AN EVALUATION OF THE APHRODISIAC AND FERTILITY ENHANCING EFFECT OF THE METHANOLIC ROOT EXTRACT OF *CISSUS POPULNEA* Guill.And Perr. (Vitaceae)

BY

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BY

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DECLARATION

I declare that this work was carried out by me OTO, Emmanuel Oga as a postgraduate student in the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria; under the supervision of Prof. O.A. Salawu and Dr. A.U. Zezi in partial fulfillment of the requirement for the award of M.Sc. degree in pharmacology and therapeutics. The work of other investigators have been acknowledged and referenced. This work is original and has not been submitted for any award for a degree or diploma.

_____ _____

OTO, EMMANUEL OGA DATE

CERTIFICATION

This dissertation titled "AN EVALUATION OF THE APHRODISIAC AND FERTILITY-ENHANCING EFFECT OF THE METHANOLIC ROOT EXTRACT OF *Cissus populnea"* by OTO, EMMANUEL OGA meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to Almighty GOD for His guidance.

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ABSTRACT

Aphrodisiac activity of *Cissus populnea* was assessed using physical, biochemical and psychological methods. Rats were randomized into five groups of six rats. The first group received normal saline (10ml/kg), the second, third and fourth groups received extract doses of 250mg/kg, 500mg/kg and 1000mg/kg respectively while the fifth group received 5mg/kg of sildenafil citrate orally for 28 days. On the 24th day, the effect of the root extract of *Cissus populnea* (CPRE) on mounting and mating frequencies was evaluated. The psychological (mood) effect of CPRE was evaluated on the 27th day by exposing the treated rats to the elevated plus maze (EPM) for five (5) minutes and the time spent in the open and close arms of the EPM were recorded. On the 29th day, the rats were anaesthetized with diethylether and blood samples were collected by cardiac puncture for biochemical analysis (testosterone, cortisol, prostate specific antigen (PSA) levels, liver and kidney function parameters). Testes were removed and an incision was made at the caudal epididymis were transferred into a petri dish containing normal saline from where sperm samples were collected using a Neubauler ruled chamber to ascertain the sperm count and morphology. The antioxidant effect of CPRE was compared with that of ascorbic acid using DPPH (2, 2- diphenyl-2- picrylhydrazyl) assay method and was measured at 518nm and the percentage antioxidant activity (AA %) was then calculated.

The acute toxicity study revealed that CPRE was relatively safe. The LD_{50} value was found to be above 5000mg/kg. The phytochemical screening revealed the presence of saponins, flavonoids, terpernoids and tannins. CPRE and sildenafil citrate significantly increased mounting frequency ($P \leq 0.05$) and mating frequency ($P \leq 0.05$) respectively compared to the negative control; maximum effect was observed at the dose of 500mg/kg of the extract. There was significant ($P \leq 0.01$) and dose-dependent decrease in sperm count in the extract treated rats. The motility and percentage of abnormal sperm cells also decreased in the extract treated

rats compared to the negative control and standard drug sildenafil citrate. The extract produced significant (P≤0.001) and dose dependent increasein testosterone level compared to the control. Significant increase (P≤0.05) in cortisol level was observed in the sildenafil and extract (250 and 500mg/kg) treated rats compared to the control. There was no significant change in prostate specific antigen level in the CPRE however sildenafil citrate treated rats showed significant ((P≤0.05) increase in PSA level compared to the control group. The administration of the extract did not show any increase in the level of hepatic andnephrotic enzymes. However there was significant increase in AST level in the extract and sildenafil treated rates compared to the control group. The percentage antioxidant activity of ascorbic acid was significantly (P≤0.05) higher than that of the extract. The extract and sildenafil significantly (P≤0.01) increased the time spent in the open arm while significantly (P≤0.01) decreasing the time spent in the closed arm in the EPM compared to the control group.

The methanolic root extract of *Cissus populnea*is relatively safe. The aphrodisiac property may be attributed to a combination of increased in testosterone level, phytochemicals such as saponins and flavonoids and its potential to relieve stress and anxiety. The extract does not have fertility enhancing effect as shown by the decrease in sperm count, motility and increase in percentage of abnormal sperm cell.

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ABBREVIATIONS

AFC	Animal Facility Centre
AHS	American Heart Society
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
c AMP	Cyclic Adenosine Monophosphate
c GMP	Cyclic Guanosine Monophosphate
CBG	Corticosteroid Binding Globulin
CPRE	*Cissus populnea* root extract
DHT	Dihydroxytestosterone
DPPH	Diphenyl – 1 - picrylhydazyl
DSM (IV)	Diagnostic and Statistical Manual of mental disorder (IV)
ED	Erectile dysfunction
EPM	Elevated Plus Maze
FIG	Figure
FSH	Follicle Stimulating Hormone
GH	Growth Hormone

GTP	Guanyl Triphosphate
HMG	Human Menopausal Gonadotropin
HRT	Hormone Replacement Therapy
HPA	Hypothalamic pituitary adrenal axis
HPV	Human Papilo Virus
ICD	International Classification of Diseases
IF	Intromission frequency
KFT	Kidney function test
LD_{50}	Median Lethal Dose
LFT	Liver function test
LH	Luteinizing Hormone
NIPRD	National Institute for Pharmaceutical Research and Development.
NO	Nitric oxide
NPT	Nocturnal penile tumescence
NSAID	Non-Steroidal Anti-Inflammatory Drugs
MF	Mounting frequency
MRA	Magnetic resonance angiography
OECD	Organization.for Economic Co-operation and Development.
PDE_5	Phosphodiesterase 5
PGE_1	Prostaglandin E1
PGF	Prostaglandin F

PSA	Prostate specific antigen
REM	Rapid eye movement
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SEM	Standard Error of Mean
SSRI	Selective Serotonin Re-uptake Inhibitor
TSH	Thyroid Stimulating Hormone
TMB	Tetra methyl benzidine
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Aphrodisiac refers to any substance that increases sexual desire arousal and performance. Their use dates back to millennia (Sandroni, 2001). They may be of plant or animal sources and can be classified based on those that increase desire and arousal, enhance or improve penile erection and those that enhance sexual pleasure. The use of aphrodisiacs is informed by the needs of the client to ameliorate a sexual disorder or dysfunction. Sexual disorders may be classified into sexual desire disorders, sexual arousal disorders, orgasmic disorders and sexual pain disorders (Hatzimouratidis and Hatzichristou, 2007). These classifications are based on International Classification of Diseases, Diagnostic and Statistical Manual of Mental Disorder-IV, The National Institute of Health Consensus Conference on impotence. Sexual dysfunction is the inability to enjoy sexual intercourse. The most occurring form of male sexual dysfunction is erectile dysfunction (ED), while data for the most occurring female sexual dysfunction (FSD) is inconsistent since sexual function involves desire, arousal and gratification (Sarit and Goldberg, 2009).

Sexual activity is essential for procreation and general wellbeing as it bonds a relationship and has a calming effect. Sex involves the psychosocial and physiological activities to maintain a normal sexual function. Endocrine disorders or imbalance (Araujo *et al*., 2000), life style patterns and substance abuse like smoking, alcohol consumption (Hammedeh *et al*., 2010), psychological stress and neurological diseases, cardiovascular diseases (Adegite *et al*., 2009), penile diseases and surgery and age affect sexual activity.

It takes different forms in men and women and may be acute or situational due to response to environment, loss of loved one or job. It may be persistent or chronic due to an underlying disease conditions. Whatever form it takes, sexual dysfunction takes a toll on the relationship and general wellbeing of the person. Most sexual dysfunction may be due to manageable

health problems. This is a concern in developing countries where access to health facilities is a challenge. Most men and women may not have the right diagnosis and poor management of a disease condition may worsen the existing situation.

