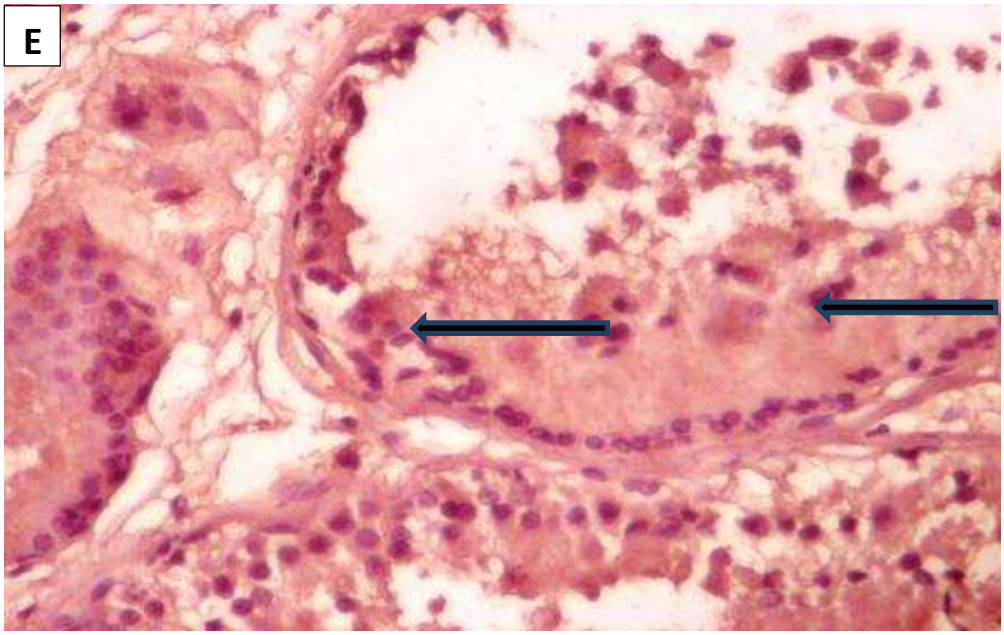
B = Control 2 (Rats that were castrated) – Gland shows weak expression (black arrows) of COX-2 in the epithelium (M X 400).



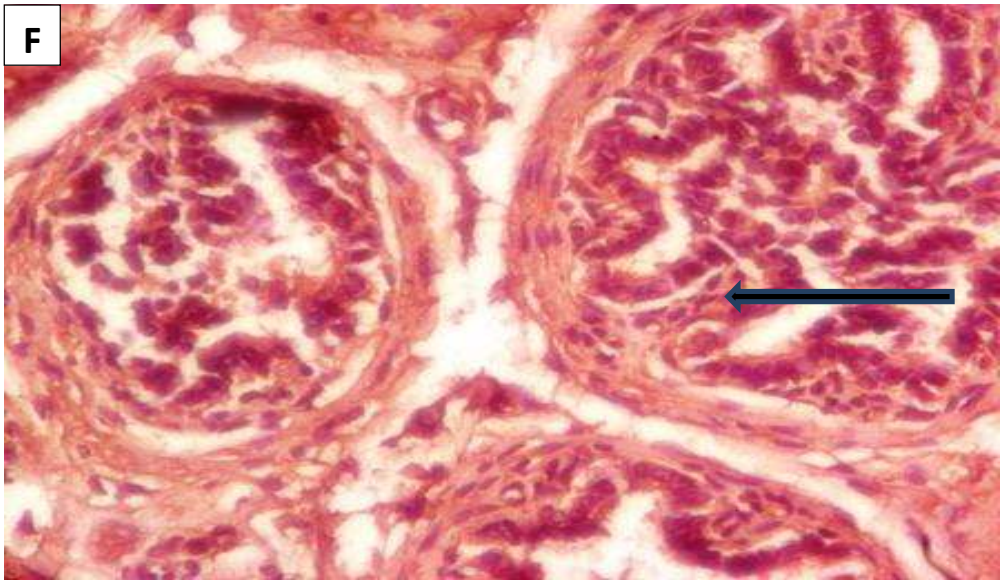
C = BpH (Castrated rats that received testosterone propionate only) – Gland shows strong expression (black arrows) of COX-2 (M X 400).



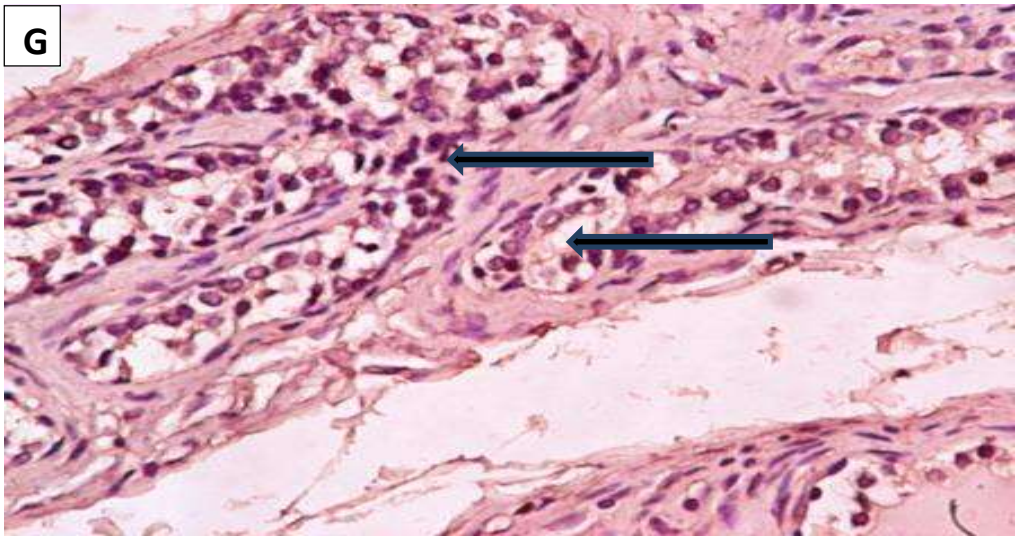
D = BpH + MeJa(Castrated rats that received testosterone propionate and methyl jasmonate) – Gland shows moderate expression (black arrow) of COX-2 (M X 400).



