EFFECTS OF AQUEOUS FRACTION OF ETHANOL LEAF EXTRACT OF *CASSIA ALATA L.*[FABACEAE] *O*N INVIVO ANTIOXIDANT STATUS IN TESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLASIA RATS

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHARMACY (PHARM.D) DEGREE, UNIVERSITY OF BENIN,

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CERTIFICATION

This research project titled' Effects of aqueous fraction of ethanol leaf extract of *Cassia alata* on invivo antioxidant status in Testosterone-Induced Benign Prostatic Hyperplasia in rats 'is an original research work carried by JENNIFER OSABUOGBE under the supervision of Dr. (Mrs.) Josephine in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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DEDICATION

I dedicate this work to my Parents, Mr. and Mrs. S. E. OSABUOGBE for their unfailing support throughout my stay in this institution and to my siblings for their continuous support and encouragement.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Benign prostate hyperplasia is a disease of ageing men. Oxidative stress is a promoter of the ageing process. This study evaluated the effect of the aqueous fraction of the ethanol leaf extract of *Cassia alata l.[fabaceae]* on antioxidant and lipid per oxidation status of male rats induced with benign prostate hyperplasia. Six groups of six rats each were induced with benign prostate hyperplasia by the subcutaneous administration of testosterone (4mg/kg). Groups 1, 2, and 3 received 50, 100 and 200 mg/kg doses of the fraction respectively. Group 5 rats (negative control) received 10 ml/kg of distilled water. Group 4 animals (standard control) were treated with finasteride (5mg/kg) while Group 5 rats (negative control) received 10 ml/kg of distilled water. Group 6 animals (normal control) were neither induced nor treated. All administration was daily for 28 days by oral gavage. Rats were sacrificed on the 29th day, blood was obtained, and serum enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured with Elisa assay test kits. Malondialdehyde concentration was equally measured. The extract did not significantly ($P<0.05$) increase the activities of SOD, CAT, and GPx compared to the negative control, while MDA concentration was also not significant.

CHAPTER ONE

1.0 INTRODUCTION

1.1 INTRODUCTION AND LITERATURE REVIEW

Many plants can induce diverse medicinal effects through the presence of certain metabolites. Plant metabolites, which are organic compounds, can be categorized into primary and secondary types. Primary metabolites, such as glucose, starch, polysaccharides, proteins, lipids, and nucleic acids, are vital for human growth and development. On the other hand, secondary metabolites found in plants encompass alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, and more. The therapeutic efficacy of plants in treating various conditions is primarily attributed to these secondary metabolites (Maurya *et al*., 2008).

Plants utilized in traditional medicine and as dietary sources are more likely to contain bioactive compounds. Recent scientific advancements worldwide have focused on exploring the medicinal properties of plants due to their potent therapeutic benefits, antioxidant activities, minimal side effects, and economic viability. Medicinal plants serve as valuable resources for the pharmaceutical industry, offering cost-effective and efficient health treatments to patients. Importantly, all plants synthesize phytochemicals, which are essential for our well-being since they cannot be synthesized within the human body. Moreover, plants are rich sources of biomolecules, vitamins, and minerals that are crucial for maintaining good health (Shakya *et al*., 2016).

Benign prostatic hyperplasia (BPH) is a prevalent global condition that affects men over 40 years old, often referred to as a disease associated with aging. The occurrence of BPH rises with age and varies across different regions worldwide. Inflammation, hormonal changes, metabolic factors, and age-related processes contribute to the development of BPH. Typical symptoms

include frequent nighttime urination, urgent need to urinate, weak urine flow, dribbling, and incomplete bladder emptying. Factors such as aging, family history, and metabolic disorders increase the risk of BPH. Complications may involve the formation of bladder stones, urinary tract infections (UTIs), and damage to the bladder and kidneys (Mallhi *et al*., 2023).

In males, BPH frequently leads to lower urinary tract symptoms and involves the nonmalignant expansion of prostate tissue. The prevalence of this condition increases with age, with histological studies showing that 50% to 60% of men in their 60s and 80% to 90% of those over 70 have BPH (Roehrborn *et al*.,1997). Various terms like bladder outlet obstruction (BOO), lower urinary tract symptoms (LUTS), and benign prostatic enlargement (BPE) are used in literature to define BPH. BPH refers to the histological changes, BPE indicates the enlarged size of the gland (usually due to BPH), and BOO signifies the obstruction to urine flow (Tammela *et al*., 1999).

1.2 BENIGN PROSTATIC HYPERPLASIA

The phrase "benign prostatic hyperplasia (BPH)" refers to the growth of cellular components within the prostate, leading to an enlarged prostate or issues with urination due to this prostate enlargement and obstruction of the bladder's outlet. From a histological perspective, BPH signifies the proliferation of both the stromal and epithelial components within the prostate gland. BPH typically occurs in the periurethral and transition zones of the prostate (McNeal 1988). It also refers to a non-cancerous increase in prostate tissue which is progressive, leading to the enlargement of the prostate and lower urinary tract symptoms (LUTS) (Roehrborn *et al*., 2009). BPH typically develops in the periurethral and transition zones of the prostate gland and is an inevitable condition in aging males (Parsons *et al*., 2008).

In histopathological terms, benign prostatic hyperplasia (BPH) manifests as nodular growth rather than a widespread increase in tissue, affecting specific regions like the transitional and periurethral zones of the prostate (Nadaf, 2019). Frequently, these nodules merge, forming adenomas. In clinical cases, adenomas from the transitional zone create the lateral lobes, whereas those from the periurethral zone form the middle lobe (Shapur, 2016). BPH causes obstruction both by compressing and distorting the bladder outlet. Distortion, akin to bending a garden hose, creates more obstruction than compression. In the context of the prostate, the lateral lobes compress the bladder outlet, whereas the middle lobe distorts it, with distortion causing more significant obstruction in terms of flow dynamics (Foo 2017).

1.2.1 PATHOPHYSIOLOGY OF BENIGN PROSTATIC HYPERPLASIA

Various possible mechanisms depicts the pathophysiological process of Benign prostatic hyperplasia including Aging, inflammation, hormonal changes, and metabolic factors.

- **Age-related tissue remodeling**: Aging is a notable contributor to the development of BPH. As men age, there is an elevation in levels of prolactin, estrogen, follicle-stimulating hormone, and luteinizing hormone (LH), alongside a reduction in serum testosterone levels. Estrogen affects the metabolism of androgens and amplifies the presence of androgen receptors in the prostate gland, which, in turn, leads to changes in prostate tissue, particularly in the transition zone. Additionally, an increase in prostate volume occurs due to imbalances in the growth factor signaling pathway and reduced apoptosis, potentially resulting in disturbances in the cellular proliferation process (Das & Buchholz, 2019).
- **Hormonal aspects**: The gradual decline in testosterone levels as men age leads to dysfunction and, eventually, the onset of BPH. Another crucial factor in the development of BPH is 5-Alpha reductase, which converts testosterone into dihydrotestosterone (DHT). DHT

accumulates in the prostate and binds to androgen receptors, contributing to the development of BPH (Madersbacher *et al*., 2019).

- **Inflammation:** Inflammation is a key factor in BPH development. Tissue damage leads to the release of self-antigens in the prostate, triggering the activation of immune cells like CD4 lymphocytes, chemokines, and proinflammatory cytokines. This process stimulates the production of growth factors and promotes angiogenesis in the prostate tissues, akin to a wound-healing response. Leukotrienes, insulin resistance, and prostaglandins also contribute significantly to prostate inflammation (Das & Buchholz, 2019).
- **Metabolic factors**: Various metabolic conditions like obesity, diabetes, and dyslipidemia play a role in the advancement of BPH. Although the specific connection between BPH and these metabolic factors remains unclear, factors such as pelvic ischemia, heightened sympathetic activity, and systemic inflammation might contribute to the development of BPH (Madersbacher *et al*., 2019).

1.2.2 EPIDEMIOLOGY OF BENIGN PROSTATIC HYPERPLASIA

The epidemiological landscape of benign prostatic hyperplasia (BPH) and male lower urinary tract symptoms (LUTS) has undergone significant changes in recent years. The term LUTS refers to a specific set of urinary symptoms, allowing for a broad description of these symptoms at the population level. Although it has become the primary term used to assess urinary symptoms in populations, LUTS is still closely linked to BPH in the literature. With the aging of the US population, the incidence and prevalence of both BPH and LUTS have been rapidly increasing. These conditions are associated with significant medical complications, a higher risk of falls, depression, reduced quality of life, and substantial healthcare expenses running into billions of dollars annually (Parsons *et al*., 2010).

The prevalence of BPH notably rises with age. BPH affects 70% of men aged 60-69 in the US, and this percentage increases to 80% in those aged 70 and above (Wei *et al*., 2005). BPH is primarily a disorder of aging, with prevalence increasing significantly with advancing age. Epidemiological data consistently demonstrate that the likelihood of developing BPH rises steadily after the age of 40, reaching a prevalence of over 70% in men aged 60 and above. As life expectancy increases globally, BPH becomes a considerable public health concern, given its substantial impact on the quality of life (Parsons, 2006).

Epidemiological studies reveal geographical variations in the prevalence of BPH. While it affects men of all ethnicities, some populations exhibit differences in disease prevalence and severity. For instance, studies suggest that Asian men may experience a lower incidence of BPH compared to their Caucasian counterparts. These variations prompt researchers to investigate genetic, environmental, and lifestyle factors contributing to these differences (Lee *et al.,* 2017).

Nigeria, like many other developing nations, faces unique challenges in healthcare infrastructure and accessibility. Limited data on BPH prevalence in Nigeria complicates the understanding of the burden of this condition (Ezeanyika *et al*., 2006). However, studies suggest that the prevalence of BPH increases with age ((Wei *et al*., 2005). similar to trends observed globally. Cultural factors, limited awareness, and underreporting may contribute to the scarcity of data in Nigeria. Factors such as genetics, lifestyle, and dietary habits are potential contributors to the development of BPH (Wang *et al*., 2022). Nigeria's diverse population and lifestyle variations between urban and rural areas further complicate the epidemiological landscape of BPH. Research initiatives and improved healthcare data collection are essential for a comprehensive understanding of BPH in Nigeria, leading to better healthcare strategies and resource allocation.

Expanding our focus to the broader African continent, BPH epidemiology exhibits both commonalities and disparities across nations. The aging population in Africa, coupled with increasing life expectancy, contributes to a rising prevalence of BPH. Socioeconomic factors, such as access to healthcare and education, influence the awareness and management of BPH in different African countries. Numerous community-based epidemiological studies have reported varying prevalence rates of Benign Prostatic Hyperplasia (BPH), ranging from 30% to 50%. In hospital-based settings, the prevalence has been documented to fall between 18.1% and 25.3% (Han *et al*., 2013; Huh *et al*., 2012). However, it is important to note that these studies are somewhat scarce in sub-Saharan Africa, with the majority of existing reports primarily originating from hospital-based settings.

In contrast to Nigeria and Africa, the United States has a more developed healthcare infrastructure and extensive epidemiological data. BPH is a prevalent condition among aging American men, with a reported prevalence of approximately 50% by age 60 and 90% by age 85. Ethnic variations exist, with African American men experiencing a higher prevalence and more severe symptoms compared to their Caucasian counterparts. In Nigeria, the sole community-based study on BPH relied exclusively on the International Prostate Symptom Score (IPSS) as the diagnostic tool for determining prevalence (Ezeanyika *et al*., 2006). Similarly, a study conducted among Ghanaian men utilized both IPSS and prostatic enlargement determined by digital rectal examination (DRE) (Chokkalingam *et al*., 2012). However, it is essential to note that these studies lacked the use of standardized tools and clinical definitions in data collection, limiting their diagnostic and epidemiological value.

1.2.3 ETIOLOGY OF BENIGN PROSTATIC HYPERPLASIA

The cause of benign prostatic hyperplasia remains unclear; however, it mainly impacts older males. Interestingly, men who undergo testicular removal before puberty do not experience benign prostatic hyperplasia. This observation has led some specialists to propose that age and factors related to the testicles might play a role in the development of this condition (Briganti *et al*., 2009).