1.1 Statement of Research

Despite advances in medical science and the discovery of the block buster aphrodisiac (sildenafil citrate), Viagra®, sexual dysfunction in men and women is still a major public health issue (Shiri *et al*., 2005; Dzeladuin *et al*., 2009). Men are more outspoken to physicians on sexual dysfunction while most women keep their concerns private. The World Health Organization (WHO) projected that, in 2025 about 322 million males will be sexually dysfunctional (Araujo *et al*., 2000). The discovery of the phosphodiesterase Vinhibitors, increased the awareness of sexual dysfunction globally. The prevalence of men sexual dysfunction is 56% in the United States of America while that of women is 63%. Prior to this development there were no data on female sexual dysfunction. Thirty-four (34%) of men between 40-70 years have one form of sexual dysfunction (Oksuz and Malhan, 2005). In a study to ascertain the prevalence of sexual dysfunction in male above 35 years in three countries, 80.8% of the study group in Pakistan had sexual dysfunction while, 57% of the men in Sub-Saharan Africa were reported to have the same sexual dysfunction (Sheer *et al*., 2004).

In Nigeria, the prevalence of sexual dysfunction is 57.4% among men above 35 years (Sheer *et al*., 2004). Fatusi *et al*., (2003), reported prevalence of sexual dysfunction of 38.5% and 63.9% among males between the ages of 31-40 and 61-70years respectively in South Western Nigeria. Adegite *et al*. (2009), also reported that, sexual dysfunction has a prevalence of 58% among males with diabetes in Nigeria. Prevalence and incidence studies of female sexual dysfunction are confounded by lack of consistent methodology. The role of cultural belief

and challenge in measuring quantitative and qualitative sexual function complicates the classification and development of concise models for female sexual dysfunction (Sarit *et al*., 2009). Reproductive and sexual dysfunction are more pronounced with increase in age (Simeon *et al*., 2004). The inability to maintain a healthy sexual and reproductive life leads to depression, nervousness, anxiety, fear and ultimately low quality of life. About 12,000 divorce cases occured annually in Nigeria since 1975 (Almanac book of facts, 1977). Alice (1996), attributed 68.9% of divorce to the inability of one partner to satisfy the other and prolonged lack of conception. Infertility among males between the ages of 35-50 years is attributed to low sperm count, low sperm motility and abnormal sperm cells (Nwafia *et al*, 2006). According to (Ozoemena *et al*., 2011), hormonal imbalance is responsible for 70% of infertile couples between the ages of 36-55 years.

In spite of these prevalence data, orthodox medical approach and research has not provided the desired treatment for the various types of sexual and reproductive dysfunction. The available drugs such as hormone replacement therapy (testosterone, estrogen, and progesterone), serotonin re-uptake inhibitors (clomipramine, fluoxetine), alpha blockers, vasodilators, phosphodiesterase V inhibitor and procedures like the use of prosthetics are expensive, not readily available and may require routine clinical checkup and monitoring which may be lacking in developing countries. The side effects of some of these agents pose a challenge in the management of existing health condition or predisposes the patient to some other health conditions. Hormone replacement therapy is associated with hepatotoxicity and hypertension while alpha blockers are associated with priapism and arrhythmia. Vasodilators can cause priapism and hypotension, with prostaglandins causing pain at injection site. Some patients may require drugs to manage sexual dysfunction all through life while they are on medications for other medical conditions that also require life-long drugs. The burden on the National Health Insurance Scheme, an already inadequate overwhelmed medical system and

the cost of living on family and friend calls for more effective drugs in the management of disease conditions.

In view of these challenges, it is pertinent that researchers and pharmaceutical companies chart a global approach to provide safe, effective, available and affordable therapeutic agents for the therapeutic management of sexual and reproductive dysfunction with the aim to reduce the incidence and prevalence of sexual dysfunction, improving sexual function and general wellbeing. This study seeks to evaluate the aphrodisiac and fertility-enhancing effects of the methanolic extract of *Cissus populnea* root.

1.2 Justification

The incidence of erectile dysfunction is on the increase. The worldwide prevalence was 152 million in 1995 and projected to be 322 million by 2025 (Aytac *et al*, 1999).

This increase may be due to substance abuse for sexual pleasure or socialization. Women increasingly have a voice to say how they feel and see themselves as equal to men. The men are therefore, pressured to satisfy their partners despite declining hormonal levels with increase in age. The intense search for medicinal plants that are cost effective, relatively safe and accessible may be due to the burden of the health condition. Sexual dysfunction is more prevalent in patients between 40 – 70 years, which coincidentally is also the period in a man's life that age predisposes him to cardiovascular diseases and endocrine disorders like diabetes that may cause sexual disorders. The prevalence of disease conditions like hypertension and diabetes is on the increase globally (American Heart Society, 2011).

Male sexual dysfunction is more prevalent due to the fact that it is most reported and the decline in male hormone naturally predisposes men to sexual dysfunction therefore, the need to focus more on it. The stigma associated with male sexual dysfunction and the resultant

effect on his productivity, self-esteem and the inability to maintain a healthy relationship make it a public health issue.

Increasing research into medicinal plant may be informed by the fact that, many people with sexual dysfunction in developing countries do not have access to medical facilities and the need for drugs that are readily available, affordable and have less deleterious side effects. Medicinal plant that have been reported to have aphrodisiac and fertility enhancing properties include: *Ambergris*, *Tribulus terrestriss*, *Tunera diffusa* and *Epimedium grandiflorum.* In Nigeria, plant parts like *Garcina kola, Carica papaya* are used to treat sexual disorders. There is need for effective corroboration of traditional medicine practice with scientific approach on information on collection, preparation, side effects, efficacy, safety and standardization of some of the plant parts. The folklore claims of some medicinal plant were unfound when subjected to scientific evaluation. Hence, the need to study more plants which are sources of new drugs.

1.3 Theoretical Frame Work

1.3.1 Acute toxicity study

Acute toxicity study in animals is usually the first test that is performed on any compound or pharmaceuticals intended for human use. It evaluates adverse effects produced by the test compound as a result of either a single exposure or multiple exposures within a short period of time (typically not exceeding 24 hours). Information obtained from these studies is therefore useful in identifying doses that cause no adverse effects; dose determination in animal efficacy and repeat dose studies and major (life-threatening) toxicity. It gives an insight into the mechanism of biological/toxic effects and the median lethal dose (LD_{50}) that provides many indices of potential types of drug activity. It may also aid in selection of starting doses for Phase 1 human studies and provide information relevant to acute

overdosing in humans. The most common conventional methods used for carrying out acute toxicity studies include the one described by Lorke's (1983), OECD 423 guidelines (2001) and acute toxic class (2002). They all have the advantage of using only a few animals to achieve all the objectives of acute toxicity studies. In this study, the OECD 423 guideline (2001) was used.

1.3.2 Animal models for studying aphrodisiac activity of drugs and medicinal plants.

Sexual dysfunction, a major cause of couple's infertility and lack of sexual activity is more prevalent in males than females thus the focus on male sexual difficulties (Yakubu *et al*., 2007). An aphrodisiac is defined as any drug or food that arouses sexual instinct, induces venerably desire and increases pleasure and performance. Several invitro and invivo models have been used to investigate the aphrodisiac properties of drugs (Yakubu *et al.*, 2007, Varsha *et al*., 2013). These can be categorized into physical (behavioral), biochemical and psychological (mood) methods.