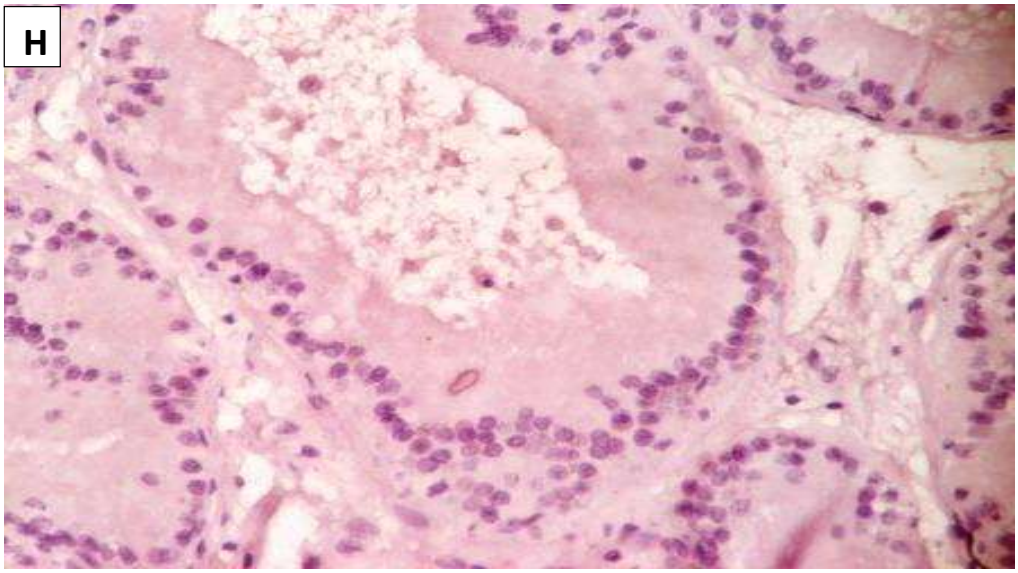
E = BpH + FN (Castrated rats that received testosterone propionate and Finasteride) – Gland shows moderate expression (black arrows) of COX-2 (M X 400).



F = CMeJa(Castrated rats that received methyl jasmonate only) – Gland shows weak expression (black arrow) of COX-2 (M X 400).



G = CFN **(**Castrated rats that received finasteride only) – Gland shows strong expression (black arrows) of COX-2 (M X 400).



H = BpH + MeJa + FN (Castrated rats that receivedTp, methyl jasmonate and finasteride)– Gland shows weak expression of COX-2 (M X 400).

Figure4.30(A-H): Photomicrograph of cross section of prostate tissue showing the effects of MeJa on the expression of COX-2 by immunohistochemistry staining

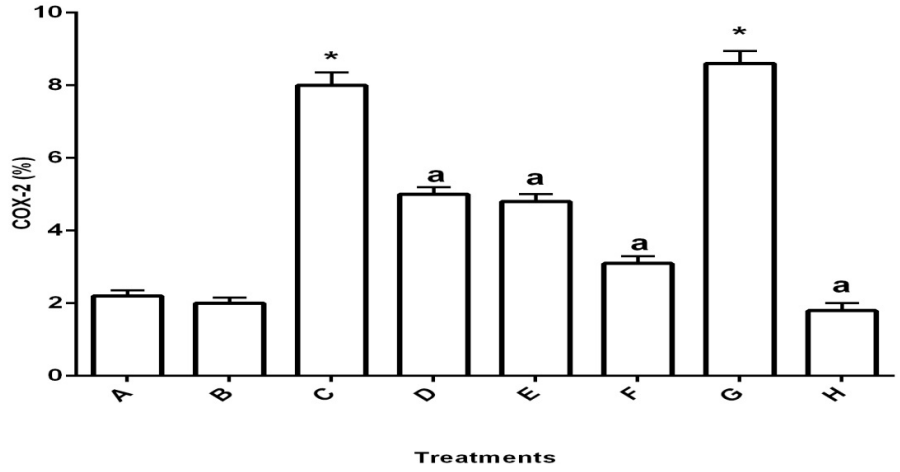
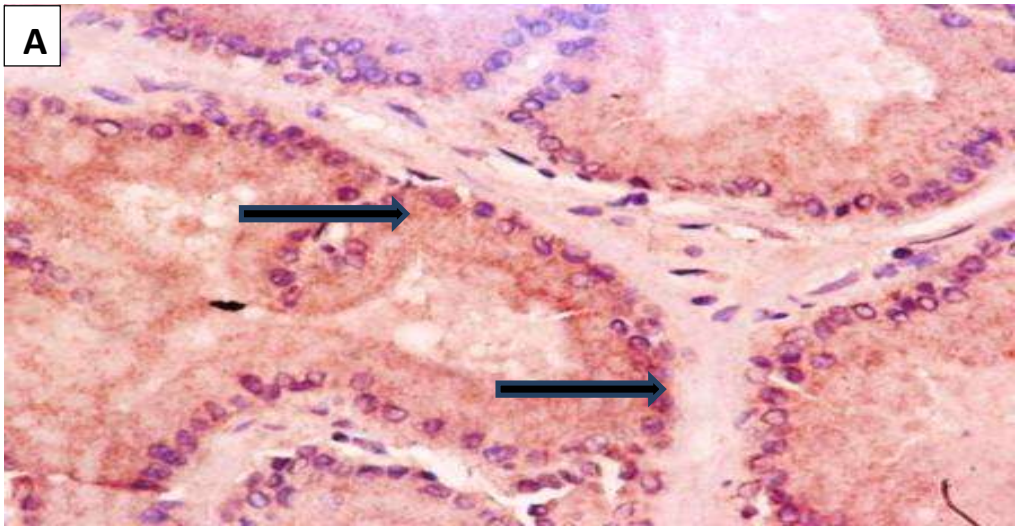
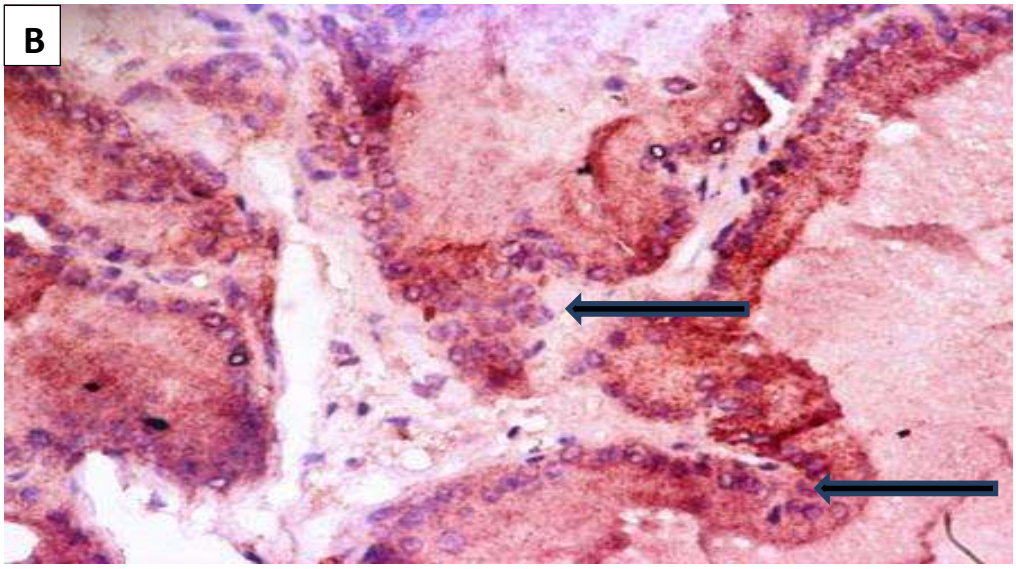


Figure4.31 Effects of MeJa on the expression of COX-2 by immunohistochemistry staining in the prostate tissue