BPH symptoms can be categorized into those directly caused by urethral obstruction and those resulting from secondary changes in the bladder. Obstructive symptoms include challenges in initiating urination despite straining, a gradual decrease in the size and force of the urinary stream, and a sensation of incomplete bladder emptying. Among the various lower urinary tract symptoms (LUTS) associated with BPH, nocturia (frequent urination during the night) is particularly bothersome. These symptoms fall into three categories: storage symptoms (such as frequent urination, nighttime awakenings to urinate, and urgency), voiding symptoms (including hesitation, intermittent urination, weak stream, straining, and a feeling of incomplete emptying), and post-voiding symptoms (like dribbling or pain during urination). Some individuals may also experience bladder discomfort or dysuria, which refers to pain or discomfort while urinating (White *et al*., 1990). Some of the causes of Benign Prostatic Hyperplasia (BPH) includes:

Hormonal Changes: Changes in hormonal levels, particularly an increase in the levels of dihydrotestosterone (DHT), a byproduct of testosterone, have been implicated in the development of BPH. The prostate gland is sensitive to the effects of hormones, and alterations in hormonal balance can lead to prostate tissue growth (Gerald and Raj, 2022).

Genetics: There is evidence to suggest a genetic predisposition to BPH. If a man has a family history of BPH, he may be at a higher risk of developing the condition himself. Genetic factors may influence hormonal regulation and prostate tissue responsiveness (Makedon *et al*., 2023).

Diabetes and Metabolic Syndrome: Conditions such as diabetes and metabolic syndrome have been linked to of BPH. Insulin resistance and chronic inflammation associated with these conditions may play a role in prostate enlargement (Ngai *et al*., 2017).

Inflammation: Chronic inflammation within the prostate gland may contribute to the development of BPH. Inflammatory processes can stimulate prostate cell growth and lead to tissue expansion (Nickel *et al.,* 2016).

1.2.4 SIGNS AND SYMPTOMS

Individuals with BPH frequently suffer changes in urine patterns. These can include increased urination frequency, particularly at night (nocturia), a sense of urgency to urinate, difficulties commencing urination, a weak urine stream, straining, dribbling at the end of urination, and the impression that the bladder is not completely emptied. These urinary symptoms might considerably impair daily activities and disrupt sleeping patterns.

Increased Frequency

Increased frequency of urination, particularly at night (nocturia) is a symptom of BPH. This occurs when the enlarged prostate places pressure on the urethra, resulting in an incomplete bladder emptying and a need to urinate more frequently.

Urgency

Men with BPH frequently have an unexpected and urgent need to urinate, which can be difficult to manage. This urgency can interrupt everyday activities and cause concern about reaching a restroom fast.

Weak Urine Stream

Another common symptom of BPH is a weak urine stream or difficulty initiating urination.

This occurs when an enlarged prostate blocks the flow of urine through the urethra, resulting in a weaker stream or unwillingness to urinate.

Incomplete Emptying

Despite the need to urinate frequently, men with BPH may have difficulty completely emptying their bladders. This can cause a sense of incomplete voiding or the need to return to the restroom immediately after urinating.

Dribbling

Following urination, some men with BPH may experience dribbling or leaking of urine due to residual urine in the bladder. This might be uncomfortable, causing embarrassment or discomfort.

Straining

Straining when urinating is another sign of BPH. The greater effort required to completely empty the bladder can result to stomach discomfort or pelvic pressure.

In some situations, BPH can cause blood in the urine (hematuria). This can be seen as pink, crimson, or cola-colored urine and could signify issues including urinary tract infections or bladder stones.

Severe cases of BPH can result in urine retention, which occurs when the bladder is unable to empty completely. This can result in pain and suffering, and medical intervention may be required to relieve the clog and empty the bladder. Chronic urine retention caused by BPH increases the risk of bladder stones, which are hard mineral deposits that build up in the bladder. These stones can cause pain and discomfort, so treatment may be required to avoid complications.

BPH can also increase the risk of urinary tract infections due to incomplete bladder emptying and urine stasis. UTI symptoms include pain or burning while urinating, murky or foul-smelling urine, and pelvic discomfort.

1.2 5 RISK FACTORS OF BENIGN PROSTATIC HYPERPLASIA

Risk factors of BPH include;

Aging: Men below the age of 40 rarely experience BPH symptoms. Around 33% of men start noticing moderate to severe signs and symptoms by the age of 60, and this percentage increases to 50% by the age of 80. The prostate gland starts at 1g at birth, grows to about 4g before puberty, and then increases to an average of 20g after virilization, post-puberty, typically around the age of 20 (McNeal 1984). Benign prostatic hyperplasia (BPH) is primarily caused by the periurethral glands in the transition zone (TZ), while prostate cancer develops in the peripheral zones (PZ). In young males, the TZ constitutes about 10% of the total prostate volume (TV), but by the age of 60, it makes up around 30% of the TV (Berry *et*

al., 2011). Both the PZ and the TZ contribute to prostate enlargement, but in older men, the TZ grows faster than the PZ. Recent studies have revealed that after the prostate volume reaches 50g, the TZ becomes the major contributor to the increase. The reason for the difference in growth rates between TZ and PZ remains unknown, although sex hormones and growth factors are believed to play a role. Throughout aging, hormonal changes occur that have been associated with the development of BPH and prostate cancer, including an increase in sex hormone binding globulin (SHBG) and estrogen relative to testosterone (Deslypere *et al*., 1984, Vermeulen *et al*., 1969).

Lifestyle: The risk of BPH is higher in individuals with obesity and lower in those who engage in regular exercise (Calogero *et al*., 2019). Recent studies have established a connection between benign prostatic hyperplasia (BPH) and an increase in the ratio of plasma estradiol to testosterone, insulin, and insulin-like growth factor-I. Regular aerobic activity, particularly when combined with a low-fat, high-fiber diet rich in whole grains, fruits, and vegetables, can reduce these plasma factors. This lifestyle approach has been demonstrated in cell culture experiments to inhibit the growth of prostate epithelial cells stimulated by serum, as well as the proliferation of prostate cancer cells dependent on androgens (Barnard *et al*., 2015).

Metabolic syndrome: a cluster of metabolic abnormalities such as obesity, glucose intolerance, dyslipidemia, and hypertension, significantly raises the risk of cardiovascular disease and is primarily influenced by the dietary and lifestyle choices of Westernized societies (Haffner *et al*., 2003). Studies have shown that men with at least three components of metabolic syndrome have an 80% higher prevalence of lower urinary tract symptoms (LUTS) compared to those without. Additionally, individuals with heart disease are at a significantly higher risk of developing clinical BPH and LUTS (Meigs *et al*., 2001). Disruptions in glucose regulation, ranging from

changes in insulin growth factor concentrations to clinical diabetes diagnosis, have been associated with an increased risk of prostate enlargement, BPH, and LUTS. Elevated levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3 have been linked to a higher risk of clinical BPH and BPH surgery (Sarma *et al*., 2009). Diabetes, high serum insulin, and elevated fasting plasma glucose levels have all been correlated with larger prostate size and an increased likelihood of prostate enlargement, clinical BPH, BPH surgery, and LUTS across various studies involving tens of thousands of men (Parsons *et al*., 2006).

The relationship between lipids (high-density lipoprotein, low-density lipoprotein, and triglycerides) and BPH and LUTS is not as well-established. Several studies have explored this connection, with three showing positive associations and two demonstrating no significant links (Parsons *et al*., 2006).

Heart Disease: The use of beta-blockers and heart disease are factors that increase the risk of prostate enlargement (Madersbacher *et al*., 2019).

Diet: Certain dietary components, such as lignans and bioflavonoids found in soy, grains, and vegetables, might have a negative impact on the development of BPH (Lu & Chen, 2014).

1.2.6 TREATMENT AND MANAGEMENT OF BENIGN PROSTATIC HYPERPLASIA

Pharmacologic therapy includes;

5-alpha-reductase Inhibitors: Currently, there are two 5-alpha-reductase inhibitors used to treat BPH: dutasteride and finasteride. Both medications work by inhibiting the conversion of testosterone into dihydrotestosterone, thereby reducing prostate size, enhancing urinary flow rate, and alleviating lower urinary tract symptoms (LUTS) within 6-9 months of treatment. Side effects primarily relate to sexual function, including reduced libido and erectile and ejaculatory dysfunction (Novara *et al*., 2006). Trials have shown that finasteride can decrease prostate size

by 20-30%, moderately improve peak urinary flow rates, and lead to approximately 15% improvement in symptom scores (Mallhi *et al*., 2023).

Alpha-Blockers: Alpha1-blockers such as doxazosin, alfuzosin, terazosin, indoramin, and prazosin, as well as alpha1A-blocker tamsulosin, are used to treat symptomatic BPH. These agents relax the smooth muscles of the bladder neck and prostate gland, improving urinary flow rate and reducing bladder outlet obstruction (BOO). Studies have reported that these drugs lead to a 20-50% decrease in symptom scores and a 20-30% improvement in peak urinary flow rates. Their effects are noticeable within 48 hours and are sustained for up to 42 months. Although all these agents have similar efficacy and side effects, they differ in cost and pharmacokinetic properties. Common side effects include postural hypotension, drowsiness, headaches, nasal congestion, asthenia, retrograde ejaculation, and dizziness (Mallhi *et al*., 2023).

Antimuscarinic Drugs: Antimuscarinic agents are used to treat moderate-to-severe BPH with overactive bladder symptoms. These agents include propiverine, trospium, oxybutynin, solifenacin, fesoterodine, tolterodine, and darifenacin. However, studies have shown a low treatment compliance rate, with 35-44% of patients discontinuing treatment due to efficacy concerns or possible side effects (Park *et al*., 2014).

Phosphodiesterase Inhibitor: Tadalafil, a phosphodiesterase inhibitor, is recommended for treating LUTS/BPH. It induces relaxation of smooth muscle in the prostate, urethra, and bladder neck. Common adverse effects include indigestion, flushing, headache, nasal congestion, and back pain (Mallhi *et al*., 2023).

Phytotherapy

Phytotherapeutic agents like *Serenoa repens* and *Pygeum africanum* have gained popularity for BPH treatment. Although their mechanism of action remains unclear, several clinical trials have

demonstrated a significant impact of Serenoa repens on increasing urinary flow rate and improving International Prostate Symptom Score (IPSS). However, these agents are not recommended by guidelines due to the lack of long-term trials (De la Rosette *et al*., 2001).

Surgical Treatment

Surgical interventions are reserved for patients with recurrent urinary tract infections (UTI) due to BPH, urinary retention, gross hematuria, or bladder stones resulting from BPH and those unwilling to use other therapies. Surgical treatments involve removing or displacing the obstructing part of the enlarged prostate, relieving the obstruction caused by BPH. Various surgical options include prostatectomy, transurethral resection of the prostate (TURP), transurethral incision of the prostate (TUIP), transurethral vaporization of the prostate (TUVP), prostatic urethral lift (PUL), photo-selective vaporization of the prostate (PVP), and aquablation (De la Rosette *et al*., 2001). The choice of procedure depends on expected outcomes, potential complications, and the severity of symptoms.

- **TURP**: It is the most commonly performed surgical method and is the recommended procedure for enlarged prostate glands. Men with LUTS secondary to BPH should be offered TURP as a treatment option. Since the innovative surgeries conducted in the 1960s and 1970s, TURP has seen significant advancements, including improvements in optics, light sources, surgical and anesthesia methods. Additionally, the introduction of bipolar technology and the use of physiological solution as an intra-operative bladder washing system have transformed the procedure. By 1980, TURP became the second most common surgical procedure, following phacoemulsification (Wei *et al*., 2005).