1.3.2.1 Physical methods

The physical methods that are used to assess the aphrodisiac effect of a substance include male sexual behavior which uses techniques such as Mount frequency, mount latency, intromission frequency, intromission latency, ejaculation frequency, post ejaculatory interval, index of libido, and computed male sexual behavior parameter. Orientation behavior, determination of hesitation time and attraction towards female, test of potency, test for libido, penile microcirculation and intracavernous pressure studies are also used.

(i) *Mounting frequency test*

Mounting is defined as an expression of sexual desire when one animal climbs another from the posterior with the aim of initiating sexual activity. Mount frequency (MF) is the number of mounts from the time of introduction of the female until ejaculation (Guathman *et al*., 2002).

(ii) *Mating frequency test*

Mating or intromission is the introduction of the reproductive organ of a sexual partner into the other partner during a sexual activity e.g. the penis into the vagina. The intromission frequency (IF) is the number of intromission from the time of introduction of female until ejaculation. Any medicinal plant with aphrodisiac property will produce statistically significant increase in mount and intromission frequencies. These indices indicate sexual arousability, motivation and vigour.

1.3.2.2*Biochemical methods*

(i)*Sperm analysis*

Fertilization requires adequate and normal sperm count, morphology and activity to occur. The most important male infertility test is a semen analysis (sperm evaluation) as more than 90% of male infertility cases are due to low sperm counts, poor sperm quality or both. Sperm analysis will provide information on the effect of the plant on sperm count, morphology and activity indicating fertility enhancing effect or otherwise.

(ii)*Hormonal determination*

(a) *Testosterone assay*

Testosterone is the main male hormone responsible for male sexual characters. It also plays a major role in spermatogenesis. Testosterone has been shown to stimulate desire for sex and helps maintain the health of the tissues of the penis enabling erections (Aversa *et al*., 2000). Increasedlevels of serum testosterone will thus, be considered as evidence of aphrodisiac property while a decreased level will be considered as lack of aphrodisiac property. It may also indicate possible mechanism of aphrodisiac action.

(b)*Corticosteroids (cortisol) assay*

Cortisol, a hormone produced by the hypothalamic pituitary adrenal (HPA) axis is released in response to oxidative stress (Sikka and Wang, 2008). HPA also controls spermatogenesis.

Acute psychosocial stress has been shown to potentiate the secretion of cortisol (Biondi and Picardi, 1999). A blood cortisol level test can thus serve as a stress barometer. An elevated circulating cortisol level (which promotes the production of reactive oxygen species (ROS)) has been reported to cause gonadal and sexual dysfunction in males. Information obtained from the study will indicate the effect of the extract on blood cortisol level and its consequences on male reproductive activities.

(c) *Prostate specific antigen (PSA) assay*

Prostate specific antigen (PSA) is secreted by the prostate gland to liquefy the semen produced by the prostate. It thus enables sperm cells to swim freely to fertilize the egg (Anitha *et al*., 2009). An elevated serum PSA level has been reported to indicate prostate disorders which have been linked with sexual apathy and semen clotting. Information obtained from the study will indicate the effect of the extract on serum PSA level and the implication on sexual and reproductive activities in males. PSA level may increase as a result of inflammation of prostate gland (prostatitis) or prostate cancer. An injury or sexual activity may also briefly raise PSA levels. PSA concentration is a marker of prostatic disease and inflammation.

(iii) *DPPH antioxidant assay*

The human body uses an antioxidant defense system to neutralize excessive levels of reactive oxygen species (ROS) that have been associated with degenerative diseases. Antioxidant scavenges these free radicals from the system and protects germ and sertoli cells which support spermatogenesis (Madhusudana *et al*., 1997). 1, 1, diphenyl-2-picryl hydrazyl (DPPH) assay is widely used to determine free radical scavenging activity of plant samples (Kato *et al*, 1988, Kumarasami *et al*., 2007). The scavenging effect of the plant sample will be compared with that of a standard antioxidant (ascorbic acid). The result will provide information on the antioxidant properties of the drug.

1.3.2.3*Psychological (mood) method*.

Male sexual dysfunction is caused by several factors including psychological disorders (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure). Therefore, any stimuli that result to a psychological burden may be associated with anxiety and may causes sexual dysfunction.

(i)*Elevated plus maze test*

Anxiety, a state of excessive fear is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes. The elevated plus maze (EPM) model, is one of the most validated tests for assaying anxiolytic substances (Pellow and File, 1986). The fear due to height induces anxiety in rats. The time spent in the open and close arms of EPM provides information on the anxiolytic or anxiogenic effect of the drug. This test will provide information on the effect of the plant extract on anxiety.

Lack of cognition may be due to disease condition which may lead to sexual challenges. It may be due to disease that affect the central nervous system. The exposure of the rats to the EPM on the 7^{th}, 14th and 21^{st} days will provide information on the effect of the drug on cognition enhancement which is key in ascertaining the effect of the extract in memory enhancement and alertness. Increased time spent in the open arm implies the drug has effect on memory while the decreased time spent in the open arm will be considered as lack of effect on memory.

1.4 Aim and Objectives

The aim of this study is to evaluate the aphrodisiac and fertility enhancing properties of the methanolic root extract of *Cissus populnea* in wistar rats.

1.4.1 Specific objectives

The specific objectives of the study are to:

(i) Carryout acute toxicity study of the methanolic root extract of *Cissus populnea* to guide in the dose selection for efficacy studies.

(ii) Evaluate the effect of the methanolic root extract of *Cissus populnea* on sexual behavior (mounting and mating frequencies).

(iii) Evaluate the effect of the methanolic root extract of *Cissus populanea* on biochemical parameters (sperm count, sperm morphology, testosterone, cortisol, prostate specific antigen levels, DPPH antioxidant assay, liver and kidney function).

(iv) Evaluate the psychological (mood) effect of the methanolic root extract of *Cissus populnea*.

1.5 Research Hypothesis

The methanolic root extract*Cissus populnea*has no aphrodisiac and fertility-enhancing properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Etiology and History of Aphrodisiacs

Aphrodisiacs are substances that are used to increase sexual desire, arousal, performance or pleasure. They are different from fertility enhancers that address issues bothering on potency (Frobose *et al*., 2006). Sexual intimacy strengthens the bond of a marriage relationship. The inability of one sexual partner to be satisfied has generated concern and caused emotional instability. The quest to maintain a stable sexual function and activity made man to sought ways to improve sexual desire and performance. Stress and fatigue from daily chore and hectic schedules takes a toll on couples, therefore, aphrodisiacs were used to improve sexual activity. The name is derived from Aphrodite, the Greek goddess of sexuality and love. Their use dates back to human existence as mentioned in literature by Shakespeare, Ovid Di Gibert and Sullivan play and documented in Egyptian papyri between 2200 and 1700 BC (Trevor and Gail , 2000).

Sex is an aspect of human life irrespective of culture for the purpose of procreation. In Nigeria, historical publications and proper documentation may not be available but different ethnic groups use aphrodisiacs and fertility enhancers. In Northern Nigeria, herbal aphrodisiacs are called '*Burantashi'* while they are known as '*Ale'* or '*Agboo*' in south western Nigeria and '*Ipi – wule*' among the Igedes' in the middle belt of Nigeria.

Berthold (1849), discovered that the removal of the testes affected sexual behaviour while the administration of testosterone and testes graft restored normal sexual behaviour. This wasan indication that the testes produce substances that regulate sexual behavior or activity.