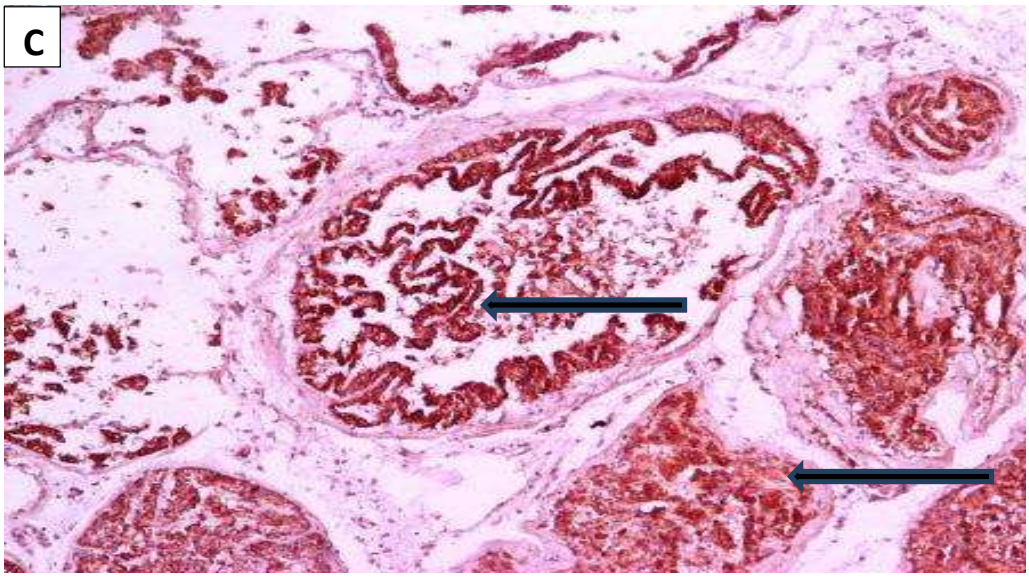
A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. castrated rats that were given TP, D=BpH + MeJa i.e. BpH rats administered with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats administered with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only. *Significantly different from B ($p<0.05$), [a]Significantly different from C ($p<0.05$).



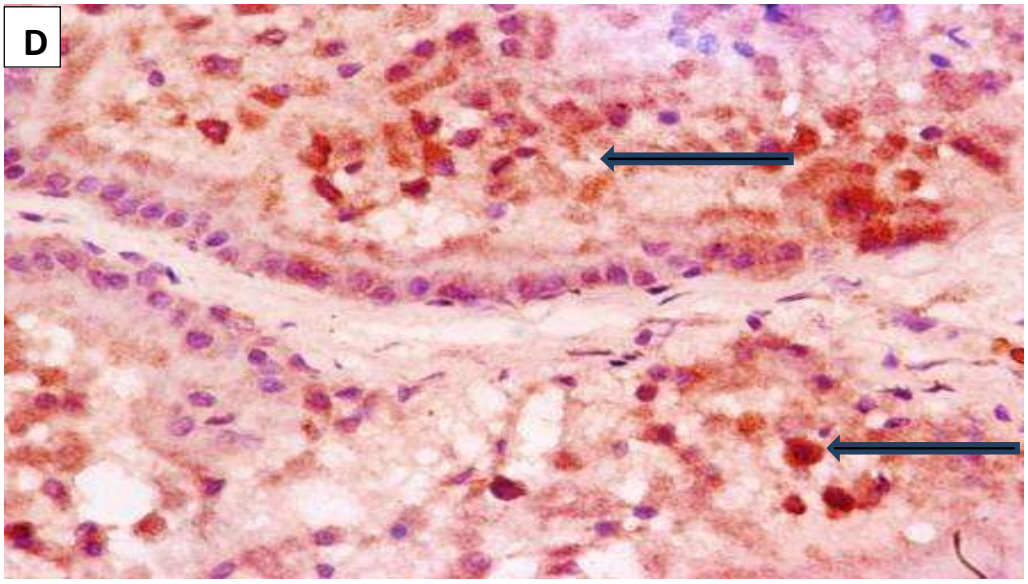
A = Control 1 (Rats that were not castrated) – Gland shows weak expression (black arrows) of iNOS in the epithelium (M X400).



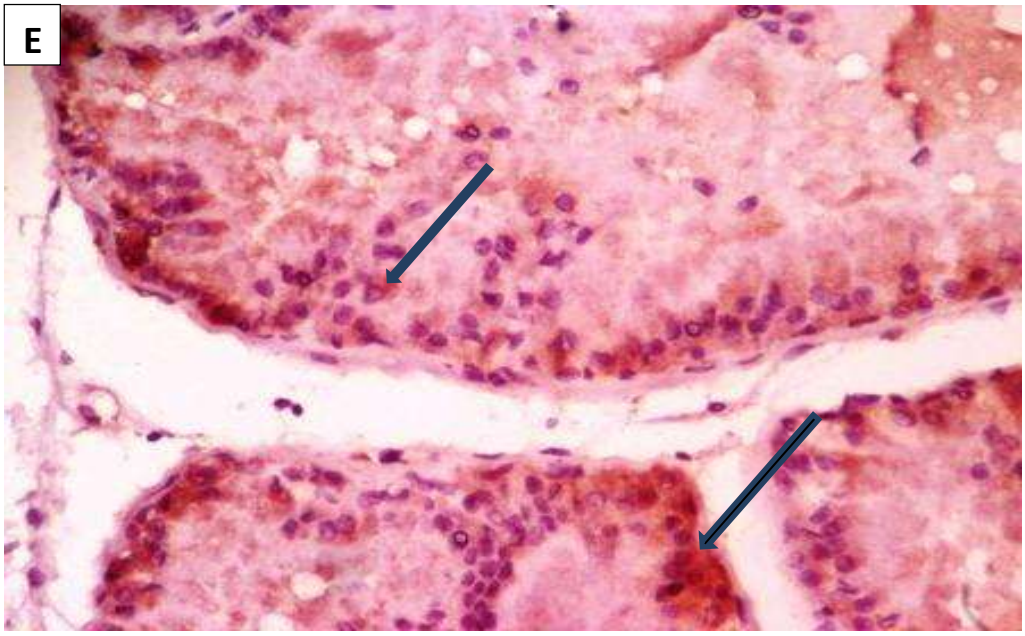
B = Control 2 (Rats that were castrated) – Gland shows weak expression (black arrows) of iNOS in the epithelium (M X400).