- **Simple prostatectomy**: This technique is used for enlarged prostates and can be laparoscopic, robotic-assisted, or open prostatectomy. The specific upper volume threshold for opting for simple prostatectomy is still a topic of debate, but typically, a volume of around 100 g is considered the limit, although experienced urologists skilled in endoscopy can perform TURP even in cases exceeding this volume. Simple prostatectomy is an attractive option when dealing with concurrent conditions that require surgical intervention, such as bladder stones, diverticula, large adenomas, and inguinal hernias. The procedure can be carried out through either a retropubic or, more commonly, a suprapubic (transvesical) approach (Mallhi *et al*., 2023; Rocco *et al*., 2011). **Aquablation**: In recent advancements, innovative methods have been introduced to reduce the post-surgery complications for patients while effectively treating BPH symptoms. One of the latest technologies is a waterjet procedure guided by ultrasound and assisted by robots, called Aquablation. This technique utilizes the Aquabeam system developed by PROCEPT BioRobotics in Redwood Shores, CA, USA. The purpose behind this surgical approach is to minimize bleeding, akin to laser procedures, but with a considerably shorter duration. Moreover, Aquablation shows potential in preserving sexual function, including both erectile and ejaculatory functions, similar to the outcomes observed in the UroLift procedure by Neotract/Teleflex in Pleasanton, CA, USA, and the Rezūm technique by Boston Scientific in Marlborough, MA, USA. Recommended for prostate volume greater than 30 g or less than 80 g (Roehrborn *et al*., 2019).

- **PUL**: Recommended for prostate glands smaller than 80 g and with no median lobe.

Laser

The use of laser techniques is not determined by the size of the prostate gland. "Thulium laser enucleation of the prostate" (ThuLEP) and "holmium laser enucleation of the prostate" (HoLEP) are advanced laser techniques for BPH treatment. ThuLEP and HoLEP should be considered for patients who wish to preserve ejaculatory function or those at risk of bleeding, such as patients on anticoagulants (Mallhi *et al*., 2023).

Nonsurgical Treatment/Minimally Invasive Therapy

Transrectal high-intensity focused ultrasound (HIFU), transurethral needle ablation (TUNA), and transurethral microwave thermotherapy (TUMT) are nonsurgical techniques used for BPH treatment.

- Transurethral needle ablation (TUNA) involves the use of a specialized catheter linked to a radiofrequency generator. The catheter tip has two needles positioned at an acute angle to each other and to the catheter itself. These needles have retractable shields that regulate the urethral temperature and the shape of the lesions created. Throughout the procedure, temperature monitoring occurs in the urethra, prostate gland, and rectum, using a dedicated probe equipped with a thermocouple (Rocco *et al*., 2011). TUNA is a safe and simple procedure and delivers radiofrequency waves to the prostate gland under local anesthesia, resulting in a 50-60% improvement in urinary symptoms based on various clinical trials (Mallhi *et al*., 2023).
- HIFU involves noninvasive tissue ablation and has reported a 50-60% improvement in urinary symptoms. However, this procedure requires general anesthesia or IV sedation and has limited long-term efficacy, making it an investigational therapy (Mallhi *et al*., 2023).

- TUMT employs microwaves to deliver heat to specific parts of the prostate gland through a catheter, destroying excessive tissue without anesthesia (Management of Benign Prostatic Hyperplasia/Lower Urinary Tract Symptoms: AUA Guideline 2021, 2021).

1.2.7 METHODS USED IN INDUCING BENIGN PROSTATIC HYPERPLASIA IN ANIMALS

Inducing benign prostatic hyperplasia (BPH) in rats is a common approach in research to study the condition and test potential treatments. Various methods have been employed to induce BPH in rats, with the choice of method often depending on the specific research goals and available resources. Here, I will outline some commonly used methods for inducing BPH in rats, along with relevant references for further reading.

- Testosterone Administration: One of the most common methods for inducing BPH in rats is through the administration of exogenous testosterone. Testosterone supplementation can be achieved through subcutaneous injection, implantation of testosterone pellets, or oral administration of testosterone or its derivatives. Excessive androgen levels contribute to the development of BPH-like symptoms in rats (Zhang *et al*., 2021).
- Dihydrotestosterone (DHT) Administration: DHT, a potent metabolite of testosterone, is another androgen used to induce BPH in rats. DHT administration can be achieved through injections or implants. DHT has a higher affinity for the androgen receptor and is more effective in inducing prostatic hyperplasia compared to testosterone (Hieble 2011).
- Hormone Manipulation with Estradiol and Testosterone: Combined administration of estradiol (a form of estrogen) and testosterone can also induce BPH-like changes in rat prostates. This method mimics the hormonal imbalances observed in human BPH patients (Afriyie *et al*., 2014).

- Intraprostatic Implantation of Tissue Fragments: Implanting prostatic tissue fragments from donor rats into the prostates of recipient rats can induce BPH. This method involves surgical procedures and allows the study of the interactions between host tissue and implanted tissue fragments (Mahapokai *et al*., 2000).

1.3 PLANTS USED ETHNOMEDICINALLY IN THE TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

1.3.1 A*lium fistulosum*

Spring onions, also known as scallions and green onions, belong to the Amaryllidaceae family. Unlike regular onions, they form smaller bulbs and do not develop a complete bulb structure. As winter approaches, the plant ceases growth, leading to withering leaves. Spring onions are native to Asia and are commonly used in Northeast India (Ijeomah *et al*., 2020). Spring onions contain bioactive compounds like quercetin and flavonoids, offering various health benefits such as anticancer, antioxidant, antimicrobial, anti-inflammatory, and anti-asthmatic effects. These properties are attributed to the presence of sulfur compounds and flavonoids. Studies have identified specific compounds within spring onions that exhibit antibacterial effects against various bacteria. Additionally, spring onions have mechanisms to regulate enzymes, inhibit mutagenesis, influence cell signaling pathways, and counteract free radicals, impacting cell proliferation and tumor formation. They also play a protective role against breast tumor cells by enhancing the activity of reductase proteins, which deactivate cancer-causing chemicals (Rodgers *et al*., 1998).

1.3.2 African cherry (*Prunus africana*)

Prunus africana .Hook.f. Kalkman, previously known as *Pygeum africanum* or *Laurocerasus africana* (Hook.f.) Browicz, is commonly referred to as the African cherry, African plum,

African prune, or bitter almond. This tree is a member of the Rosaceae family, specifically within the subfamily Amygdaloideae (syn. Prunoideae), and belongs to the subgenus Laurocerasus (Gurib-Fakim, 2006). The name "Prunus" is derived from the plum-like appearance of its fruit, and "africana" signifies its endemic presence in the montane forests of Africa (Graham, 1960).*Prunus africana* is distributed across various Afromontane Forest regions in Africa, including Angola, Burundi, Cameroon, South Africa, Ethiopia, Ghana, Kenya, Lesotho, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zambia, the Democratic Republic of Congo, Zimbabwe, and Madagascar, as well as certain offshore islands. The tree holds diverse traditional uses, encompassing medicinal and household applications (Cunningham and Mbenkum, 1993; Stewart, 2003). Of notable significance is its role in the treatment of benign prostatic hyperplasia (BPH), as evidenced in numerous studies (Keehn and Lowe, 2015; Komakech *et al*., 2017; Komakech and Kang, 2019; Nambooze *et al*., 2022).

1.3.3 Pumpkin (Cucurbita pepo)

Cucurbita pepo L. belongs to the Cucurbitaceae family and is one of the 15 species in the Cucurbita genus. In Urdu, Saraiki, and Hindi, it is known as 'Kadoo,' while in English, it is referred to as squash. This plant grows as a large annual vine and features vibrant yellow-orange flowers that are pollinated by insects. Its leaves are round and lobed, often covered in fine prickles. The terms "pumpkin" and "squash" are used interchangeably even though the species differ, as their fruits are similar in appearance (Adnan *et al*., 2017).*Cucurbita pepo*, commonly known as pumpkin, is utilized globally both as a vegetable and in the treatment of various illnesses. Traditional practices in countries like Pakistan, Nigeria, Bangladesh, East Africa, and Foumban involve the use of pumpkin, particularly its seeds, to address health issues such as hyperplasia, prostate cancer, urinary disorders, nephritis, bronchitis, hemorrhoids, and anemia

(Omotayo *et al*., 2012). In Pakistan, the fruit, which is the edible part, is employed in treating a wide range of ailments, from simple issues like eye and stomach problems to more complex conditions such as hyperplasia, rheumatism, blindness, miscarriage, and prostate cancer (Rahman, 2013).

Furthermore, the fruit of *C. pepo* contains a mixture of triglyceride fatty acids, along with isolated compounds such as Tetrahydro-thiophene, linoleic acid, calotropoleanly ester, cholesterol, 13(18)-oleanen-3-ol, and 13(18)-ursen-3-ol (Badr *et al*., 2011).

1.3.4 Fireweed (Epilobium augustifolium)

Epilobium angustifolium has been used in traditional medicine by various indigenous cultures for its medicinal properties. The plant is believed to have therapeutic effects on conditions like gastrointestinal disorders, respiratory ailments, and skin problems. Additionally, it has been used as a remedy for urinary tract issues, such as benign prostatic hyperplasia (BPH) and inflammation of the prostate gland. Fireweed has also been employed in herbal teas and tinctures for its potential diuretic and anti-inflammatory properties (Granica *et al*., 2014).

1.4 *Cassia alata*

Cassia alata, a member of the Fabacea family and Caesalpinioideae subfamily, goes by various names such as King of the forest, emperor's candlesticks, candle bush, candelabra bush, Christmas, Ringworm Bush, Dadrughna, Dadmardan, Dadmari (Daad=Ringworm) Desay, Fleur, Impetigo bush, Ringworm tree, Candelabra bush, Guajava Empress candle plant, Seven Golden Candlestick, and Christmas candle. This plant is a moderate-sized medicinal shrub, growing up to 6-8 feet in height. Typically found in open wastelands near water sources, it has a soft wooden structure. Cassia alata is renowned as one of the oldest medicinal plants in Central America and is widely recognized globally, especially in Asian Pacific regions. People have historically used it for treating various ailments such as skin diseases, worms, fever, insect bites, ringworm, goiter, hookworm infestations, sexually transmitted diseases, constipation, as well as skin issues like blemishes, scabies, ringworm, and other fungal infections (Kavipriya *et al*., 2018).

1.4.1 Scientific Classification

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae

- Subfamily: Caesalpinioideae

- Tribe: Cassieae

- Subtribe: Cassiinae

- Genus*: Senna*

- Species: Senna alata

1.4.2 Botanical description and habitat

This plant is commonly found in tropical and sub-tropical regions, including countries like India, Pakistan, Burma, Sri Lanka, the Philippines, and various African nations. It thrives in diverse environments such as roadsides, riverbanks, edges of rainforests, lake shores, pond and ditch margins, open forests, orchards, and around villages. It can grow at elevations up to 1,400 meters and is known for its rapid growth, although its lifespan is relatively short. Typically, it flourishes in moister tropical areas, capable of withstanding mean annual rainfall ranging from 600 to 4,300mm and average yearly temperatures between 15 to 30°C. This adaptable plant prospers in well-drained, sunny locations with soil that can be heavy or sandy, ranging from acidic to slightly alkaline. It can be found in wastelands, flood plains, and exhibits resilience to both drought and waterlogged soils (Kavipriya *et al*., 2018).

The plant itself is an upright shrub, reaching heights of 3 to 4 meters. Its foliage comprises pinnately compound leaves with 6 to 12 pairs of leaflets, measuring 30 to 60 cm in length. The oblong leaflets are smooth and thinly leathery, ranging from 6 to 15 cm in length and 3.5 to 7.5 cm in width, with a rounded tip and a slight indentation in the middle. The flowers are arranged vertically in a column and bloom from the base upwards. The inflorescence resembles a lit, yellow candle due to its yellow flowers at the base and unopened flower buds covered by orange bracts at the top. The plant produces winged pods with a dark purple to black color and a smooth,

4-sided shape. Each pod contains 50 to 60 flattened, triangular to squarish seeds (Kavipriya *et al*., 2018).

1.4.3 Ethnomedicinal Uses

A concoction made from the leaves, and occasionally the flowers, roots, or stems, is commonly ingested for its laxative effects, primarily in regions such as India, Indonesia, various African countries, and Sierra Leone (Chatterjee *et al*., 2012; Pushpangadan *et al*., 1984). In Tanzania, pounded leaves are steeped to make a tea, which is used to alleviate constipation (Chhabra *et al*., 1984). In Nigeria, fresh sap is applied topically to the skin to treat fungal infections like ringworm, and for chronic lichen dermatosis, a leaf decoction is used or leaves are pounded and directly applied to the skin with palm oil in some African countries (Oliver 1960; Akendengue *et al*., 1984). Additionally, a potent decoction made from dried leaves is utilized as an abortifacient. In veterinary medicine, leaf decoctions are employed to address various skin issues and external parasites like mites and ticks, particularly in livestock (Chatterjee *et al*., 2012).