It was misconstrued that male sexual behavior was elicited by the distention or swelling of the seminal vesicle (Carter, 1974). Others like Ball, (1934) and Nissen, (1929), held that pressured sensation from accessory organs gives rise to sex drive. Tarchnioff, (1887), supported the distention of seminal vesicle theory. It was later discovered that, male frogs continued to mate even after the removal of their testes implying that, neural impulses were responsible for the observed sexual behavior. Steinarch, (1894), reported that inexperienced male rats exhibit mating behavior post castration, for two (2) weeks. Tomcats with mating experienced were able to copulate for months even after the removal of their testes (Dunbar, 1975). Differences in sexual behavior of humans to that of other animals is the well-developed psychic qualities. This is an indication of the psychological aspect of sexual behaviour (Pfuger 1877). Tsai, (1925) and Warner, (1927) in their work asked if a subject will prefer hunger, thirst or sex. They also investigated thelevel of deprivation, pain, and stress that will affect sexual behavior and make a subject satisfy an appetite over another and concluded that sex is an appetite that can be satisfied or ignored due an overriding urge. Beach (1948), reported that some male rats with brain damage continued to mate while others do not. The brain lesions or damage must have interfered with the normal mating behavior. However, on the administration of testosterone normal mating was restored.

The mesolimbic tract is important in appetitive behavior such as sexual behavior (Hull *et al*, 1997). Sex can be divided into appetitive phase and the consummatory phase (Hinde, 1970 cited by Nelson, 1999). Hormones affect sex drive and sexual performance but to a different degree (Sach, 1995). Androgens have effects on the nervous system at the neurotransmitter levels and the proteins that affect their function (Hull *et al*, 1997). Androgens are therefore, essential for the expression of normal sex drive in men but, their role is not independent of other mediators in erectile response (Lugg *et al*, 1995).

2.1.1 Classification of aphrodisiacs

Aphrodisiacs may be classified into those that:

1. Increase libido (sexual desire and arousal). They may achieve this through their effect on the endocrine system.

2. Improve erection. These may act through effects on neurotransmitters and some enzymes involved in sexual function.

3. Enhance sexual pleasure. These act on the psychologically-mediated pathway of sexual function.

2.2 Physiology of the Male Reproductive System

The male reproductive system (figure 2.1) consists of the testis, a fibrous void capsule within the cavity of the scrotum. It is about 5cm in length and 3cm in diameter. The capsule elongates posteriorly, the connective tissues lining it thicken and divideinto tiny 250 tubules that coil to form the seminiferous tubules. These tubules form channels that join the epididymis. The epididymis coils on the outer surface of the testis and continues to form the vas deferens which links the urethra. The corpus carvenosa dorsally line the urethra while ventrally, it is lined by the corpus spongiosum. The corpus spongiosum extends to form the glan. The external shaft consisting of the urethra, corpus carvenosa, corpus spongiosum, glan and the prepuce is called the penis(Shier *et al*., 2000).

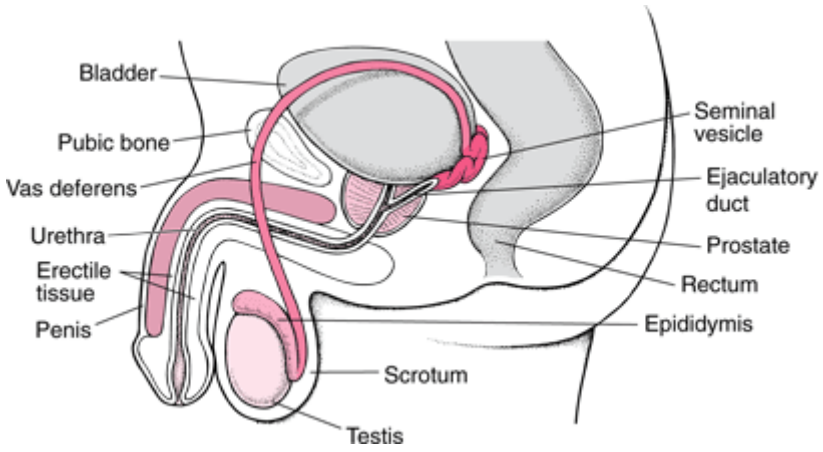


Figure 2.1: The male reproductive organs by Harvin (2013).

2.2.1 Functions of the male reproductive system

Stratified epithelial spermatogenic cells line the seminiferous tubules and they give rise to sperm cells. Interstitial cells within the space between the seminiferous tubules produce and secrete male sex hormones. The epididymis, a coiled thread- like tube connects the ducts in the testes with the vas deferens. Immature sperm cells reaching the epididymis are held since they are non-motile and on attaining maturity, they are capable of swimming when released as ejaculates. The vas deferens connects the duct of the seminal vesicle to form the ejaculatory duct which passes through the prostate gland and emptiesinto the urethra. The prostate gland is a chestnut shape structure that secretes alkaline fluid which neutralizes the acidic fluids containing sperm cells. The fluid enhances the motility of sperm cells and helps neutralize the acidic secretions of the vagina. The bulbourethral gland is responsible for the secretion of fluid in response to sexual stimulation, this fluid also lubricates the ends of the penis in preparation for sexual intercourse. The scrotum is a pouch with subcutaneous tissues which encloses the testis. It contains serous membrane which cushions the testes and aids the

smooth movement of the testes within it. The penis is a cylindrical organ that conveys urine and sperm through the urethra. The glan of the penis is thin and hairless containing sensory receptors for sexual stimulation. Parasympathetic nerve impulse from the sacral region of the spinal cord releases nitric oxide which causes dilation of penile arteries. The filled penile arteries enlarge erectile tissues; the pressure of the arterial blood flow compresses the veins reducing venous blow flow. When sustained, erection will be established. The erect penis is use for sexual penetration of the vagina and ejaculation of semen (Shier *et al*., 2000).

2.3 Types of Sexual Disorders

Sexual disorders are caused by different factors which may be physiological or psychological. They often result in sexual dysfunction and a state of low self-esteem. These disorders can be classified based on International Classification of Diseases (ICD-10) which focuses on physical factors that influence sexual response and American Psychiatric Association Statistical Manual of Mental Disorders (DSM-IV) whichfocuses more on the emotional and psychological factors affecting sexual function and activity.

2.3.1 Sexual interest/ desire disorder

This involves the absence of sexual interest or desire. It also involves lack of sexual thoughts and total lack of sexual response. This is common with women and it is referred to as hypoactive sexual desire disorder. It may be acute or chronic and the tendency for treated patients to relapse is high in the presence of the stressor (ICD-10, WHO, 1992).

2.3.2 Sexual arousal disorder

This is the inability or diminished feeling for sexual activity. It occurs in different forms in men and women and includes:

I. Erectile dysfunction- this is the consistent or recurring inability to achieve, attain or maintain a penile erection sufficient for the completion of sexual activity.

II. Female sexual arousal disorder- this is the absence of sexual excitement and pleasure. It involves minimal vulva swelling and vaginal lubrication and can lead to distress. In some women, it is the persistent genital arousal dysfunction characterized by spontaneous, unwanted genital arousal in the absence of sexual stimulation. The feeling is not relieved by episodes of orgasms and may persist for hours or days.

2.3.3 Orgasmic disorders

Orgasm is the climax of sexual activity experienced by men and women. Delayed, absent or premature orgasmfollowing normal sexual activity is a concern and the right medical attention is required to properly probe and rule out any medical cause such as azospermia, or hormonal imbalance like hyperprolactinemia.Sexual dysfunctions associated with orgasm include disorders like:

I. Premature ejaculation is the ejaculation even with minimal sexual stimulation (brief ejaculatory latency). It occurs before or immediately after penetration. This form of dysfunction is persistent or the frequency of occurrence induces sexual phobia and relationship anxiety.