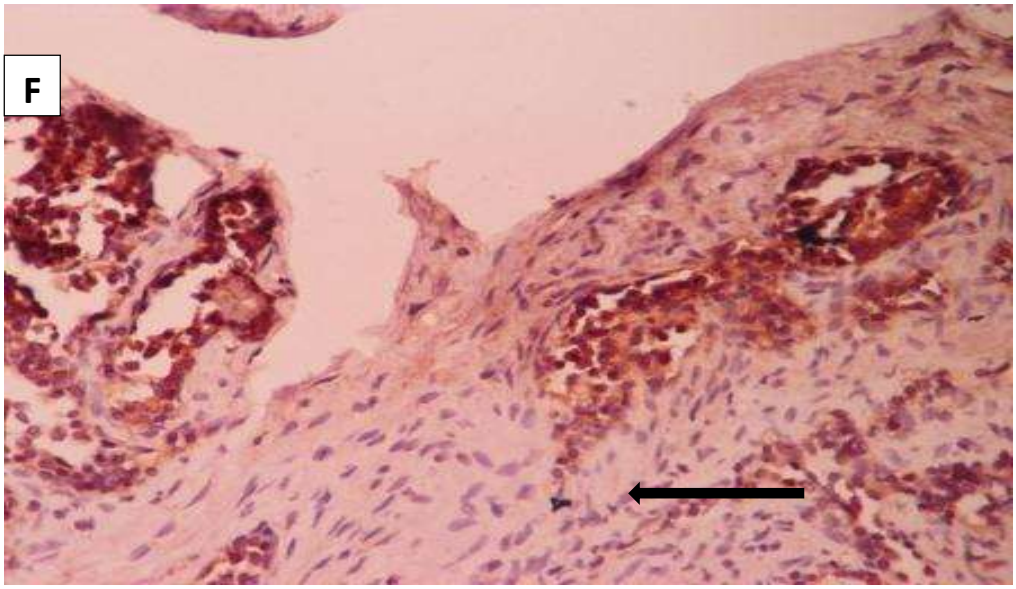
C = BpH (Castrated rats that received testosterone propionate only) – Gland shows strong expression (black arrows) of iNOS in the epithelium (M X400).



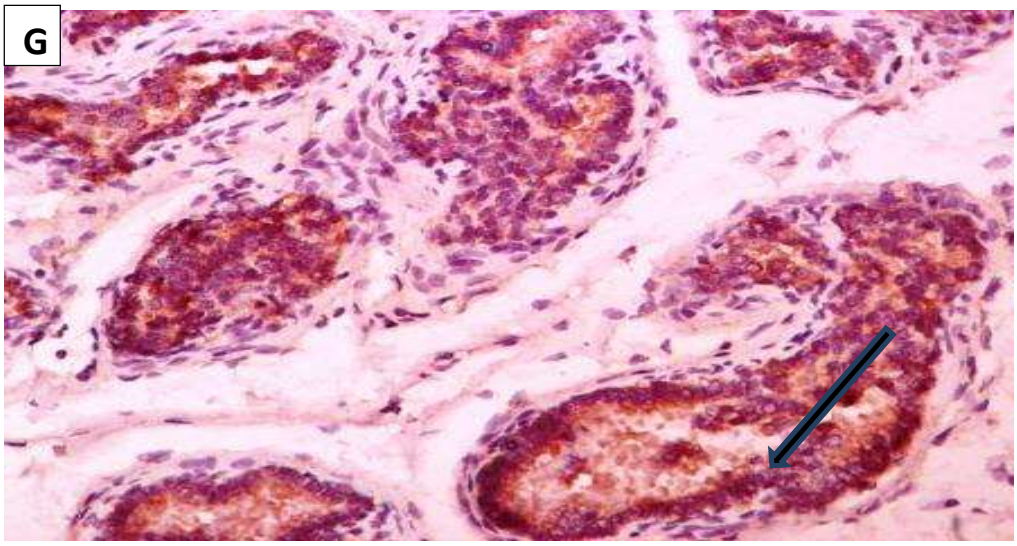
D = BpH + MeJa (Castrated rats that received testosterone propionate and methyl jasmonate) – Gland shows weak expression (black arrows) of iNOS in the epithelium (M X400).



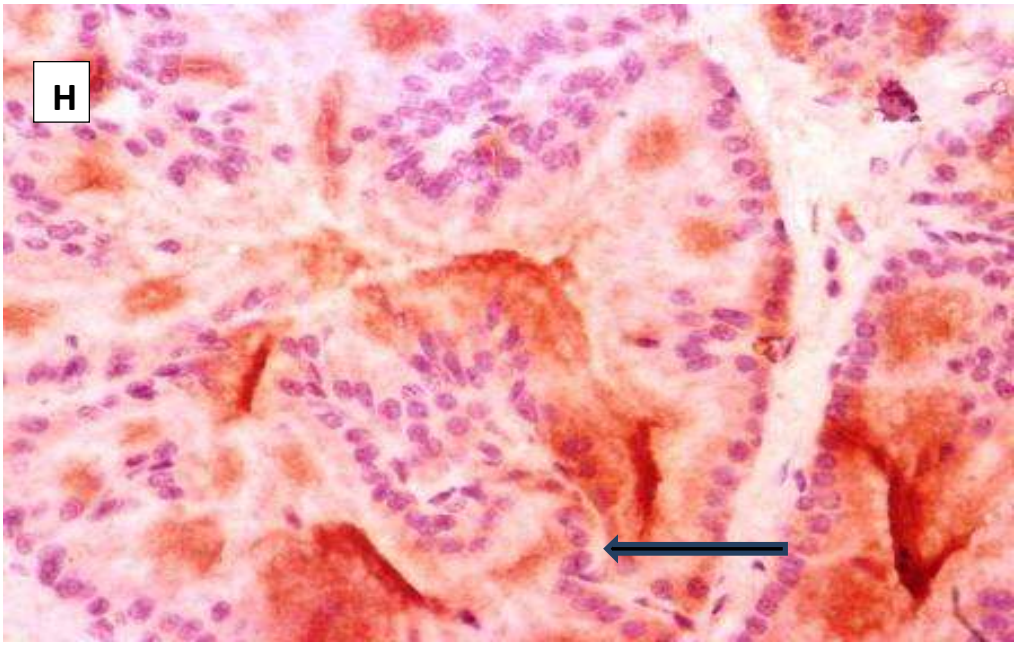
E = BpH + FN (Castrated rats that received testosterone propionate and Finasteride) – Gland shows moderate expression (black arrows) of iNOS in the epithelium (M X400).



F = CMeJa **(**Castrated rats that received methyl jasmonate only) – Gland shows mild expression (black arrow) of iNOS in the epithelium (M X400).



G = CFN = (Castrated rats that received finasteride only) – Gland shows moderate expression (black arrow) of iNOS in the epithelium (M X400).



H = BpH + FN + MeJa (Castrated rats that receivedTp, mthyl jasmonate and Finasteride) – Gland shows moderate expression of iNOS in the epithelium (M X 400).