In India, leaf decoctions are used as expectorants for bronchitis and dyspnea, as astringents, mouthwashes, and washes for eczema. In Brazil, the leaves are regarded as emmenagogues, stimulating blood flow in the pelvic area and uterus, with some promoting menstruation (Chatterjee *et al*., 2012).

Regarding the flowers, the Tikuna Indians of the Amazon prepare a purgative decoction, consuming one cup every morning. In Peru, the flowers are infused to treat urinary infections and promote urination. Interestingly, the flowers act as diuretics, increasing urination, while the leaves are believed to have anti-diuretic properties (Chatterjee *et al*., 2012).

As for the wood, decoctions are made to address liver problems, urticaria, rhinitis, and loss of appetite caused by gastrointestinal issues (Chatterjee *et al*., 2012).

1.4.5 REPORTED PHARMACOLOGICAL ACTIVITY

Cassia alata is acclaimed to possess a lot of medicinal properties. Some of them are discussed below:

1.4.5a Antibacterial activities

The antibacterial potentials of medicinal herbs are appraised using zone of inhibition (ZOI) or minimum inhibitory concentration (MIC). The *in vivo* antibacterial potential of *Cassia alata* was assessed against methicillin-resistant *S. aureus* (MRSA), extended spectrum beta-lactamase, and carbapenemase-resistant. Enterobacteriaceae was isolated from infectious patients, via Mueller–Hinton broth via the microdilution technique. The extract showed significant activities at 512 mg/ml due to the flavonoids, quinones, tannins, sterols, alkaloids, and saponins analyzed (Wikaningtyas and Sukandar, 2016). *Cassia alata* leaves collected from an Indian aboriginal tribe, displayed significant ZOI of 21 to 27 mm against clinical isolates of multidrug-resistant (MDR) bacteria, obtained from infected patients, when subjected to ASTs via Kirby Bauer's disc diffusion assay (KBDD) (Swain and Padhy, 2015).

Ciprofloxacin (30 μg/disc) and vacuum liquid chromatographic (VLC) fractions of *S. alata* methanolic extract were assessed against *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus* (Gram positive), *Shigella boydii, Shigella dysenteriae, Pseudomonas aureus, Vibrio mimicus*, *Salmonella paratyphi, Vibrio parahaemolyticus*, and *Salmonella typhi*, (Gram negative) using the disc diffusion assay. The fractions exhibited significant inhibitory activities against bacteria isolates at 100 μg/ml (Saha, *et al.,* 2009)

Anthraquinone and flavonoid glycosides detected in *S. alata* extracts significantly inhibited the growth of *E. coli* and *S. aureus* with ZOI between 9.7 and 14.8 mm (Khan, *et al.,* 2001) (Somchit *et al.,* 2003). The crude extracts and isolates obtained from purified fractions of *S. alata* flower extract (anthraquinone glycosides, steroids, tannins, and volatile oils) were assessed on selected bacteria isolates. Strong inhibitory activities were displayed at MIC of 500 mg/ml against the clinical isolates of *S. faecalis, B. subtilis, S. aureus, Pseudomonas putida*, and *M. Luteus.* At 500 mg/ml, ZOI of 10 to 25 mm was observed, with close proximity to the inhibitory activities displayed by streptomycin, penicillin, and methicillin (Adedayo *et al.,* 2001). The synergic effect of *Eugenia uniflora* and *S. alata* leaves was examined on clinical isolates of *B. subtilis* and *S. aureus.* The dried leaves were processed into a local antiseptic herbal soap and the antibacterial potential was assessed via hole-in-plate agar diffusion assay. The herbal soap significantly inhibited the growth of the tested organisms. (Oyedele, *et al.,* 2017).

1.4.5b Antifungal Activities

Several bioactive compounds isolated from *C. alata* exhibit strong *in vitro* and *in vivo* antifungal activities. The antifungal activities of cannabinoid alkaloid (4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol), 1,8-cineole, caryophyllene, limonene, α-selinene, β-caryophyllene, germacrene D, hexadecanoic acid methyl ester, hexadecanoic acid, (6Z)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene, octadecanoic acid methyl ester, cinnamic acid, 3,7-dimethylocta-1,6-diene, pyrazol-5-ol, flavonol and gallic acid, methaqualone, and isoquinoline have been explored (Oladeji, *et al.,* 2016) (Tcheghebe, *et al.,* 2017) (Ogunwande, *et al.,* 2010).

Volatile oils extracted from *C. alata* flowers were assessed against standard strains and clinical isolates of *Candida* and *Aspergillus* species. The oils significantly inhibited the growth of the tested microbes (Essien, *et al.,* 2011). The methanolic extract and purified n-hexane and

ethanolic fractions of *S. alata* flower displayed strong inhibitory activities against *A. Niger, C. utilis, G. candidum, A. brevipes*, and *Penicillium* species with an MIC of 0.312 to 5 mg/ml. However, at different concentrations, the purified fractions exhibited prominent inhibitory activities than the methanolic extracts. Likewise, mycelia growth was significantly inhibited by the purified fractions, with a total suppression of sporulation for 96 h at 2 mg/ml, compared to less sporulation after 48 h for methanolic extracts. (Adedayo, *et al.,* 1999).

The aqueous and ethanolic leaf and bark extracts of *S. alata* was assessed via disc diffusion assay against *M. canis*, *C. albicans*, and *A. fumigatus.* The bark extracts displayed prominent concentration-dependent susceptibility against *C. albicans.* On the contrary, the aqueous extract showed a strong ZOI of 12 and 16 mm, while ethanolic extracts exhibited 10 and 14 mm. However, tioconazole displayed strong inhibitory activities of 18 mm which are significantly higher than the extracts at equivalent concentration (Somchit and Mutalib, 2003).

S. alata flowers and leaves collected from Ogbomoso, Southwest Nigeria, were examined in an attempt to justify the indigenous claims of its antifungal efficacy. *In vitro* antifungal activities of ethanolic and methanolic extracts were investigated using the disc diffusion approach. Ethanolic extracts showed pronounced inhibitory activities when compared to methanolic extracts. The IC_{50} value of the ethanolic extract was two folds higher than the methanolic extracts against the fungi isolates. The inhibitory activities displayed could be due to methaqualone, cinnamic acid, isoquinoline, and toluidine detected. (Adelowo and Oladeji, 2017).

1.4.5c Dermatophytic Activities

Currently, the leaves, flowers, and bark of *S. alata* are used for treating various kinds of skin infections and diseases. In Thailand, the plant was mentioned as one of the 54 medicinal plants

used for treating scabies, shingles, urticarial, itching, pityriasis versicolor, and ringworm. (Neamsuvan, 2015). The dermatophytic activities displayed by *S. alata* are linked to the bioactive compounds such as anthranols, anthrones, flavonoids, phenols, tannins, and anthracene derivatives. (Ramstad, 1959).

Cannabinoid alkaloid (4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol) and apigenin isolated from *S. alata* seeds were incorporated in a local antiseptic soap. The soap significantly hindered the spread of ringworms, eczemas, carbuncles, boils, infantile impetigo, and breast abscess. (Okwu and Nnamdi, 2011). The leaf, stem-bark, flower exudates, and ethanolic leaf extract examined against clinical isolates of *T. jirrucosum, M. canis, T. mentagrophyte, E. jlorrcosum, B. dermatitidis, A. flavus,* and *C. albicans* displayed strong inhibitory activities against the causative organisms. (Makinde, *et al.,* 2007)

1.4.5d Antimalarial Activities

Chemotherapy reported the antimalarial activities of *S. alata* could be linked to quinones, alkaloids, and terpenes (Federici, *et al.,* 2001) (Fowler, *et al.,* 1994). Quinones isolated from *S. alata* significantly displayed *in vitro* antiplasmodial activity, against *Plasmodium falciparum* via the microdilution test of Desjardin. (Kayembe, *et al.,* 2010). Terpenes isolated from *S. alata* leaves displayed pronounce *in vitro* antiplasmodial assays against *P. falciparum* in ethylene glycol-water fractions. Significant activity was observed at concentration below 1 μg/ml (Kayembe, *et al.,* 2012)

The appraisal of aqueous leaf extract justifies the ethnomedical applications of *S. alata* as remedy for malaria and fever. The leaf extract considerably inhibited 3D7 strain of the *P. falciparum* parasite in Wistar mice (Vigbedor, *et al.,* 2015)

1.4.5e. Anthelminthic Activity

Traditionally, *S. alata* leaf and flower decoctions are used in treatment of intestinal worm infestation and stomach disorder. The anthelmintic potency of alcoholic leaves extract of *S. alata* and *T. angustifolia* at 10 to 100 mg/ml were assessed in clinical isolates of *Ascardia galli* and *Pheretima posthuma* by observing time of paralysis and point of death of the worms. The leaves extract significantly inhibit the worms (test organisms) more than piperazine citrate (standard anthelmintic drug). (Anbu, *et al.,* 2013).

1.4.5f Antiviral Activities

S. alata is an indispensable bactericidal and fungicidal natural therapy. However, the justification of antiviral activities is not properly documented. The antiviral efficacy of n-hexane, ethyl acetate, butanol, and aqueous leaf extracts was assessed on dengue virus (DENV) obtained from an infected pregnant woman in Indonesia via focus assay. The extracts significantly inhibited DENV-2 with IC_{50} (<10 μg/ml), CC_{50} (645.8 μg/ml) and SI (64.5 μg/ml). (Angelina, *et al.,* 2017)

1.4.6 PHYTOCHEMISTRY

Leaves

C. alata has been reported to have diverse bioactive compounds in the leaves. (Alam, *et al.,* 2009) (Veerachari, 2012) (Kundu, *et al.,* 2012). Chemical constituents of *C. alata* leaves have been reported to include; kaempferol and Kaempferol-3-O-β-D-glucopyranoside) has also been identified using HPLC in the leaf extract of the plant (Saito, *et al.,* 2012) (Panichayupakaranant *et al.,* 2004).

In addition to flavanols, flavone compounds have been isolated from *C. alata* leaf ethanol extracts (Rahaman *et al.,* 2006) (Rahman *et al.,* 2008).Also, 3,5,7,40-tetrahydroxy flavone and 2,5,7,40-tetrahydroxy isoflavones were obtained from an ethyl acetate fraction of the leaf (Rahaman, *et al.,* 2006) (Rahman, *et al.,* 2006). Other researchers also reported other types of flavonoid compounds, namely anthraquinone and kaempferol 3-O-gentiobioside (Adiana, *et al.,* 2011).

Other compounds identified in the leaf extract of *Cassia alata* include ((6Z)-7,11-dimethyl-3-methylidenedodeca-1, 6,10-triene), (4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene), (4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one), (3,7-dimethylocta-1,6-diene), (hexadecanoic acid methyl ester), (hexadecanoic acid), and (octadecanoic acid methyl ester) (Igwe, *et al.,* 2015).

Alkaloid compounds from C. alata leaves have also been identified, namely adenine (Moriyama, *et al.,* 2003), Chrysoeriol, **(**quercetin, 5,7,40 -trihydroflavanone), (kaempferol-3-O-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranoside), (n-dotriacontanol), (n-triacontanol), (stearic

acid), palmitic acid, diomestin (Promgool, *et al.,* 2014), luteolin (Tatsimo, *et al.,* 2017) and (1,3,5-trihydroxy-7-methylanthracene-9, 10-dione) (Prasenjit, *et al.,* 2016).

Seeds

C. alata seeds were reported to have many bioactive compounds (Mannan, *et al.,* 2011). These include chrysoeriol-7-O-(200-O-β-D-manno pyranosyl)-β-D-allopyranoside and **27** rhamnetin-3-O-(200-O-βD-mannopyranosyl)-β-D-allopyranoside (Gupta, *et al.,* 1987).