II. Retrograde ejaculation is a condition in which the ejaculate is forced back into the bladder rather than through penile urethra.

III. Absent or Anejaculation is the when there are no ejaculates maybe due to azospermia.

2.3.4 Sexual aversion disorder

This is an extreme state of sexual phobia and anxiety at any attempt or suggestion of sexual activity. In men with history of ejaculatory disorders, sexual panic disorders are common. The main cause of sexual aversion is the presence of penile disease conditions like:

I. *Pyronies disorders*

A plague or hard lump forms on the penis. It can develop into a hard scar reducing the elasticity of the penis and causes pain during an erection. It is a major cause of sexual dysfunction, can be permanent and affects the quality of life. Treatment can be initiated from 18 months into disease condition or mayrequire surgical intervention (WHO, 1992).

II. *Balanitis*

This is the inflammation of the head of the penis. Symptoms include swelling, redness, itching and rashes. It is associated with a foul smelly discharge. It is seen in uncircumcised individual, dermatitis, infections like *Candida albicans*, syphilis, gonorrhea, herpes and diabetes. It can lead to phimosis and paraphimosis.(Richard, 2009).

III. *Phimosis*

This is a disease condition in which the foreskin of the penis is so tight that it cannot be pulled back to reveal the head of the penis (WHO, 1992).

IV. *Priapism*

This is a condition which derived its name from Priapus, the Greek god of fertility. It is a prolonged painful (more than 4 hours) erection without sexual stimulation. It can lead to scarring of the penis and permanent erectile dysfunction. Drugs like

papavarine, opium alkaloids, canthanide (Spanish fly) have been associated with this condition (WHO, 1992).

V. Penile cancer

This is cancer of the penis which is caused by the human papilovirus (HPV), smoking and aging (Richard, 2009).

2.4 Causes of Sexual Dysfunction/Disorder

Underlying disease conditions and lifestyle habits may predispose males to or cause sexual dysfunction. Some of these include:

2.4.1 Diabetes

Sexual dysfunction can occur as a result of damages to smooth muscles, nerves and arteries resulting from diseases like diabetes. Hyperglycemia resulting from diabetes increases intracellular adenosine triphosphates (ATP) level which closes the ATP dependent potassium channels, decrease outward potassium efflux with consequent depolarization of β cells and opening of voltage gated calcium channels to release insulin. This process damages arteries, nerves and fibrous tissues resulting in sexual disorders like erectile dysfunctions.

2.4.2 Atherosclerosis

Atherosclerosis is the cause of approximately 40% of erectile dysfunction in men more than 50 years (America Heart Society, 2011). It is a disease condition that impairs direct stimulation of receptors or blood flow by nitric oxide which dilates pulmonary vasculature with minimal effect on the heart. Any condition that impairs blood flow will ultimately prevent flow to the genitals resulting in sexual dysfunction. Cardiovascular disease conditions that impair normal blood flow with consequent flaccidity and weak penile erection of the genitals have been associated with sexual dysfunction.

2.4.3 Central nervous system diseases

Disease conditions like Parkinsonism and Alzheimer's are associated with sexual dysfunction (Gutman *et al,* 2008). Neurological disorders affecting the sacral portion of the spinal cord ($S_2 - S_4$) leads to sexual dysfunction. Parasympathetic nerve impulse from the sacral portion of the spinal cord releases the vasodilator nitric oxide and produces dilation of the arteries leading to the penis. However, injuries or impairment of this process lead to sexual dysfunction.

Multiple sclerosis, a demyelinating inflammatory disease of the central nervous system white matter is caused by infiltration of mononuclear cells, demyelination and scarring (fliosis) or may be an autoimmune disorder with genetic predisposition. T cells are involved and antibodies may cause demyelination of the fibres exposing the voltage- dependent potassium channels leading to nerve damage and blockade of nitric oxide form eliciting its vasodilatory effect on penile nerve terminals.

2.4.4 Penile diseases and injury.

The tunica albuginea is a connective tissue surrounding the testis and houses the tunica septa lobules and the somniferous tubules. Cavernosal disorder (pyronies disease), a chronic inflammation of *Tunica albuginea* results in sexual dysfunctions (Levine *et al,* 2003).Disease conditions like orchitis and torsion of the testis may also cause sexual dysfunction due to pain until treated.

2.4.5Drugs:

Drugs used in the treatment of hypertension and the antidepressant, selective serotonin re-uptake inhibitor have been associated with sexual dysfunction. Some antihypertensives tend

to induce venous flow from the penis which is usually reduced during an erection but vasodilation enhances venous flow from the penis. The selective serotonin re-uptake inhibitors (SSRI) such as fluoxetine, paroxetine caused decreased libido. Drugs like ketoconazole, cimetidine inducesexual dysfunction due to activity on the hypothalamic pituitary testicular axis. Cimetidine inhibits dihydrotestosterone and other androgens. Inhibition of the metabolism of oestrogenaffects spermatogenesis and sex drive. Cimetidine increases the level of prolactin resulting in gynecomastia and impotence in men (McQuiad, 2004).

2.4.6Hormonal effects:

Sex hormones regulate the development and maintenance of sexual characters. Their activities in a sexually matured adult complement that of the follicle stimulating hormone (FSH) and the luteinizing hormone (LH).

i) Follicle stimulating hormone:

Follicle stimulating hormone is a glycoprotein produced by gonadotrophs in the anterior pituitary. It acts on gonadal function by increasing cyclic adenosine monophosphate (cAMP) in tissues which stimulates transactivation of P450 gene in granulosa cells (Jayes *et al*., 2013). Its main function is to induce gametogenesis and follicular development which makes it important in spermatogenesis, production of quality sperm cells and fertility. FSH converts androgens to oestrogen by granulosa cell in women and in men acts on sertoli cells of the testis to initiate production of androgen binding proteins.

i) Luteinizing hormone:

Luteinizing hormone is produced in the Leydig cells of the testis and the adrenal cortex. It acts on the Leydig cell to stimulate the production of androgens including testosterone. Testosterone is converted to dihydrotestosterone (DHT) which is responsible for the production of nitric oxide synthase and cetrulline from argininewhich is required for theattainment of penile erection (Lugg *et al*, 1996). Inhibition of LH synthesis or release due to disease of the hypothalamic pituitary axis may lead to hypogonadism and sexual dysfunction.

ii) Prolactin:

Prolactin, an amino acid peptide hormone stimulates breast development, the regulation of milk production (lactation) and relaxation of the penis after an erection. Its activities are affected by the presence of other hormones like oestrogen, corticosteroids, progestin and insulin. Increased level of prolactin (hyperprolactinemia) which might be due to inhibition of dopamine by dopamine antagonist like phenothiazine or due to disease of the hypothalamus can result in galactorrhea, gynecomastia hypogonadism causing sexual dysfunction.

iii) Hyperthyroidism and Hypothyroidism:

The secretion and metabolism of cortisol, oestrogen, testosterone, insulin and catecholamines are affected by the status of the thyroid that is hyperthyroidism or hypothyroidism. Hypothyroidism slows down body function and this can also affect sexual health. Cushing's syndrome is implicated in erectile dysfunction due to excess production of cortisol. Corticosteroid interplays with other hormones and when given chronically, it suppresses the pituitary release of thyroid stimulating hormone (TSH)

responsible for normal thyroid function, and metabolic activities. The luteinizing hormone (LH) is also inhibited and this drastically affects the level of testosterone and other androgen. Ultimately, fertility and erection might be impaired leading to sexual dysfunction. The testes have both gametogenic and endocrine functions in which the follicle stimulating hormone plays a major role.