Figure 4.32(A-H): Photomicrograph of cross section of prostate tissue showing the effects of MeJa on iNOS expression via immunohistochemical staining (MX400)

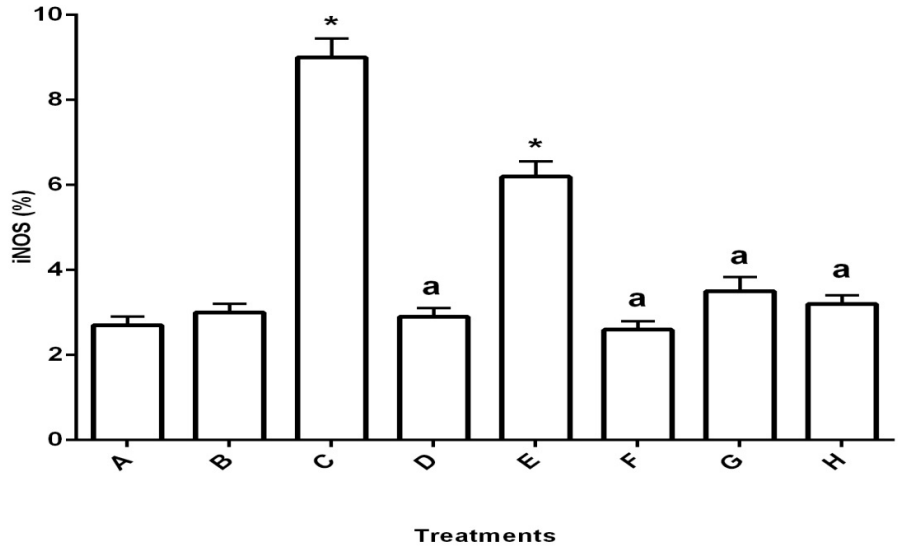


Figure4.33 Effects of MeJa on iNOS expression via immunohistochemical staining of the prostate tissue

A=Control 1 i.e. rats that are not castrated, B=Control 2 i.e. rats that are castrated, C=BpH i.e. castrated rats that were given TP, D=BpH + MeJa i.e. BpH rats administered with methyl jasmonate (MeJa), E= BpH + FN i.e. BpH rats administered with finasteride (FN), F=CMeJa i.e. castrated rats that were given MeJa only, G=CFN i.e. castrated rats that were given FN only.

CHAPTER FIVE

DISCUSSION

Several types of therapeutic agents such as dutasteride and finasteride commonly used to treat BpH are known to have adverse side effects like impotence, loss of libido, heart and lungs complications (Bullock and Andriole, 2006).Therefore, the search for better drug candidates from natural source is on the increase. Inspite of the anticancer actions of methyl jasmonate,information on its chemopreventive role on the development of BpH is very rare. Thisstudy therefore was aimed at determining the role of methy jasmonate in Tp-induced BpH in catsrated Wistar rats.

The prostatic weight of BpH rats was increased significantly when matched with castratedcontrol group and this support the discoveries of Wang and Olumi (2011) who discovered that the increase in the prostate size was connected with stromal–epithelial relations within the tissue. Oral administration of Finasteride andMeJa injection (singly or combined) significantly reduced prostatic weight thereby repressed the development of hyperplasiain the prostate tissue. Li *et al*. (1993) reported higher amount of zinc in human prostate gland than in some other tissues. The accumulation of zinc in the prostatic epithelial cell is needed for the mainbiologicalroles of production and secretion of citrate.

From our result, castration decreasedthe zinc level significantly relative to non-castrated rats whereas the zinc level was raisedin BpH rats relative to its castrated control. Chyan *et al.* (2014) detected that the concentration of zinc islowered in prostate carcinoma patients whereas high concentration of zinc was found in BpH. The increased zinc level observed in BpH rats in our study correlated with the workof Wu *et al*. (2015). Methyl jasmonate capacity to decrease zinc level in BpH rats indicatesits ameliorative effects. Besides, there have been inconsistent reports in therole of prostatic selenium in relation to prostate pathologies. For instance, higher level of plasma selenium was observed in BpH(Guttenplan *et al*., 2001),whilelower level was observed in some prostatic pathology (Lopez-Fontana *et al*., 2010). Our study howeverrevealed

insignificant changesin the selenium level of BpH rats when compared with other groups.

Part of the proteins made by the secretory cells of the prostate columnar epithelium,in human being after puberty,is prostatic acid phosphatase (Graddis *et al*., 2011). This protein has been reported to be around 0.5 mg/g wet weight of prostate glandand 1.0 mg/mL seminal fluid. Also, the serum levels of prostatic acid phosphatase are usually fairly low in unaffected individuals, while in people with advanced prostate carcinoma, higher levels of prostatic acid phosphatase (PAP) were observed**(**Ahmann and Schifman, 1987).Hence, the raisedlevels of total, prostatic and non-prostatic acid phosphatases in BpH rats indicateabnormality and irregularbiologicalactivities in the prostatic tissue.In this study, administration of methyl jasmonatereducednon-prostatic acid phosphatase activity in the rats. Also, the activity of prostatic alkaline phosphatase in BpH ratsincreased significantly relative to castrated control rats and this suggest tissue damage.

The main antioxidative enzymes thatremove free radicals are catalase, glutathione reductase,superoxide dismutase, glutathione peroxidase, and peroxiredoxins. Superoxide dismutase is the enzyme that first attack free radicals especially superoxide radical and reduce it to hydrogen peroxide (Singh, 2015). This H_2O_2 isconvertedto oxygen and water by GPx or CAT. Glutathione peroxidase (a selenoproteinenzyme)eliminates H_2O_2 by using it to oxidize GSHinto its oxidized form while it is being regenerated by glutathione reductase usingreduced nicotinamide adenine dinucleotide phosphate as reducing agent. Also,GST,containsa family of enzymes that inactivate noxious endogenous derivatives and xenobiotic agents, known to be associated with cellular opposition to oxidative stress. Prostatic luminal epithelial cells of BpH have been reported to express high levels of GST. In this study, the elevated activity of GST observed in the BpH rats was reduced by MeJa.

Increased lipid peroxidation levels with concomitant decrease in antioxidants were detected in the prostate of BpH (Lee *et al*., 2012). Also, certain plant extractswere reported to reduce oxidative damage in BpH rats. The extracts of saw palmeto, used often to treat BpH, exhibited antioxidant properties. Thus, antioxidants can be regarded as potent candidates to suppress theprogress of BpH. From this study the reduction in the activities of CAT, SOD, GST, GPx and GSH suggest that the administration of TP to castrated rats for 4 weeks might have caused the generation of ROS that overwhelm the activities of the antioxidant enzymes. In contrast, MeJa restored the antioxidant

status of BpH rats. Therefore, the ability of MeJato restore the prostatic antioxidant condition of BpH rats near normalcy is suggestive of its antioxidative properties.As a result, methyl jasmonatemight be an effectual antioxidative agent against prostatic oxidative stress induced by testosterone propionate.

Radicals and non-radical ROS are known initiator and promoters of lipid peroxidation (oxidative injury). Ayala *et al*. (2014) discovered that peroxidation of the lipid bilayers can be initiated when the hydroperoxide formed originally in tissues are broken down by enzymatic reactions. Also the aldehyde end product of the reactions (e.g. malondialdehyde and 4-hydroxynonenal) is known to attack macromolecules like deoxyribonucleic acid and protein (Uchida *et al*., 1999). Our resultestablished thatinduction of BpH by Tpin castrated rats elevated the prostatic malondialdehyde level relative to the control. Interestingly, treatment with MeJa significantly reduced the prostatic malondialdehydelevels when compared with the BpH rats and this effect couldpossiblyemanate from its antioxidant potential.