Chemical compounds of C. alata seeds analyzed by GC-MS were (n-hexadecanoic acid), (15-tetracosenoic acid), (oleic acid), (octadecanoic acid), (2-methyl-1-octanol, pentanoic acid), and (2-ethyl-1-decanol) (Isah, *et al.,* 2015). In addition, α-D-galactopyranosyl has also been identified (Gupta, *et al.,* 1991).

Twigs

The chemical constituent of C. alata twigs has been reported. They isolated compounds were (lunatin), (7,40 -dihydroxy-5- methoxyflavone), (luteolin), and (trans-dihydrokaempferol) (Promgool *et al.,* 2014). Some anthraquinone compounds have been identified. The types of anthraquinone compounds were (aloe-emodin), (rhein), (emodin) and (chrysophanol) (Chatsiriwej *et al.,* 2006). Others anthraquinone that have also been isolated were (1,3,8-trihydroxy-2-methyl-anthraquinone), (1,5-dihydroxy-8-metoxy-2-methyl-anthraquinone -3-O-(þ) glucopyranoside) (Tiwari *et al.,* 1971), and emodin (1,6,8-trihydroxy-3-methyl-anthraquinone) (Tiwari *et al.,* 1971)

Root

In addition, (physcion) has been identified from *C. alata* root (Fernand, *et al.,* 2008) Five compounds, (ω-hydroxyemodin), (ziganein), (apigenin), and (trans-resveratrol) were isolated from the C. alata roots (Promgool *et al.,* 2014).

Flower

Isolation of the compound on C. alata flower has been reported (Yadav, 2013). *C. alata* flowers were extracted with hot methanol solvent and analyzed to identify stearic acid compounds, (alanonal) and (β-sitosterol-β-D-glucoside) (Yadav *et al.,* 2013).

Furthermore, chemical compounds of flower of C. alata that analyzed by GC-MS were (oleic acid), (nonadecanoic acid), (3, 11-tetradecadien-1-ol), and (octadecanal) (Isah, *et al.,* 2015).

1.4.6 ISOLATED COMPOUNDS

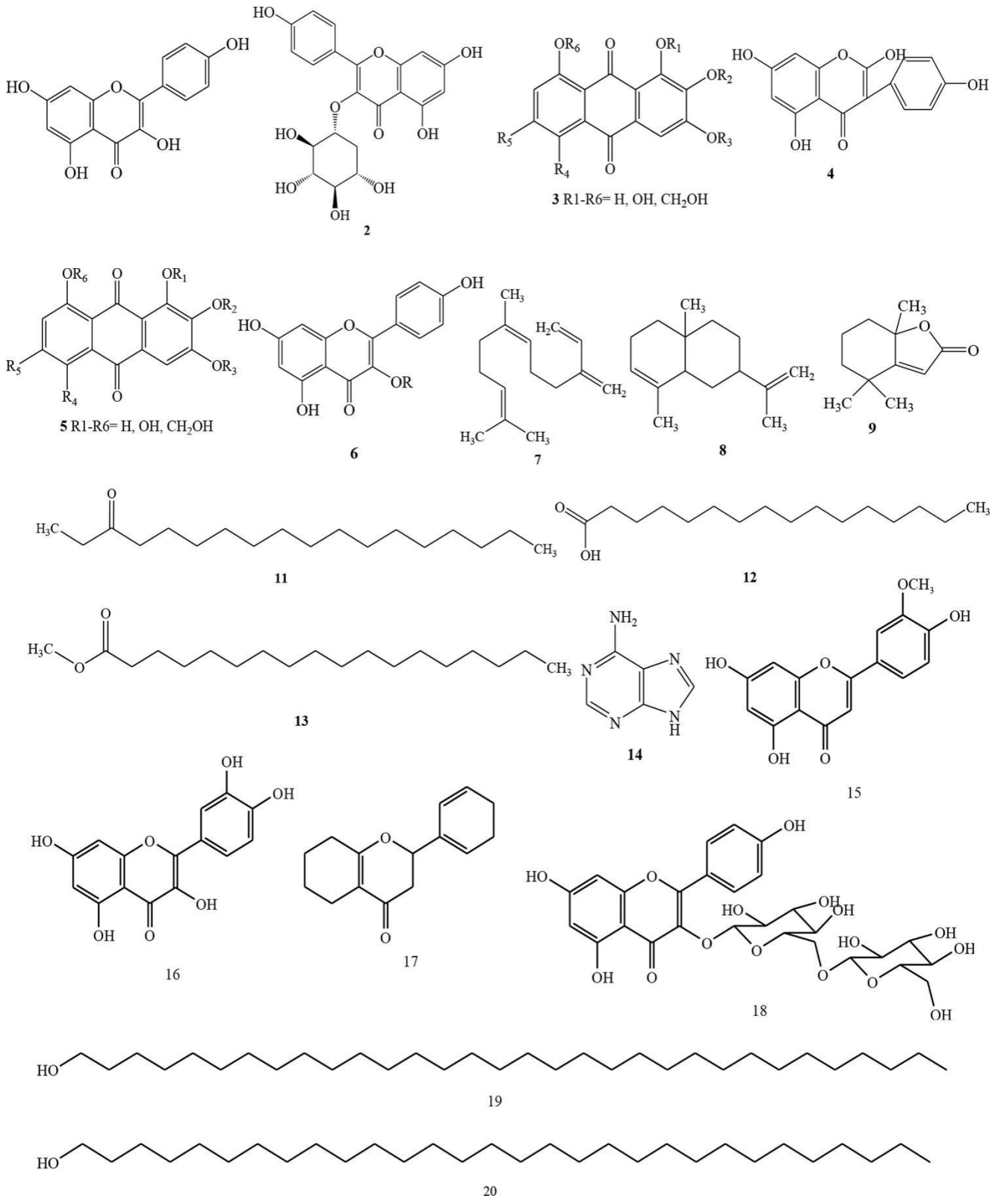

Fig 3: Some compounds identified in leaves of *Cassia alata*

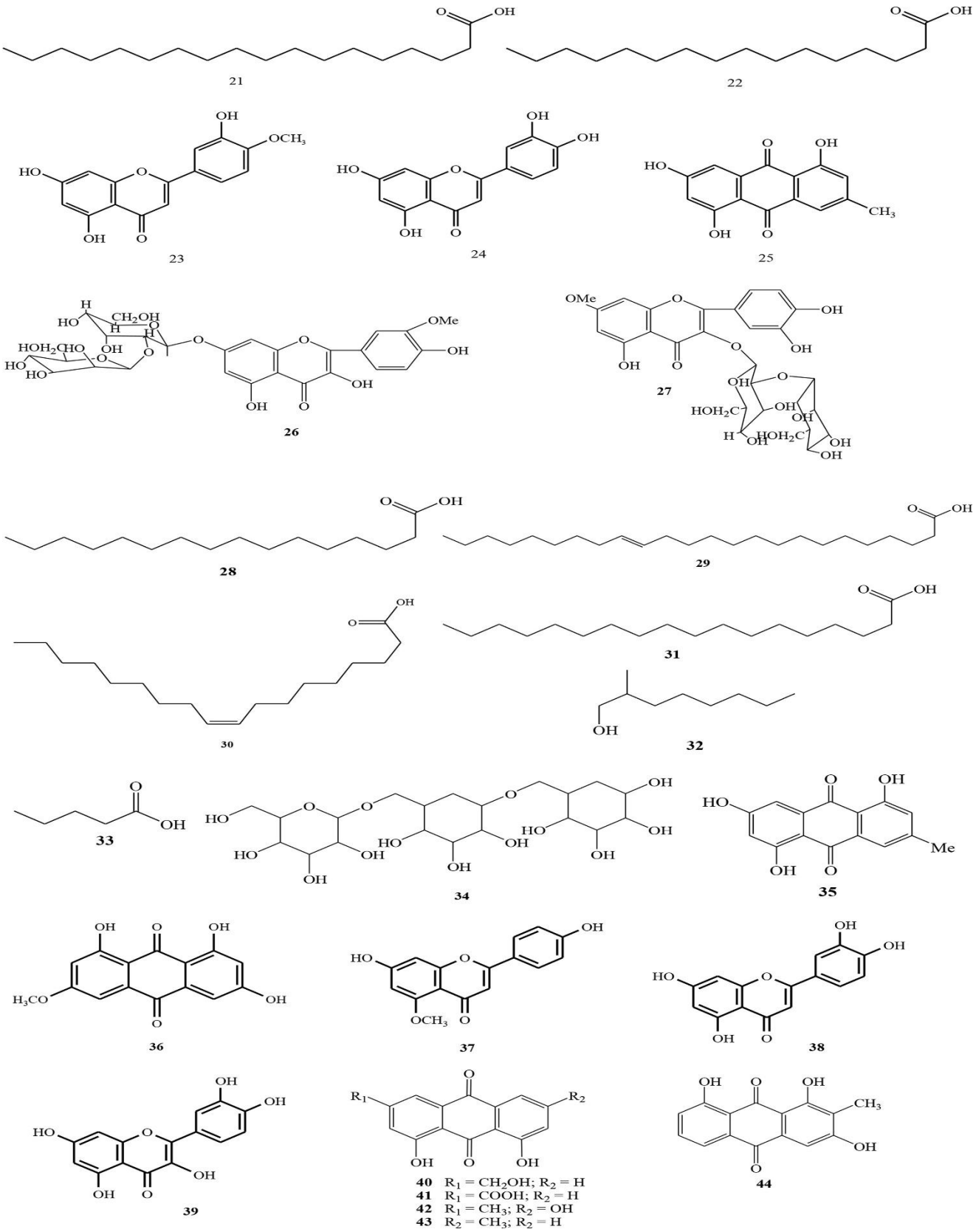

Fig 3: A diagram showing the bioactive compounds obtained from *Cassia alata* seeds and stem

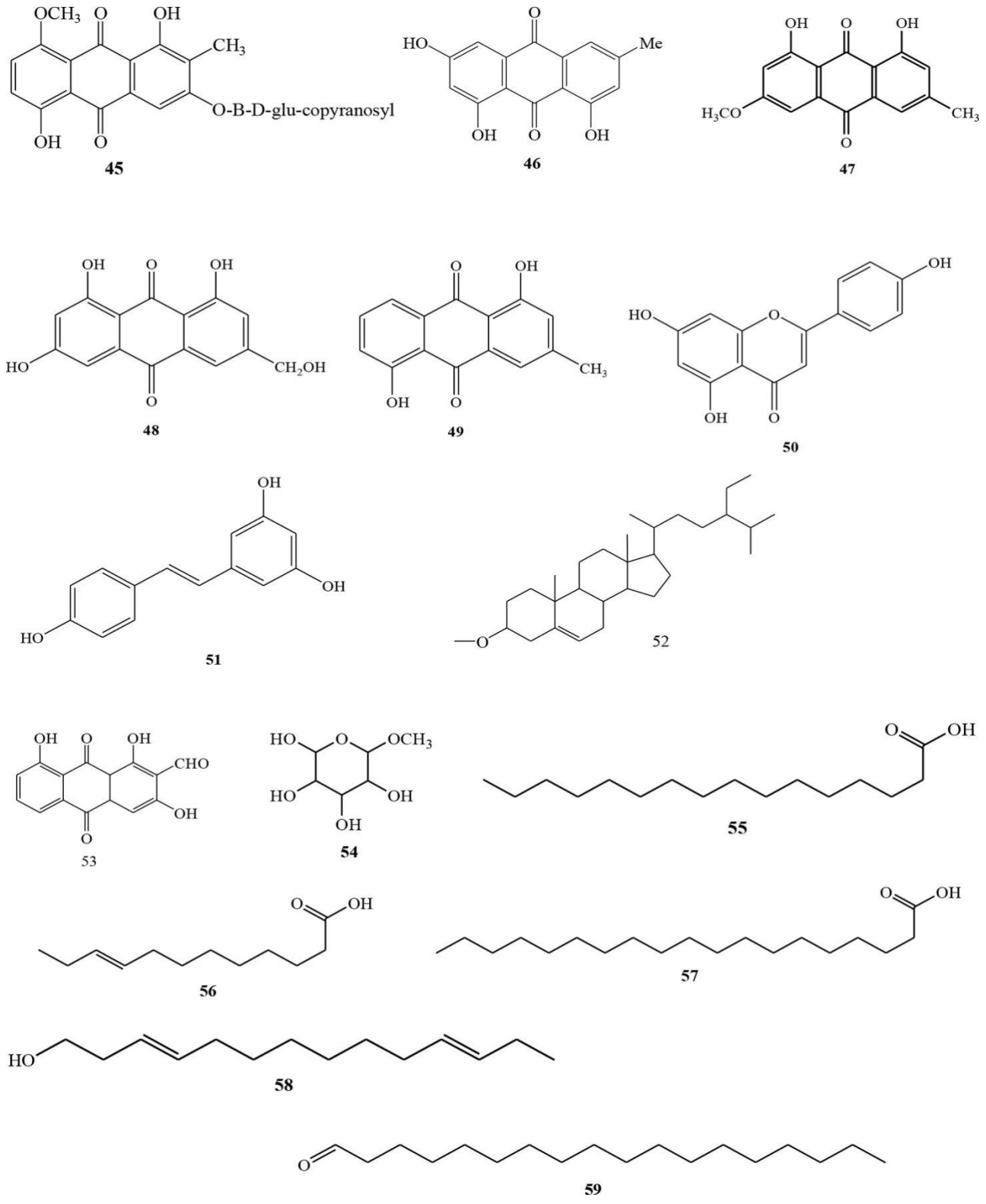

Fig 3: A diagram showing the bioactive compounds obtained from *Cassia alata* flower