2.4.7 Free radicals effect

Normal metabolic processes result in generation of free radicals. Cytochrome P450 enzyme metabolize many chemicals in the liver to generate reactive oxygen species (ROS) like hydrogen peroxide, superoxide and also reactive nitrogen species (RNS) like nitric oxide, peroxynitrite. The activities of ROS and RNS limit the normal body functions. The natural antioxidants like catalase, superoxide dismutase, glutathione andvitamins help to counter the negative effects of free radicals. Nitric oxide (NO) is a key mediator of penile erection. It is a reactive free radical that acts as a vasodilator and as a regulator of mitochondrial respiration in skeletal muscle and organs. It can also act as a neurotransmitter which when released from neuronal and endothelial sources (Nelson *et al,* 1999), combines with guanylate cyclase and is converted from guanyl triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) which produced vasodilation and increase blood flow to penile blood vessels. Increased level of free radical impairs cell mitochondrial activity and may lead to cell death and damages to arteries resulting in sexual dysfunction.

2.4.8 Lifestyle effect

Alcohol consumption has been implicated in muscle paralysis and general weakness. Sexual act involves coordinated rhythmic skeletal muscle contraction to generate energy to drive the process. Weak or paralyzed muscle may not be effectively mobilized for sexual activity.

Chronic alcoholism can affect muscle of the penis due to alteration of whole body potassium leading to increase in potassium level and secondary aldosteronism. Elevated levels of alcohol lead to increase Apo lipoproteins A_1 and A_{11}levels which are risk factors for atherosclerosis, hypertension, cerebral and coronary vasospasm, and ischemia. These conditions predispose an individual to sexual dysfunction.

Smoking cigarette is also implicated in sexual dysfunction. Exposure to smoking decreases sperm count (Ogunfeibo *et al*., 2005) and paralyses skeletal muscles required for sexual activity. Nicotine, a substance found in cigarette is a vasoconstrictor which reduces blood flow to the penis leading to decreased sexual activity.

2.5 Diagnosis of Sexual Dysfunctional

Most cases of sexual dysfunction or interference to sexual pleasure or activity are recognized by the individual or the partner. The clinician commences diagnosis by taking a complete history of the symptoms, followed by a thorough physical examination. An evaluation of attitude to sex and other contributing factors like fear, anxiety, past sexual trauma or abuse, relationship concerns, medications, life style pattern like alcoholism, drug abuse and/or the existence mental health disorders like depression or schizophrenia will help the clinician in understanding the underlying cause of the problem. The presence of diabetes, hypertension, hyperlipidemia, coronary artery disease, spinal cord compression, pituitary tumors and associated medical conditions are verified by conducting a variety of laboratory tests which include complete blood chemistry, urine test, full blood count, lipid profile test, serum creatinine test, serum testosterone, prolactin, corticosteroid levels and prostate specific antigen test.

Advanced tests for sexual dysfunction include:

1) Duplex ultrasound which is used to evaluate blood flow, venous leaks, and signs of arteriosclerosis, scaring or calcification of erectile tissues (Allen *et al*., 1994).
2) Penile nerve function is ascertained using the bulbocavenosus reflex test to determine sufficient nerve sensation in the penis. The glan is squeezed and this makes the anus to contract (Broderick, 1998).
3) Nocturnal penile tumescence (NPT) test: It is normal for a man to have three to fiveNPT per night lasting for 30 – 60 minutes each. Nocturnal erection occurs in about 80% of healthy male of all ages during the rapid eye movement (REM) phase of sleep. It is generally conducted with a simple outpatient electronic device which records the number, duration, rigidity and circumference of penile erections. This is used as a pointer to normal nerve function and blood supply (Allen *et al*., 1994).
4) Penile biothesometry, -This involves the use of electromagnetic vibrations to evaluate sensitivity and nerve function in the glan and shaft of the penis (Broderick *et al*., 1998).
5) Dynamic infusion cavernosometry – This is a technique in which fluid is pumped into the penis at a known rate and pressure. It gives a measure of the vascular pressure in the corpus cavernosum during erection (Broderick, 1998).
6) Corpus cavernosometry measures the vascular pressure at the corpus cavernosum (American Urological Association report, 2007).
7) Magnetic resonance angiography (MRA) - is similar to magnetic resonance imaging. A contrast agent that causes vascular tissue to stand out against other tissues is injected into the individual's blood stream and magnetic fields and radio waves are used to provide detailed image of the blood vessels. The contrast provides enhanced information regarding blood supply and vascular anomalies (Broderick, 1998).

2.6Management of Sexual Dysfunction

Sexual dysfunctions (decreased libido, erectile and ejaculatory dysfunction) will occur at a point in an adult's lifetime due to hormonal decline and imbalance with increasing age. Information on the use of aphrodisiac dates back to millennia because these conditions are almost as old as man. The management of sexual disorders require the use of psychotherapy and specific therapeutic agents.

2.6.1 Psychotherapy and life style changes in the management of sexual dysfunction

1) Behavioural psychotherapy (sexual anxiety and phobia) requires expert counsellors and sexologist to encourage couple to communicate, be more honest, tolerate and accept the partner.
2) Life style changes which involve adopting a healthier lifestyle such as quitting smoking, exercising regularly and reducing stress are recommended and may be all that are needed to find relief. For those who require more intensive treatment, lifestyle changes is added to the therapeutic intervention.

2.6.2 Therapeutic management of sexual dysfunction

The goal of therapy in male sexual dysfunction is to restore and maintain the normal physiological components of sexual function and processes. Drugs used to achieve these goals include:

a) Phosphodiesterase V inhibitors (PDE5)

The phosphodiesterase V inhibitors are the first line treatment for erectile dysfunction. Members of the group include sildenafil, vardenafil, avanafil and tadalafil.

They act by selectively blocking the degradative (hydrolytic) action of phosphodiesterase V on cyclic guanosine monophosphate (cGMP) in the smooth muscle lining the blood

vessels supplying the corpus carvenosum of the penis. They thus promote the cGMP-dependent smooth muscle relaxation that is essential for normal erection.

Phosphodiesterase V inhibitors are metabolized in the liver and are absorbed 30 – 120 minutes after oral administration. With the exception of tadalafil (Cialis®), they should be taken on an empty stomach at least 1 hour before sexual intercourse. Peak concentration is attained 60 minutes after oral administration. They are 96% bound to plasma protein and undergo N- demethylation in the liver.

Adverse effects of PDE5 inhibitors include flushing, visual abnormalities, hearing loss, dyspepsia and headache. Sildenafil and vardenafil may cause abnormal colour perception (blue haze) while tadalafil use has been linked with myalgia.

Contraindication: Concomitant use of nitrates and PDE5 inhibitors can be dangerous and should be avoided. All PDE5 inhibitors should be used cautiously and at lower initial dosages to patients receiving α-blockers (e.g., prazosin, terazosin, doxazocin) because of the risk of hypotension.

Drug interaction: Hepatic metabolism of phosphodiesterase V inhibitors implies that interaction with other drugs is inevitable. Drugs like HIV protease inhibitor (ritonavir), ketoconazole, itraconazole, nitric oxide releasing drugs like glyceryl trinitiesthat induce or increase CYP3A4 will increase the metabolism of PDE5 inhibitors.

b) Androgens (Testosterone)

Testosterone has long been used as replacement therapy (Aksam *et al.*, 2007). It is a hormone produced by the testicle and is responsible for the proper development of male sexual characteristics. It is also responsible for maintaining muscle bulk, adequate level of red blood cells, bone growth, a sense of well-being and sexual function. Free testosterone is metabolized in the gut and inactivated in the liver which prevents it from

reaching the target organs unless it is esterified or bypasses first pass effect. There are thus various dosage forms of testosterone that enables the drug gets to the point of delivery and elicit the desired action.