In addition, prostatichistopathologyinBpH rats showed severe hyperplasia of the gland andmany secretions. Conversely, reduced hyperplasiaof the tissue was detected inBpH rats treated with MeJa which is an indication that MeJa can impede the growth of prostatic cells.Webber *et al*. (1995) reported that Prostate Specific Antigen (PSA) improves sperm mobility by degrading the proteins of the extracellular matrix. The PSA also engender multiplication of the stromal fibromuscular cells in the prostate and have higher expression in the epithelial cells of BpH patients (Sutkowski, 1999). As the PSA increasedthe epithelial barrier integrity decreased, the basement membrane becomes degraded, PSA escapes into the stromal stall and stimulates the appropriate response. In this study, PSA expression was upregulated in the BpH group and there existed the stack of nuclei on the epithelial layer. One of the hallmarks of malignancy is unrestrained proliferation and immunohistochemical evaluation of the expression of Ki67 protein has beengenerally employedto determine the ability of cancer cells to proliferate (Desmeules *et al*., 2015). From this result, Ki67 expression was upregulatedin BpH rats as compared with the castrated control and thisis suggestive of increased prostatic cell volume in BpH rats. On the other hand, the expressions of PSA and Ki67 were downregulated by the administration of MeJa to BpH rats.

Androgenslevel, besides age, is vitalrisk factor in BpH development.The key androgen linked with prostatic growth is testosterone and is required for the sustenance of prostate physicalfeature and functional integrity (Jarvis *et al*., 2015). Testosterone

stimulates the multiplication of cells in the prostatethrough theaction of type 2 5α-reductasethat converts testosterone toDHT. Elevated levels of serum testosterone and DHT levels were discovered in BpH subjects and these correlate with the prostate size (Carson and Rittmaster, 2003). The result of this study is in line with previous reports (Carson and Rittmaster, 2003), as increased serum testosterone levels was detected in BpH rats whereasadministration of finasteride and methyl jasmonate (when combined) to BpH rats markedlyreduced the level of the hormone. This result suggests that methyl jasmonate or finasteride can mop-up excess testosterone thusreducing the quantity of DHT formed. Decrease in the levels oftestosterone or DHT is oftenaccompanied withalterations in the growth and weight of prostate. Prostate gland relieson steroid androgens to maintain its architecture and its receptor (androgen receptor) is significantin the maintenance of healthy prostatic growth. Also testosterone and its active metabolites (DHT)couldaffect cell signals by its receptor that participates in biochemical activities like growth factors activation, apoptotic and inflammatory processes (Chen *et al*., 2013).Such role of androgen receptor in the prostate supports its ability to transform normal and healthy prostate to hyperplastic prostate or prostatic cancer (Chen *et al*., 2013).

Furthermore, inflammatory infiltrates and their mediators are implicated in BpH development (McLaren *et al*., 2011). Mast cells are involved in inflammatory diseases, several tumor developments (including prostate tumor) and are also considered to be the keycontrollers of inflammatory progressions in tumors. Moreover, medicalopinions suggest that prolonged inflammation is implicated inBpH progression, because BpH tissues usually have insinuating lymphocytes and macrophages near the glandular elements (Chughtai *et al*., 2011). In this study, myeloperoxidase activity and the level of nitric oxide in the prostate were elevated together with strong expression of COX-2 and iNOS (inflammatory markers) in the prostates of BpH rats and thus suggest prolonged inflammation in BpH rats. The leukocyte enzyme (myeloperoxidase), becomes unrestricted when leukocyte is activated (Malech and Nauseef, 1997),usually forms diffusible oxidants and free radicals, hencestimulateperoxidation of the lipid membrane and movement of neutrophil to the inflamed site. Investigation of the prostate tissue via histology of BpH rats revealed extensive infiltration of inflammatory cells. Methyl jasmonate and finasteride showed their protective ability by alleviating the observed inflammatory reactions in the prostateof hyperplasia rats.

Kyprianou *et al*. (1996) reported that increase in cell proliferation followed by reduced apoptosis can lead to pathologic enlargement of the prostate. Therefore, apoptosis may possibly protects the buildup and spread of faulty cells. This work reportsthat expression of Bcl-2 proteinwas upregulated, while Bax protein was downregulated in benign prostatic hyperplasia rats. These results are supported by the findings of Kyprianou *et al.* (1996) who stated that Bcl-2 in BpH patients was strongly expressed in the basal and luminal epithelial cells when compared to healthy prostate tissue. We therefore suggest thathigher expression of Bcl-2 downregulate apoptosis in human prostate, leading to growth disparity in favour of cell proliferation, which eventuallystimulates increase in prostate size. Interestingly, MeJa and FNconsiderably mitigated Bax and Bcl-2 expressions.

The buildup of cholesterol, synthesizedin the prostate at a level nearly similar to that of the liver, is dependent on the age of the individual. The increased serum triglyceride and total cholesterolwith concomitant decrease inhigh density lipoprotein cholesterol detected in this studyis in line with the report of Freeman and Solomon (2004). These researchers stated vice versa that the amount of cholesterol in the serum of BpH patients doubles that of anormal subject. Moyad and Lowe (2008) reported similar results and suggested thattreatmentoptions that lower cholesterol could reduce the riskof BpH development. Hence, this study suggests that administration of finasteride and methyl jasmonate to BpH subjects can protect against lipid disorder of BpH. In addition, xenobiotic metabolism is improved when cytochrome P450-dependent isoenzymes are induced and these usually support the development of metabolic product from parent drugs that are highly reactive. From the study, the activity of aniline hydroxylase was increased in BpH rats while administration offinasteride and methyl jasmonate (singly or combination) reduced the activity of aniline hydroxylase in benign prostatic hyperplasia rats.

5.2 Conclusion

Methyl jasmonate is a promising chemopreventive agent with strong antioxidant and pharmacological properties. Hence, it is necessary to explore its mechanism of action and establish its health protective properties. Although, abundant in plants, it can also be used to improve the health value of several nutraceuticals. Hence, the study confirms that methyl jasmonate improved the antioxidant status, inhibited inflammation and reduced the testosterone levels of BpH rats. It also modified lipid disorders, reduced the induction of phase 1 enzyme and induced apoptosis in benign prostatic hyperplasia rats.

5.3 Contributions to knowledge

The study on the effects of methyl jasmonate on Tp-induced BpH rats has contributed the following to the body of knowledge in this field:

1. Methyl jasmonate decreased the weight of prostate in BpH rats.
2. Methyl jasmonate has protective effects on Tp-induced prostatic oxidative stress by increasing the antioxidant enzymesactivities whichwere significantly reduced in BpH rats.
3. Methyl jasmonate modified the androgen status in rats by decreasing the serum testosterone level in benign prostatic hyperplasia rats.
4. Methyl jasmonate also ameliorated the lipid disorder observed in the BpH rats.
5. Methyl jasmonate showed protective effects in BpH via anti-inflammatory reactions.
6. Methyl jasmonate also protect against Tp-induced cell proliferation in BpH rats by inducing apoptosis.

5.4 Recommendation

From the findings of this study we recommend that:

a) Plants rich in methyl jasmonate should be consumed by men with BpH in order to prevent the progression of BpH.
b) Methyl jasmonate when combined with the conventional drug (e.g Finasteride) in the management of BpH has better effect due to synergism of the two compounds.

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APPENDICES

Appendix 1: Preparation of Reagents for Homogenizing Buffer

Phosphate buffer (0.1M: pH 7.4)

To prepare the buffer, 7.16 g of $Na_2HPO_4.12H_2O$ was dissolved in 200 mL of water (distilled) while 1.56 g of $NaH_2PO_4.2H_2O$ was dissolved in 100 mL of water (distilled). Lastly, the two solutions were mixed and adjusted to pH of 7.4.