1.5 BACKGROUND OF THE STUDY

Plants hold a crucial significance in our lives, not only for their essential role in the environment but also for their longstanding use in treating various human ailments and enhancing organ health over centuries. A significant portion of the world's population, approximately 80%, relies heavily on traditional remedies for their healthcare requirements. Currently, there are around 70,000 to 80,000 plant species utilized globally for medicinal or aromatic purposes. This extensive usage is attributed to the presence of biologically active and naturally occurring phytochemicals found in different parts of plants. These chemical compounds are produced by plants as a part of their regular metabolic processes, serving as a defense mechanism against environmental threats like pollution, stress, drought, UV exposure, and pathogenic attacks (Gibson *et al*., 1998; Izhaki, 2002). Beyond their nutritional value, these phytochemicals offer health benefits to humans (Hasler and Blumberg, 1999).

1.6 PROBLEM STATEMENT

Benign prostatic hyperplasia or Benign prostate enlargement (BPH/BPE) and PC, two frequent urological disorders in Western civilization, were thought to be uncommon among African people. Recent, if scant, clinical and epidemiological data show that these diseases are relatively frequent among African men. In reality, clinical statistics reveal that, comparable to the Western population, prostate cancer is the most prevalent malignancy in African males in most African nations (Yeboah *et al*., 2016)

1.7 JUSTIFICATION OF THE STUDY

Cassia alata through various researches has been identified as a plant with potentials to inhibit tumor cells and serve as an anticancer agent. It contains bioactive chemicals that may decrease inflammatory, cellular, and molecular markers implicated in apoptosis (Cell death). As a result, *Cassia alata* may have anti-cancer properties against colon tumors. Nonetheless, more detailed research is needed to investigate the potential of *Cassia alata* (Kavipriya *et al*., 2018).

1.8 AIM AND OBJECTIVES

The objective is to evaluate the claimed anti-BPH activity of the leaf of *Cassia alata* in rats.

The aims of the study include:

To determine the activity of the aqueous fractions of *Cassia alata* ethanolic extract in testosterone induced BPH rats

CHAPTER TWO

2.0 MATERIALS

2.1 REAGENTS AND EQUIPMENT

Ethanol, chloroform, finasteride, testosterone acetate, olive oil, distilled water, ice, syringes.

Soxhlet apparatus, measuring cylinder, weighing balance, cotton wool, hot water bath, sample bottles, plain bottles, separation funnel, rat cages, forceps, beakers, thimble, evaporating dish, grinder, ELISA kits for hormone determination, round bottom flask, crucible, mortar and pestle.

2.2 METHODOLOGY

2.2.1 PLANT COLLECTION AND PREPARATION

The leaves of the plant *Cassia alata* were collected from Ekosodin community, ovia north east local government of Edo state in October, 2023. Identication and authentication were carried out by Dr. Henry Akinibosun of the Department of Plant Biology and Biotechnology, Hebarium unit, Faculty of Life Sciences, University of Benin, where the hebarium number UBH-S491 was issued.

The leaves were removed from the branches dried and dried under shade till it was completely dried and milled to powder using the attrition mill and then weighed. The weight obtained was 1.7kg, It was then put into timbles and extracted in batches with absolute ethanol (2.5L) in the Soxhlet apparatus. The extract was concentrated using a rotary evaporator and was preserved in glass jars at 4℃ till needed.

2.2.2 FRACTIONATION

A weighed amount of the concentrated extract (0.5kg) was fractionated with chloroform (2.5L) to yield the chloroform and aqueous fractions with the aid of a separating funnel, after which it was subjected to freeze drying to obtained the dried aqueous (16.5%) and then preserved in a refrigerator at 4℃ till needed.

2.3 ANIMALS

Thirty-six (36) male albino rats weighing between 147- 257g were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin and the Animal House of the Anatomy department, Faculty of Basic Medical Sciences, University of Benin and kept in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.

Animals were acclimatized in the animal house for one week prior to initiation of experiments. They were maintained under the standard conditions of humidity (63% ±5%), temperature (26 – 28 °C), and natural day/night light cycle, the animals were kept in ventilated polypropylene cages, they were provided with clean water and given pelletized animal feed (Chikun® finisher pellet) when necessary.

The Ethical Review Committee of the Faculty of Pharmacy granted an ethical approval for the study.

2.4 EXPERIMENT

The animals were randomly divided into six groups (1-6) consisting of six male rats in each group. The sixth group served as the normal control and the animals were not induced, but only received water and feed throughout the experiment. All the remaining five groups were induced

with benign prostatic hyperplasia by daily subcutaneous administration of testosterone in olive oil at a dose of 4mg/kg.

The animals in groups one, two, and three were treated with 50, 100, and 200 mg/kg body weight of ethanol leaf extract of *Cassia alata respectively*. The animals in group four were treated with Finasteride at a dose of 5mg/kg/day orally: this group served as the positive control. Group five animals served as the negative control and were treated with only Testosterone acetate. The animals were weighed weekly throughout the experiment.

After 28 days of the administration, the animals were fasted overnight and then anesthetized in a chloroform-saturated chamber. They were then dissected and the needed samples were collected.

2.5 COLLECTION OF BLOOD

Blood samples were collected through the inferior vena cava into plain bottles. The blood samples were centrifuged at 3000 rpm for 10 minutes and the blood serum (supernatant) was collected into plain bottles. The collected serum samples were analyzed for enzymes indicating antioxidant activity which include superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GPx).

2.6 EVALUATION OF SERUM ANTIOXIDANT ACTIVITY

2.6.1 EVALUATION OF SERUM SUPEROXIDE DISMUTASE ACTIVITY

An evaluation of SOD in serum was carried out using the Misra and Fridovich (1972) technique, but with minor adjustments. This approach results in the production of free oxygen radicals that serve as chain-propagating species through autoxidation and spectrophotometric analysis of the final product, which is typically colored.

In order to prepare for this test, a test sample tube was prepared by pipetting 2.5ml of carbonate buffer into it, then filling it with 0.2 ml of the sample and 0.3ml of adrenaline. A blank tube was prepared in the same manner, but a buffer was used instead of the sample. The blank tube was tested for content at 480nm after 1 minute.

An autoxidation of adrenaline resulted in the formation of adenochrome, which was measured using a spectrophotometer at 480nm. SOD catalyzed this reaction in accordance with the following equation:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The calculation of activity was done using the formula:

% Inhibition = $(O.D_{ref} - O.D_{test} \times 100) / O.D_{ref}$

Where 1 unit of enzyme activity = amount of the enzyme that caused 50% inhibition of the reaction

Unit⁄mg wet tissue = (% inhibition ⁄ 50) × S

Where S = tissue weight ⁄ volume

$O.D_{ref}$ = absorbance of reference sample

$O.D_{test}$ = absorbance of the test sample

2.6.2 EVALUATION OF SERUM CATALASE ACTIVITY

The assessment of catalase activity was done in accordance with the approach described by Cohen *et al* (1970). In accordance with this procedure, 5ml of hydrogen peroxide was added to 0.5ml of the sample and mixed by inversion, followed by acidification with 1ml of dilute sulphuric acid, then left to rest for 30 minutes at room temperature. A further 7ml of potassium permanganate was added. Before the absorbances of samples at 480nm were measured using a blank prepared with the reagents mentioned earlier, the absorbance of the sample was read and the reagents replaced with water.

The activity of catalase in each sample was calculated using the formula:

Unit⁄mg tissue = [(ΔOD⁄min) × Vi × 1000]⁄[M × V×L×Y]

Where:

OD = absorbance of the test sample at 480nm

V = total volume of the reaction mixture

M = molar extinction coefficient of H_2O_2 = 40 $m^{-1}cm^{-1}$

L = light path = 1 cm

Vi = volume of the sample serum used = 0.5 ml

Y = mg tissue used

1 unit of enzyme activity = 1 mole of H_2O_2 consumed per minute

2.6.3 EVALUATION OF SERUM GLUTATHIONE PEROXIDASE ACTIVITY

The approach described by Ryan et al (2019) involved using a test tube, which was filled with a pyrogallol phosphate buffer and then added glutathione, hydrogen peroxide, and the sample. After 10 minutes of incubation at 37°C, the preparation was added trichloroacetic acid and then

passed through centrifugation (3000 rpm for 15 minutes). The supernatant was gathered and the working reagent was added. By substituting glutathione and hydrogen peroxide with buffer, the control tube was prepared, while the standard tube was prepared using buffer and the blank was prepared using buffer, glutathione, sample, and phosphate. Following this, the absorbance of the sample was compared to its absorption at 450nm using the blank.

The principle for this procedure is represented as:

$2\ \text{Pyrogallol} + 3\ H_2O_2 \rightarrow \text{Purpurogallen} + 5\ H_2O + CO_2$

The spectrophotometric appearance of purpurogallin was measured at 450nm.

The formula used is as follows:

$\text{Unit/mg} = [(\Delta OD_{450nm}/30sec) \times Vi \times Df] / (M \times V \times L \times S)$

Where:

OD = Absorbance of the test sample at 450nm

V = Total volume of the reaction mixture

Df = Dilution factor = 1

M = Molar extinction coefficient of purporogallen = 12 m^{-1} cm^{-1}

L = light path = 1cm

V = volume of sample homogenate used

S = mg of protein in the tissue used

The result was expressed in unit/mg where 1 unit of enzyme activity = 1 mole of pyrogallol oxidized per minute

2.6.4 EVALUATION OF SERUM MALONDIALDEHYDE LEVELS

The method proposed by Gutteridges and Wilkins (1980) was utilized in this scenario. Thiobarbituric acid reactive substance (TBARS) mixed with a test tube, which was heated in boiling water for 15 minutes, and then centrifuged at 3500 rpm for 10 minutes, resulted in the recovery of supernatants. At 535 nm, the absorbance was measured by a spectrophotometer.

The concentration of MDA was calculated using the formula:

Unit/mg = (OD × Vi × 1000)/(A × V × L × Y)

Where OD = Absorbance of the test sample

Vi = Total volume of reaction mixture = 3.6ml

A = molar extinction coefficient of the product

L = Light path

V = Volume of sample homogenate used

Y = mg of tissue used

2.7 DETERMINATION OF PROSTATE WEIGHT AND VOLUME

The prostate gland from each animal was harvested and placed in a tiny beaker on a weighing balance (which had already been zeroed with the dish). The prostate gland's weight was recorded, and the prostate was then put into a measuring cylinder containing water, with the water displacement observed and documented as the prostate's volume. This procedure was followed for all animals involved.