Unesterified testosterone has a half-life of 4 days while the esterified forms have an approximate half-life of 8 days. It is 90% excreted as glucuronic and sulfuric acid conjugates in urine and 6% in feaces. The maximum doses are 200mg and 40mg/ml for testosterone cypionate andundecanoate respectively.

Testosterone deficiency is treated giving testosterone supplement three times weekly in combination with HCG 2000 IU twice daily for at least four months.

Testosterone enanthate is administered at a dose of 250mg to reach the maximum concentration within 2 -3 weeks before the next dose.

Sublingual testosterone is attached to a hydrophobic molecule, hydroxyl propyl- β-cyclodextrin which ensures its absorption. This dosage form elicits same effect as 200mg daily parenteral administration of testosterone. The gel dosage forms (10mg T gel) are applied on the genitals in doses of 5 – 10g daily.

Adverse effects: The extensive hepatic metabolism and eventual inactivation of testosterone has been associated with elevated liver enzyme. In some patients prostate enlargement and increased serum PSA were reported (Nieschlag et al., 2005). Prostatic carcinoma and hypertrophy may occur in the elderly. Hypertension, polycythemia, erthrocytosis are associated with testosterone administration.

Contraindication: Testosterone is contraindicated in hypertensive, hepatic, benign and malignant prostate diseases.

Drug interaction: Testosterone is metabolized in the gut and liver. Its co- administration with leflunomide (which produces liver injury and elevation of hepatic enzymes) will

potentiate the effect of testosterone. Its administration with anticoagulants results in increased bleeding as androgens increase sensitivity to warfarin like drugs.

Testosterone decreases serum glucose levels thus reduces the insulin required in diabetics and Concurrent administration with insulin will lead to hypoglycemic state and sometimes coma.

c) Vasodilators

Some agents when injected directly into the penis exert their relaxant effect directly on the smooth muscle of the corpora cavernosa. They can be used alone or in combination with other medications. The most commonly used agents are alprostadil (prostaglandin E1 [PGE1]), papaverine, and phentolamine. The optimal dosages and the most effective combination of these agents must be determined based on periodic review of the patient.

Alprostadil is identical to naturally occurring PGE1 and has various pharmacologic effects, including vasodilation and inhibition of platelet aggregation. When injected into the penile shaft, it relaxes trabecular smooth muscle and dilates cavernosal arteries, thereby, in turn, promoting blood flow and entrapment in the lacunar spaces of the penis, causing penile erection. The most common adverse effect is pain and penile lumps at injection site.

Papaverine is a benzylisoquinoline derivative with a direct non-specific relaxant effect on vascular, cardiac, and other smooth muscles. In the treatment of erectile dysfunction, it is injected intracavernosally into the penis to increase blood flow and produce an erection. Adverse effect is hypotension due to reduced pressure in blood vessels.

Phentolamine is an alpha1- and alpha2-adrenergic blocking agent that blocks circulating epinephrine and norepinephrine, reducing the hypertension that results from catecholamine effects on the alpha-receptors. Injected into the penis, it causes an erection. Its adverse effects include tachycardia, arrhythmia and myocardial ischemia.Erection producedmay be sustained, lasting over four hours (Priapism). Priapism may lead to death.

2.6.2 Penile prosthetics are used to achieve erection for sexual activity.

a) Vacuum penile pump. This is employed to increase the pressure in the penis to achieve an erection. It used any time the couple intend to have sexual intercourse.

b) Penile implants are a form of prosthetic which makes the penis erect for sexual activity.

2.7 The Role of Medicinal Plants in the Treatment of Sexual Dysfunction

The skill, facilities and cost of providing proper diagnosis of sexual dysfunction is a challenge in many developing countries including Nigeria. Most people in resource poor countries resolve to the use of alternative medicine or traditional herbal medicine. The increasing demand of plants with aphrodisiac and fertility properties may be due to accessibility, cost effectiveness, reduced side effects and good therapeutic outcome (Kotta *et al*., 2013). Research on medicinal plants is on the increase due to evidence of their use as sources of therapeutic agents. Several plants have been reported to have aphrodisiac and fertility properties (Cao *et al*., 2012). Yohimbine used by West Africans as an aphrodisiac was later developed scientifically to a therapeutic agent licensed as an aphrodisiac. Many useful medications have been discovered due to research into medicinal plants; this has led to development of safe and effective drugs like artemisinin obtained from *Artemisia annua* and quinine from cinchona for malaria treatment. In Nigeria, medicinal plants have been screened and found to be have aphrodisiac effect and these include: *Garcinia kola* (Chukwuma, 2013) and *Corynanthe yohimbe* (Kotta *et al*., 2013). Other plants used as aphrodisiacs include Gingko (Kim *et al*, 1976*). Indian ginseng, Litsea chinensis*, *Ochis maculata*. The dry fruit

extract of *Piper guineense* (Mbongue *et al,* 2003). *Tunera aphrodisiaca* (Damiana), (Suresh *et al*, 2009), *Lepidium meyenii* (maca)(Gonzales *et al,* 2003).

2.8 Description of the plant *Cissus populnea*

Cissus populnea (Plate 2.1) belongs to the family *Vitaceae,*it is a strong woody liane, 2-3 m high and 7.5cm in diameter. It is native to savanna Africa of Senegal, Nigeria, Sudan and Uganda (Ibrahim *et al.,* 2011). It is a medicinal plant that is used as staple food in Nigeria. Other species of the plant include *C. quinquangularis, C.incisa, C. adnate, C.alata, C.anisophylla, C.antarctica, C. campestris* and *C.cucurbitina, C.erosa.*

It is called '*daafearaa*' in Hausa, '*Ogbolo'* or '*Ajara*' in Yoruba, '*Odada*' in Igede and '*Okoho*' in Idoma.

2.9 Ethnomedicinal Uses of *Cissus populnea*

Cissus populnea stem has been used in Africa and Nigeria for skin infection (Ibrahim *et al*., 2011), the root as antibacterial (Belman *et al*., 2000) and as antidote in poisoning (Osibote *et al.,* 2010).

The stem and branches have been used as sauce and food thickener in the middle belt region for about three centuries. The leaves are rich sources of proteins, carbohydrates, crude fat and minerals like calcium, iron, zinc, copper, magnesium, sodium and potassium (Onojah, 2007).



Plate 2.1.*Cissus populnea* plant in its natural habitat

2.9.1 Scientific studies on*Cissus populnea*

Cissus populnea has been used locally to treat ailment such as venereal, stomach and skin infections. The plant also has purgative properties (Ibrahim *et al*., 2011). Soladoye and Chukwuma (2012), reported the presence of saponins, anthraquinones, and flavonoids in the stem and root of *Cissus populnea*. Its flavoring properties were observed to be due to the presence of proteins, carbohydrates, crude fat, calcium, zinc, iron, copper, magnesium, sodium and potassium (Onojah, 2007).

Ogunfeibo *et al*., 2005 reported that *Cissus populnea* extract at 200mg/kg increased sperm cell concentration and reduced testicular and alkaline phosphatase activity in rabbits exposed to cigarette. The aqueous stem bark extract neitheraltered sperm count in rats nor had activity against *Staphylococcus aureus, Salmonella paratyphi, E. coli and Proteus mirabilis* (Ojekale *et al*., 2006).