Appendix 2: Preparation of Reagents for Protein Determination

Sodium hydroxide (0.2M)

This was prepared by dissolving eight grams of sodium hydroxide in 900 mL of water (distilled) and made up to 1000 mL.

Biuret reagent

The was prepared by adding 9 g of sodium potassium tartarate and 5 g of KI in 500 mL of 0.2 M NaOH solution and mixed thoroughly. 3 g of copper sulphate dissolved in 300 mL of water (distilled) was added to the mixture and made up to 1000 mL.

Bovine Serum Albumin

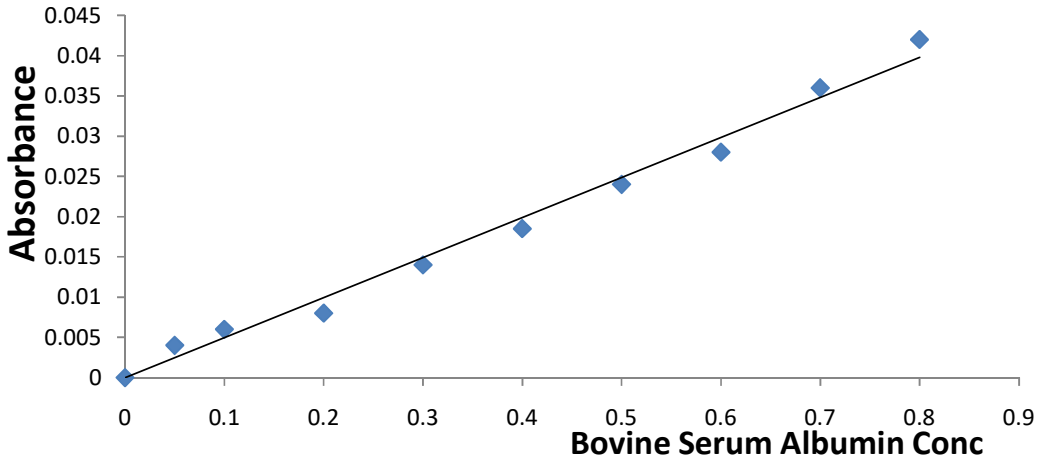
To prepare the stock BSA, 7.4 g of BSA was dissolved in 100 mL of 0.9% NaCl to give final concentration of 74 mg/mL.

Standard BSA curve by the Biuret method

After preparing 2-10 mg protein/mL of BSA solution, several dilutions of the stock were made. Into 1.0 mL of each dilution was added 4 milliliters of the Biuret reagent, incubated at 25°C for thirty minutes and read at 540 nm using spectrophotometer.

Table for Protein Determination

Test tube number	1	2	3	4	5	6	7	8	9
Stock BSA(mL)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Distilled water (mL)	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Biuret reagent (mL)	4	4	4	4	4	4	4	4	4
BSA concentration (mg/mL)	0.05	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80
Absorbance (540nm)	0.004	0.006	0.008	0.014	0.0185	0.024	0.028	0.042	0.042



Calibration curve for protein determination

Appendix 3: Preparation of reagents for Lipid Peroxidation

30% Trichloroacetic acid (TCA)

To prepare 30% TCA, 30 g of trichloroacetic acid was dissolved in 100 mL of water (distilled) and kept at 4°C**.**

Thiobarbituric acid (0.75%) in 0.1 M HCl

To prepare 0.75% TBA, 0.075g of thiobarbituric acid was dissolved in of 0.1M HCl (10 mL) and the liquid was agitated in water bath, so as to help dissolution, at 90°C.

Tris- KCl buffer (0.1 M: pH 7.4)

The preparation was by the addition of KCl (1.12 g) and Tris base (2.36 g) in 90 mL of water (distilled), pH was adjusted to 7.4 and made up to 100 mL.

Appendix 4: Preparation of Reagents for Reduced Glutathione Determination

Reduced Glutathione (working standard)

The preparation was by weighing, 40 mg of reduced glutathione and dissolved in hundred mililitre of phosphate buffer.

Phosphate buffer (0.1M: pH 7.4)

To prepare the buffer, 7.16 g of $Na_2HPO_4.12H_2O$ was dissolved in 200 mL of distilled water while 1.56 g of $NaH_2PO_4.2H_2O$ was dissolved in 100 mL of distilled water. Lastly, the two solutions were mixed and adjusted to pH of 7.4.

Ellman's Reagent [5,' 5,'-dithiobis-(2)-nitrobenzoic acid]

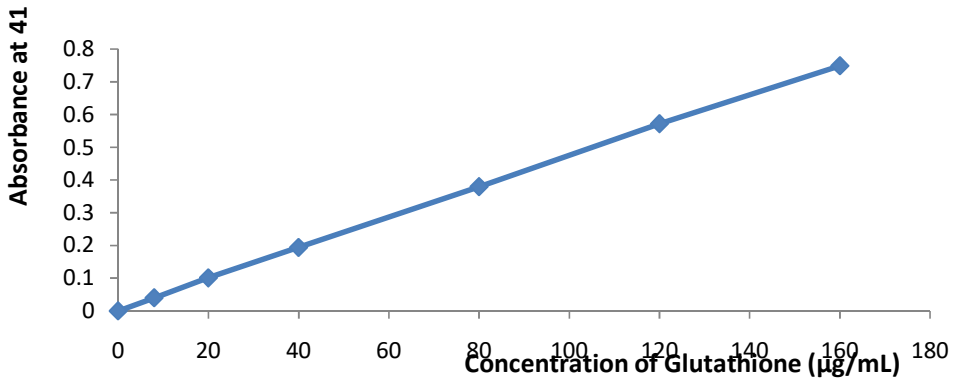
The preparation was by dissolving Ellman's reagent (40 mg) in 100 mL of phosphate buffer.

4% Sulphosalicyclic Acid (SA).

The preparation was by dissolving SA (4 g) in 80 mL of distilledwater and filled up to 100 marks in a standard volumetric flask. This reagent is stable for approximately 3 weeks at 4°C.

Table of the calibration curve for determination of the level of reduced glutathione

Stock	Phosphate Buffer	Ellman's Reagent	Absorbance (412nm)	GSH Conc.(ug/mL)
0.02	0.48	4.50	0.033	8
0.05	0.45	4.50	0.099	20
0.10	0.40	4.50	0.246	40
0.20	0.30	4.50	0.346	60
0.30	0.20	4.50	0.505	80
0.40	0.10	4.50	0.683	100



Calibration Curve for GSH

Appendix 5: Preparation of Reagents for Catalase Activity

Phosphate buffer (0.05 M: pH 7.4)

The preparation was by weighing 0.696 g and 0.265 g of dipotassium hydrogen phosphate trihydrate and 0.265 g of potassium dihydrogen phosphate, respectively and dissolving it in 90 mL of distilled water and made up to 100 mL. The pH was adjusted to 7.4.

Hydrogen peroxide (19 mM)

This was prepared by adding194 µL of 30% H_2O_2 in 50 mL of phosphate buffer and filled up to 100 mL flask with the same.