2.8 ASSESSMENT OF PROSTATE INDICES

The rats' prostate weight (PW), prostate volume (PV), and final both weight (BW) was measured in all groups, and the PW and BW were used to calculate the prostate index (PI), as stated in the formula below:

$$PI = PW/BW \times 100$$

The mean PI for all groups was computed. The percentage of inhibition of PW and PI was also calculated using the formula:

$$\% \text{ inhibition} = 100 - [(PI_T/PI_N) \times 100]$$

Where:

PI_T = Prostate index of treatment group

PI_N = Prostate index of negative group

CHAPTER THREE

3.0 RESULT

3.1 EFFECT OF AQUEOUS FRACTION OF ETHANOL EXTRACT OF C.*alata* ON SUPEROXIDE DISMUTASES

From the result of the experiment, it was observed that there was a slight decrease in the activity of SOD in the negative control group in comparison with the normal. However, at a dose of 50 mg/kg it was observed that the activity of SOD was lower than the activity produced by the negative control group, the activity at the dose of 100 mg/kg was slightly higher than the activity produced at 50 mg/kg but was lower than that produced by the negative control group. The activity of SOD produced at 200 mg/kg was the highest observed, it was slightly higher than the activity produced by the positive control group and significantly higher than the activity of the negative control group. The activity of SOD produced by the positive control was slightly higher than the activity produced by the negative control. However, the effects was not statistically significant at $P < 0.05$. The observed effect is presented in Figure 3.1.

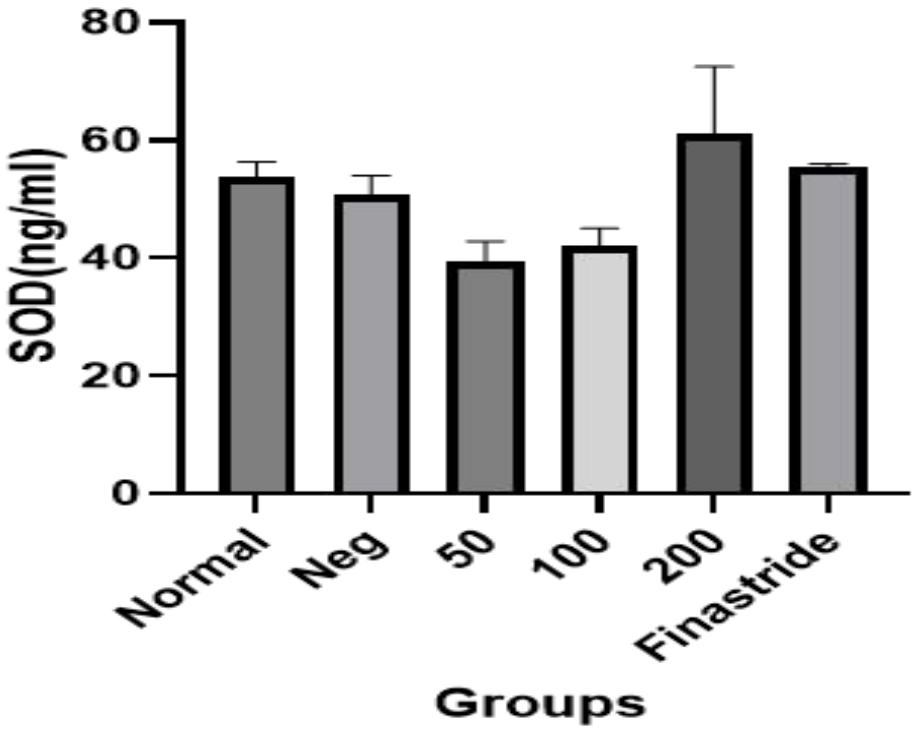

Fig. 3.1: Effect of aqueous fraction of ethanol leaf extract of *C. alata,* on serum SOD level (ng/ml): Values are expressed as Mean ± SEM

3.2 EFFECT OF AQUEOUS FRACTION OF ETHANOL LEAF EXTRACT OF C.*alata* ON SERUM CATALASE

From the result of the experiment, it was observed that there was a slight decrease in the activity of CAT in the negative control group in comparison with the normal. However, at a dose of 50 mg/kg it was observed that the activity of CAT was lower than the activity produced by the negative control group, the activity at the dose of 100 mg/kg was slightly higher than the activity produced at 50 mg/kg but was lower in comparison to that produced by the negative control group. The activity of CAT produced at 200 mg/kg was the highest observed, it was slightly higher than the activity produced by the positive control group and significantly higher than the activity of the negative control group. The activity of CAT produced by the positive control was slightly higher than the activity produced by the negative control. However, the effects was not statistically significant at $P < 0.05$. The observed effect is presented in Figure 3.2

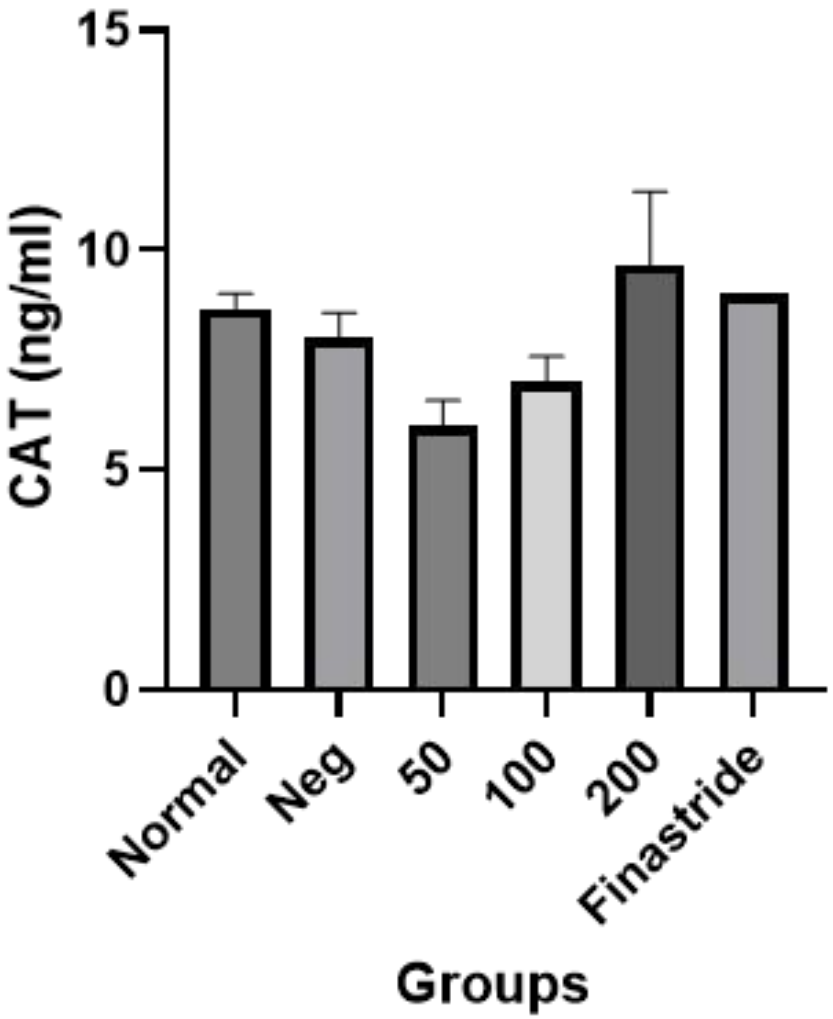

Fig 3.2: Effect of aqueous extract of *C. alata,* on CAT level (ng/ml): Values are expressed as Mean ± SEM

3.3 EFFECT OF AQUEOUS FRACTION OF ETHANOL LEAF EXTRACT OF C.*alata* ON SERUM GLUTATHIONE PEROXIDASE

It was observed that at the various doses (50,100,200 mg/kg) there was a dose-dependent increase in the concentration of the serum level of GPx. The animals treated with finasteride had an higher GPx activity in comparison with the negative control. The activity in the normal group was slightly higher than the activity seen at the negative control group. The activity seen at 50 mg/kg was lower than that of the negative control group, the activity at 100 mg/kg was also lower than that of the negative control group. The dose of 200 mg/kg had the highest GPx activity. The observed effect is presented in Figure 3.3.

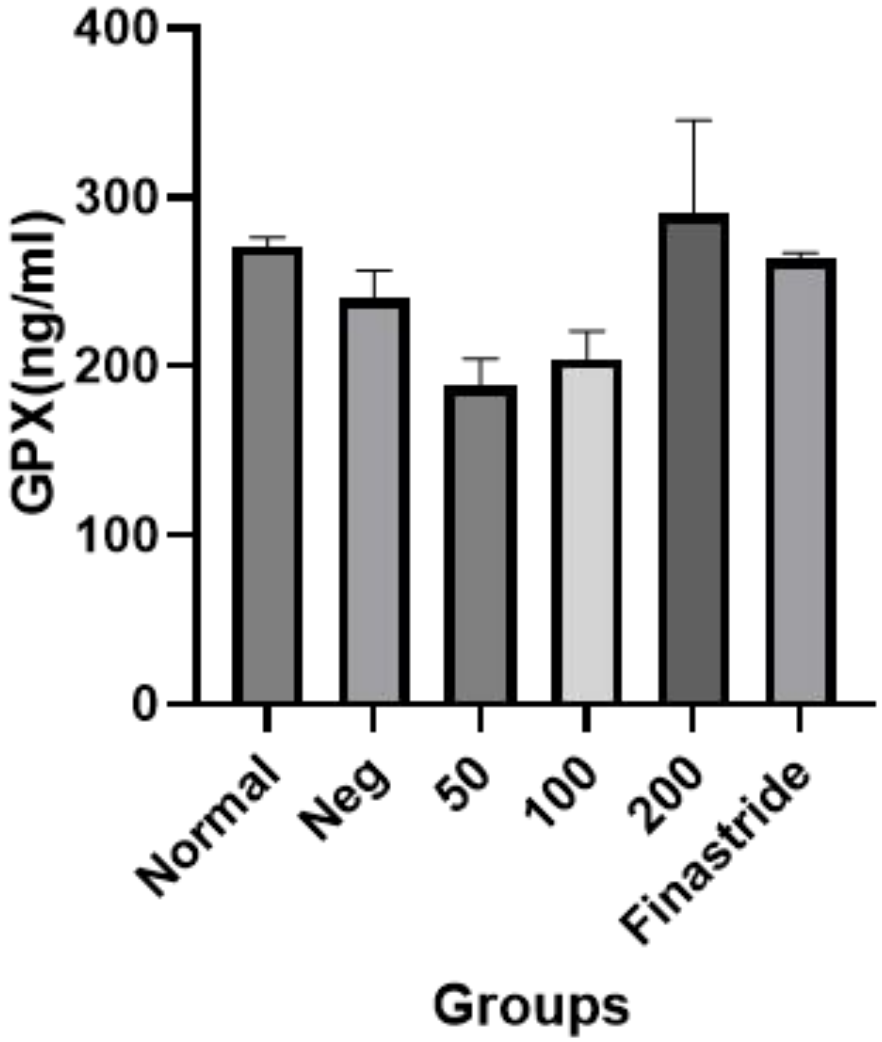

Fig 3.3: Effect of chloroform fraction of ethanol leaf extract of *C. alata,* on gluthathion peroxidase serum level (ng/ml): Values are expressed as Mean ± SEM

3.4 EFFECT OF AQUEOUS FRACTION OF ETHANOL LEAF EXTRACT OF C.*alata* ON SERUM MALONIDIALDHYDE LEVEL

The fraction caused a dose-dependent increase in the concentration of the serum level of MDA. The animals treated with finasteride had an higher MDA serum concentration level in comparison with the negative control. The concentration in the normal group was slightly higher than the activity seen at the negative control group. The concentration seen at 50 mg/kg was lower than that of the negative control group, the concentration at 100 mg/kg was also lower than that of the negative control group. The dose of 200 mg/kg had the highest MDA serum concentration level. The observed effect is presented in Figure 3.4.