Appendix 6: Preparation of Reagents for SOD Activity

Carbonate Buffer (0.05 M: pH 10.2)

To prepare carbonate buffer, 4.2 g of $NaHCO_3$ and 14.3 g of $Na_2CO_3.10H_2O$ were liquefied in 900 mL of distilled water and rounded up to 1000 mL by same.

0.3M of adrenaline.

To prepare adrenaline, 0.0137 g of adrenaline was dissoved in two hundred mililitre of distilled water and filled up to two hundred and fifty mililitre mark in the flask with the same and this was prepared just as when needed.

Appendix 7: Preparation of Reagents for GST Activity

1-Chloro- 2, 4, - dinitrobenzene (CDNB)

To prepare 20 mMCDNB, 3.37 mg of CDNB was liquefied in 1mL of ethanol.

Reduced Glutathione (0.1M)

To prepare this, 30.73 mg of reduced glutathione was dissoved in one milliliter of phosphate buffer.

Phosphate buffer (0.1 M: pH 6.5)

The preparation was made by disolving K_2HPO_4 (4.96 g) and KH_2PO_4 (9.73 g) in distilled water, adjusted to pH 6.5 and made up to 1000 mL.

Appendix 8: Preparation of Reagent for GPx Activity

Sodium azide (NaN_3 10 mM)

The preparation was by dissolving 0.0325 g of NaN_3 in 50 mL of distilled water.

Reduced glutathione (GSH 4 mM)

This was prepared by weighing 0.0123 g of GSH and dissolved in phosphate buffer (10 mL).

Hydrogen peroxide (H_2O_2, 2.5 mM)

The preparation was by dissolving 0.028 mL of H_2O_2 in 100 mL of distilled water.

Tricholoroacetic acid (10%)

This preparation was by dissolving 2 g of trichloroacetic acid in 20 mL of distilled water.

Dipotassium Hydrogen orthophosphate K_2HPO_4 (0.3 M)

The preparation was by dissolving 5.23 g of K_2HPO_4 in 100 mL of distilled water.

Ellman's reagent

This preparation was by dissolving Ellman's reagent (0.04 g) in 100 mL of phosphate buffer.

Phosphate buffer

The preparation was by dissolving K_2HPO_4 (0.992 g) and KH_2PO_4 (1.946 g) in 200 mL of distilled water and adjusted to pH 7.4.

Appendix 9: Preparation of Reagents for DNA Fragmentation

Tris-EDTA Buffer TE (5mM Tris-HCl and 20mM EDTA)

The preparation was by weighing 0.61g of Tris (hydroxymethyl) aminomethane (Tris) and 7.45g EDTA and dissolved in 900 mL of distilled water. (This was adjusted to pH of 8.0 and made up to a litre.

Tris-EDTA Buffer TE[1] (5mM Tris-HCl and 20mM EDTA)

The preparation was by weighing 0.61g of Tris (hydroxymethyl) aminomethane (Tris) and 7.45g EDTA and dissolved in 900 mL of distilled water followed by the addition of triton X-100. The pH was adjusted to 8.0 and total volume made up to a litre.

Diphenylamine reagent

This was prepared by dissolving 1.5g of Diphenylamine (DPA) in acetic acid (100 mL) to which concentrated H_2SO_4 (1.5 mL) was added. This reagent is photo-sensitive, hence it was prepared fresh and kept in an amber-colored bottle before use.

Appendix 10: Preparation of Reagents for Nitric Oxide

Griess Reagent

This was made by dissolving N-(1-naphthyl) ethylenediamine dihydrochloride (0.1%) and sulfanilamide (1%) in phosphoric acid (5%).

Appendix 11: Preparation of Reagents for MPO Activity

O-dianisidine

The preparation was by dissolving O-dianisidine dihydrochloride (0.0167 g) in 100 mL of phosphate buffer.

Phosphate Buffer (0.1 M: pH 7.4)

The preparation was by dissolving 496 mg of K_2HPO_4 and 973 mg of KH_2PO_4 in distilledwater and rounded up to 100 mL.

Diluted H_2O_2

The preparation was by adding 4 µL of H_2O_2 to 96 µL of distilled water to make 100 µL of diluted H_2O_2.

Appendix 12: Preparation of Reagents for AnH Activity

Reduced nicotinamide adenine dinucleotide (NADPH)

This was prepared by dissolving 0.025 g of NADPH in 2 mL of distilledwater.

Aniline hydrochloride (27.98mM):

To prepare this, 0.18 g of aniline hydrochloride ($C_6H_5.NH_2.HCl$; MW = 129.59) was dissolved in 40 mL of distilled water and made up to 50 mL.

***P*-Aminophenol (14mM):**

To prepare this, 0.15 g of *p*-aminophenol ($NH_2C_6H_4.OH$; MW = 109.13) was dissoved in 90 mL of distilled waterand made up to 100 mL with 0.1M Na_2CO_3 solution.

Trichloroacetic acid (20%):

To prepare this, 20g of TCA ($CCl_3.COOH$; MW = 169.39) was dissolved in 90 mL of distilled water and made up to 100 mL with same.

Sodium hydroxide (0.5N) in 1% phenol:

To prepare this, 2.0 g of NaOH and 1.0 g of phenol were dissolved in distilled water and made up to 100 mL.

Sodium carbonate (1.0M):

To prepare this, 52.99 g of anhydrous sodium carbonate (Na_2CO_3; MW = 105.99) was dissolved in 450 mL of distilledwater and made up to 500 mL with the same.

Appendix 13: Preparation of Reagents for AmD Activity

Reduced nicotinamide adenine dinucleotide

This was prepared by dissolving 0.025 g of NADPH in 2 mL of distilledwater.

Semicarbazide solution:

To prepare this, 6 g of semicarbazide was dissolved in 50 mL of distilled water and was adjusted to pH of 7.0 with dilute NaOH solution and made up to 100 mL with distilled water.

***P*-Aminopyrine (20mM):**

To prepare this, 0.231 g of *P*-aminopyrine (4-dimethyl aminoantipyrine) was dissolved in 50 mL of 1.15% potassium chloride solution.

Formaldehyde standard (1.2 mM):

To prepare this, 1 mL of concentrated formaldehyde solution (360 mg) was diluted to 1 litre with distilled water, and used immediately. 1 mL of this diluted formaldehyde solution was rediluted to 10 mL with distilled water and used as the standard in the enzyme assay.

Zinc Sulphate (20%):

To prepare this, 20 g of Zinc Sulphate ($ZnSO_4$) was dissolved in 100 mL of distilled water.

Saturated Barium hydroxide solution:

To prepare this, Barium hydroxide was dissolved in distilled water, until no more of the solute could dissolve at the room temperature.

Saturated Sodium tetraborate solution:

Disodium tetraborate (Borax) was dissolved in distilled water, until no more of the solute could dissolve at the room temperature.

Double-strength Nash reagent (Nash, 1953):

This is 4.0 M ammonium acetate containing 4 mL acetylacetone per litre and this was made by liquefying ammonium acetate (15 g) in 50 mL of distilled water containing 0.2 mL acetylacetone (2,4-pentene-dione).

PUBLICATIONS FROM THIS WORK