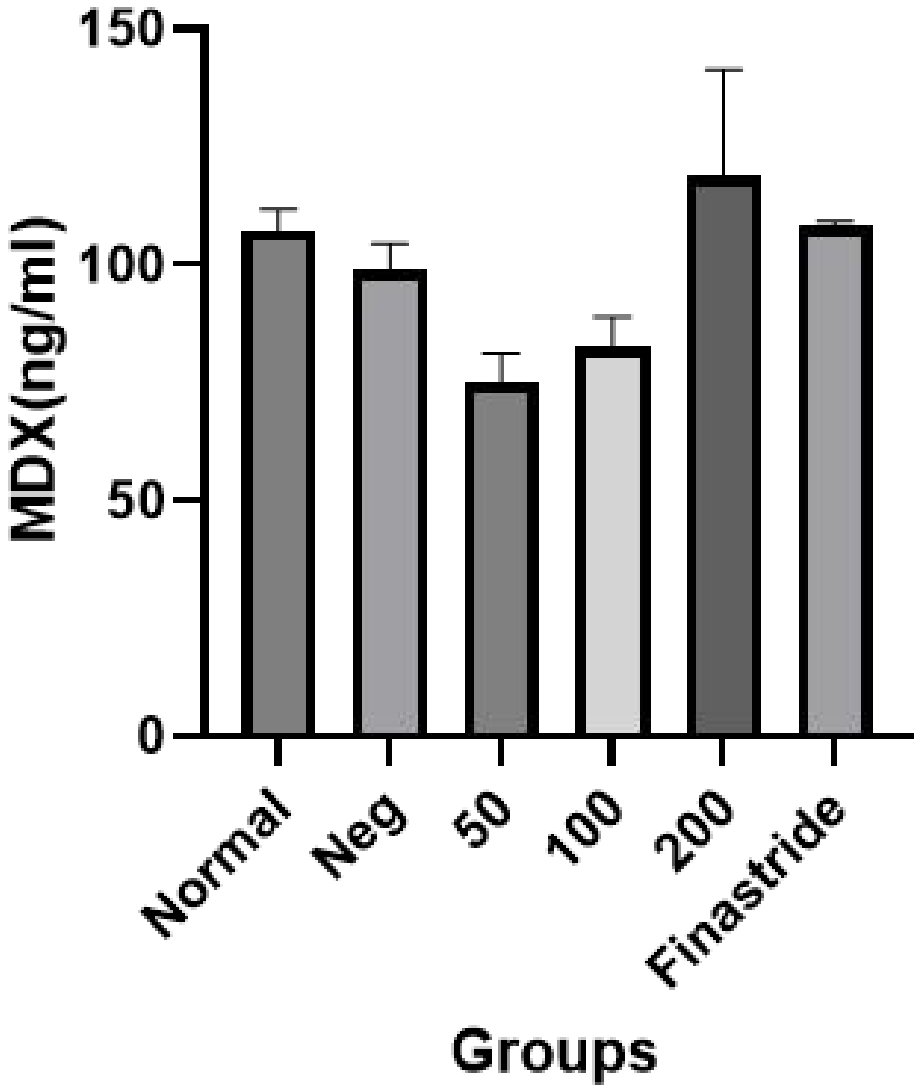

Fig 3.4: Effect of aqueous fraction of ethanol leaf extract of *C. alata,* on serum Malonidialdhyde level (ng/ml): Values are expressed as Mean ± SEM

3.5 EFFECT OF AQUEOUS FRACTION OF ETHANOL EXTRACT OF C.*alata* ON PROSTATE INDICES

The fraction caused a dose dependent increase in the prostate index at 50 mg/kg, 100 mg/kg, and 200 mg/kg respectively. Similarly, a dose dependent increase of the prostate volume was also observed at 50 mg/kg, 100 mg/kg, and 200 mg/kg. Prostate indices for the study groups are presented in table 1.

Table 1: Effect of aqueous FRACTION of *C.alata* on prostate parameters

Treatment Group	Mean body Weight (g)	Mean prostate weight (g)	Prostate Index	Mean prostate volume (ml)
50 mg/kg Extract	243.52 ± 28.29	0.64 ± 0.11	0.27 ± 0.08	0.68 ± 0.11
100 mg/kg Extract	230.93 ± 20.98	0.75 ± 0.12	0.32 ± 0.08	0.77 ± 0.15
200 mg/kg Extract	204.95 ± 25.42	0.8 ± 0.13	0.39 ± 0.03	0.87 ± 0.16
Finasteride	236.68 ± 33.33	0.5 ± 0.08	0.21 ± 0.06	0.45 ± 0.1
Normal Control Group	209.97 ± 27.67	0.31 ± 0.19	0.15 ± 0.01	0.4 ± 0.18
Negative Control Group	219.78 ± 32.08	0.48 ± 0.11	0.21 ± 0.05	0.72 ± 0.11

CHAPTER FOUR

4.1 DISCUSSION

Benign prostatic hyperplasia (BPH) is known to be intimately linked with increased oxidative stress which increases with age (Aryal *et al,*2007). Oxidative stress occurs when there is a disparity in antioxidant to pro-oxidant levels, with the level of pro-oxidant overwhelming the antioxidant (Aryal *et* al,2007). Studies carried out by Ripple *et* al (1997) demonstrated an increase in oxidative activities caused by physical levels of androgens that is in part due to an increase in mitochondrial activity. Androgens initiate an increase in cellular metabolism in prostatic cells and this result in the production of reactive oxygen species (ROS) that damage the prostate tissue and lead to alteration in enzymatic activities and level of antioxidant. The activities of these enzymes play a significant role in defending the prostate tissue from oxidative stress (Ashish *et al,* 2016) and oxidative stress has been implicated in the etiology of BPH. This relation established the foundation for the evaluation of serum antioxidants to determine the effect of the aqueous fraction of the ethanolic leaf extract of *C.alata* on BPH. Some enzymes implicated are superoxide dismutase, catalase, and glutathione peroxidase. Malonidialdehyde is an indicator for the level of lipid peroxidation in cells and the effect of the fraction on this indicator was also investigated. Increased lipid peroxidation and decreased level of superoxide dismutase, catalase and glutathione peroxidase have been found to be associated with BPH (Ahmad *et al,*2012; Eze *et al*, 2009; Minciullo *et al*, 2015).

Superoxide dismutase (SOD) are metal-containing enzymes that catalyze the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide. The enzyme has been found in all aerobic organisms examined, where it plays a major role in the defense against toxic-reactive oxygen species which are generated as byproducts of many biological oxidations. The generation of oxygen radicals can be further exacerbated during environmental adversity and consequently SOD has been proposed to be important for stress tolerance (Bowler *et al,* 2011). Lower activity of SOD were found in the majority of BPH tissues compared to the surrounding disease-free prostate tissue(Olinski *et al*, 1995) .This establishes the relation between BPH and SOD. From the study, aqueous fraction of the ethanolic leaf extract of *Cassia alata* at a dose of 200 mg/kg increased the serum level of SOD compared to the negative control and this effect was also observed to be higher than the serum level produced by the positive group (finasteride). At 50 and 100 mg/kg doses respectively, there was no significant increase in the serum level of SOD in comparison with the negative control. From the result observed, it can be inferred that at 200 mg/kg dose the aqueous fraction of the ethanol leaf extract of *Cassia alata* may likely have anti BPH activity.

However,this variation in activity was not statistically significant, indicating that though the fraction showed activity at the 200 mg/kg dose, the fraction of ethanolic leaf extract of *C.alata* did not affect the activity of SOD at the doses studied. This study is in dissonance with the findings reported for the seed extract of *Proposis Africana* (Nnaemeka *et al*, 2018). The findings reported that treatment with of the extract lead to a significant ($P < 0.05$) increase in tissue SOD activity when compared with the BPH positive control.

Catalase is an enzyme that is found among all living organisms that are expose to oxygen, where t functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Cohen *et al*,1970). It plays an important role in the breaking down of hydrogen peroxide which is a vital ROS produced in the postrate tissue in BPH. Lower activity of catalase have been shown in testosterone- induced animals with BPH (Ashish *et al*,2016). From the result obtained in this study, the fraction caused a slight decrease in the tissue CAT activity as seen in the rats in the negative group in comparison with the control group. There was a decrease in tissue CAT activity in the rats treated 50 mg/kg dose of the aqueous fraction in comparison with the negative control group, but at 100 mg/kg the serum level of catalase was increased in comparison to the 50 mg/kg dose, but in comparison with the negative control, the activity was lower. The fraction at a dose of 200 mg/kg increased the serum level of the catalase enzyme in comparison to the negative control. Also, the effect produced at 200 mg/kg was slightly higher than the effect produced by the positive control. The activity of serum catalase in the positive control group was higher than the negative control group. However, these observations among the various groups were not statistically significant indicating that the fraction of ethanolic leaf extract of *C.alata* did not affect the activity of catalase at the doses studied. This study does not harmonize with the findings reported for the seed extract of *Punica granatum* (Nwachuku *et al*, 2021). The findings reported that treatment with of the extract lead to a significant ($P < 0.05$) increase in tissue CAT activity when compared with the BPH positive control (dutasteride).

Glutathione peroxidase (GPx) is an enzyme that serves as a catalyst in the reduction of radicals to alcohol and oxygen. It functions in the reduction of lipid hydroperoxides to their various

corresponding alcohols and causes a decrease in free hydrogen peroxide to water (Flohe and Gunzler, 1984). It has been reported that there is a significant decrease in the activity of GPx in patients with BPH in comparison with healthy patients (Jeon *et al,* 2017), this helps us to establish that there is an existing relationship between BPH and GPx. In this study, the rats that were treated with testosterone (negative group) had a slight decrease in the activity of GPx in comparison with the normal control, while this activity was actually significantly decreased at 50 mg/kg dose of the fraction in comparison with the negative control, the activity at 100 mg/kg was slightly higher than the activity at 50 mg/kg but less than the activity produced by the negative group. The effect produced at 200 mg/kg was slightly higher than the effect produced by the positive control and the remarkably higher than the negative control. However, this variations in activitywere not statistically significantly , indicating that the aqueous fraction of the ethanolic extract of *C.alata* has no significant effect on the activity of GPX at the doses studied. The result observed here is in desonance with the findings reported for the extract of *Ziniber officinale* (Obisike *et al, 2019).*The findings reported that treatment with of the extract lead to a significant increase in tissue GPx level activity when compared with the BPH positive control.

Malondialdehyde which is produced from the disintegration of polyunsaturated fatty acids acts as a relevant determinant of the level of lipid peroxidation (Vershney and Kale,1990). An increase in the production of free radicals results in an overproduction of MDA whose level can be determined experimentally (Gawel *et al, 2004).* MDA levels has shown to significantly increase in oxidative stress (Kowalezuk and Stryjecka-Zimmer, 2002) as well as in BPH (Sadeghimanesh *et* al, 2021). In this study, a slight decrease in MDA level was observed for the animals in the

negative group in comparison with the normal group, in the rats treated with 50 mg/kg dose of the extract, the MDA level was lower compared to that of the negative control group. MDA level in the rats treated with 100 mg/kg dose of the fraction was observed to be slightly higher than the activity at 50 mg/kg dose but lower than the level produced in the negative control group. Also, the level produced in the rats treated with 200 mg/kg dose of the fraction was slightly higher than the level produced in the positive control group and remarkably higher than the negative control. However, this variations in level was not statistically significant, indicating that the aqueous fraction of the ethanolic leaf extract of *C.alata* has no significant effect on the level of MDA at the doses studied. This study is in dissonance with the findings reported for the seed extract of *Proposis Africana* (Nnaemeka *et al*, 2018). The findings reported that treatment with the extract and finasteride significantly ($P < 0.05$) reduced the MDA concentration in the kidney and liver of the treated groups.

Prostate volume and weight are a reflection of the size and with the proliferation of prostatic cells, there is a resultant increase in the volume and the weight of the prostate gland. The prostate index is a measure of the weight of the weight of the prostate to body weight indicating that an increase in prostate size will increase the prostate index. The prostate gland is line with secretory epithelial cells, basal cells, and some neuroendocrine cells. The gland is divided into lobes and the lobes and the lobes are surrounded by stroma cells (Ittmann, 2017). The effect of the fractions on the prostate gland was determined and it was discovered that the fraction caused a dose-dependent increase in the prostate index at the varying doses of the fraction.

4.2 CONCLUSION

The result obtained from this study showed that the aqueous fraction of ethanol leaf extract of *C.alata* has effect on the antioxidant and lipid peroxidation status associated with BPH at the studied doses, however the effects were not significant ($P < 0.05$). This suggest that aqueous fraction of ethanol leaf extract of *C.alata* does not possess the capacity to scavenge free radicals.

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