The stem bark extract of *Cissus populnea*showed antioxidant effect by scavenging DPPH (2, 2-diphenyl-1- picrylhydrazyl) and chelates Fe^{2+} (Akomolafe *et al*., 2012).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1Collection and identification of Plant material

The root of *Cissus populnea* was collected from a farm in Suleja in April, 2012 by Mallam Muazams Ibrahim, of the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria It was identified and authenticated by Mrs. Grace Ugbabe a taxonomist in the same Department. Voucher sample (NIPRD/H/6604) was prepared and deposited at the Herbarium of the Institute for future references.

3.2Crude Extract Preparation

The root of *Cissus Populnea* was, cleaned, shade dried and reduced to fine powder using pestle and mortar. The dried powder (500gm) was macerated in 70% methanol for 72 hours and the resulting mixture was filtered by suction using a Buckner flask. The filtrate was concentrated to 50ml using a rotary evaporator at low temperature. The concentrate was evaporated to dryness on water bath at 40 - 45^{o}C to obtain the methanolic root extract of *Cissus populnea*. The extract was then stored in the dessicator prior to use.

3.3Animals

Wistar rats of either sex weighing 150g – 200g obtained from the Animal Facility Center (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja were used for the study. The animals were kept at room temperature of 22 ± 3^{0}C and a 12 hour natural light and dark cycle. The rats were feeds with rodent chow and given water *ad libitum*.Theanimals were housed in well ventilated cages with a relative humidity of (30–

70)%. The animals were marked for identification. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985) and NIPRD Standard Operating Procedures (SOPs) for pharmacological studies involving whole animals.

3.4 Drugs and Chemicals

3.4.1 Drugs and chemicals

Sildenafil citrate, ethinyl estradiol, progesterone and methanol were supplied by Sigma Aldrich Chemical, USA and obtained locally from the company's country representative, Zayo Sigma Ltd. Assay kit for prostate specific antigen (PSA), testosterone (DE-EIA ASSAY KIT) and corticosteroids (EIA ASSAY KIT) were obtained from Alpha laboratories Ltd Abuja, Nigeria.

3.4.2 Equipment and apparatus

Stop clock, elevated plus maze, microscope, animal cage.

3.5 Phytochemical Analysis

Preliminary phytochemical screening of the root extract of *Cissus populnea* was carried out using standard methods described by Trease and Evans (1989) and Sofowora (1993). The extract was screened for the presence of flavonoids, alkaloids, tannins, terpernoids, saponins and glycosides.

3.6 Acute Toxicity Study

The acute toxicity study was carried out according to OECD 423 guideline (2001). Nine overnight fasted wistar rats were randomized into two groups of three rats each. Doses of 300

and 2000mg of extract/kg body weight were given to the rats orally. The rats were closely observed for signs of toxicity which included paw/licking, change in skin color, change in fur, eye lacrimation, nostril discharge, salivation, diarrhoea, lethargy, tremor, convulsion and death within four (4) hours after administration of extract and thereafter daily for 14 days.

The result from the first phase determined the dose used for the second phase. The same procedure was repeated in which a single group of three rats were given a limit dose of 5000mg/kg of the extract.

3.7 Pharmacological Screening

3.7.1 Experimental design

Thirty wistar rats randomized into five groups of six rats each were used for study. The first group was given normal saline (10ml/kg) orally, served as the control group. The second, third and fourth groups were given 250, 500 and 1000mg per kg body weight of the methanolic extract respectively. The fifth group received the standard drug, (Sildenafil citrate 5mg/kg body weight). The drugs were administered daily for 28 days.

3.7.2 Physical method of assessing plant with aphrodisiac activity

3.7.2.1 *Mounting frequency test*

On the 24th day of the treatment each male rat in each group was put in a cage with two estrous female rats. Oestrous was induced in the female rats using 1mg progesterone and 100µg ethinylestradiol 6 and 48 hours respectively before the pairing (Varsha et al., 2013). The rats were observed for mounting behavior. The number of times the male rat mounts the female within five minutes time frame was counted and recorded

3.7.2.2 *Mating frequency test*

On the 26th day of treatment the sexual episode/intromission is usually established when a male rat mount a female rat and lick its penis. The number of times each male rat in all the groups mounted a female and licked its penis was recorded for a period of five minutes (Varsha *et al*., 2013).

3.7.3 Biochemical method of assessing plants with aphrodisiac and fertility effects.

3.7.3.1 Collection and separation of sera samples for biochemical analyses.

On the last (29th) day of the experiment, overnight fasted rats were anaesthetized by diethyl ether inhalation. Blood samples were collected via cardiac puncture into plain sera tubes and allowed to clot. Serum was separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 minutes and then assayed for levels of biochemical parameters. The testes and seminal vesicles were thereafter dissected and an incision was made at the caudal epididymis from where semen samples were collected for analyses.

3.7.3.2*Sperm count*

The caudal epididymidis was cut open and washed with physiological saline, sperm were collected by diffusion of sperm. The epididymidis was put in petri dishes containing physiological saline preheated to 37^0C and the epididymis were extruded gently and fluid flow into the petri dishes which is then incubated for 10 min in 37^oC in a constant temperature incubator.

Each semen sample collected was evaluated microscopically for sperm count, viability, motility and morphology by filling a Neubauler ruled chamber.

3.7.3.3*Corticosteroid assay*

The corticosteroid assay was carried out according the method of Gonzales et al. (2003), using EIA assay kit.It is a quantitative analysis of cortisol level in biological fluid. it is based on completion between hormone conjugate and cortisol to binding site in the antibody coated plate. The sample and standard solution are added to the microplate then the hormone conjugate is added, mixed and incubated at room temperature for one hour. The plate is washed and the result is obtain by comparing the absorbance of the sample, standard at 450nm.

3.7.3.4*Prostate specific antigen (PSA) assay*

The prostate specific antigen (PSA) assay was also carried out using the method described by George*et al.,* (2013). DIASPOT PSA assay kit, a prostate specific antigen semi-quantitative rapid test trips was used. It is a chromatographic immunoassay for the quantitative determination of prostate specific antigen in whole blood serum or plasma. The test strip contains prostate specific antigen monoclonal antibodies and PSA monoclonal antibody coated on the membrane. The serum reacts with the particles coated with PSAantibody and theresulting mixture migrates upward by capillary action on the membrane and generates a colour line. The result was compared and read within 5 minutes.

3.7.3.5*Testosterone assay*

The method described by Gonzales et al., 2003 in which the serum is used to ascertain the testosterone level. The DS-EIA STEROID-TESTOSERONE-RT immunoenzymo-metric assay kit was used. This immunoassay was used to determine testosterone level, with the aid of competitive microplate enzyme immunoassay. The plates were coated with antitestosterone antibodies calibrators. The sample serum or control serum was added first to

the 96 microplate titre well. The enzyme testosterone in the sample competes with the enzyme testosterone conjugate for binding with the anti-testosterone coated microplate to form an antigen – antibody complex. The unbound conjugate was removed by washing. The enzyme activity in the antibody bound fraction is inversely proportional to the native testosterone. A colour change in tetramethylbenzidine (TMB) substrate solution revealedactivity of the enzyme. All components for the assay were maintained at room temperature. The micro plate wells for the control, standard and test serum were formated and 0.025ml of each was pipetted into the appropriate well. 0.1ml of the conjugate was pipetted into each well and swirled gently for 20 – 30 seconds to mix. The resulting mixture was incubated at room temperature for 60 minutes. 0.3ml of the washing solution was added to the well and decanted or aspirated. This was repeated four times. About 0.1ml of TMB – substrate was added to each well at time intervals and incubated for 15 – 20 minutes at room temperature in a dark place and 0.15ml of stopping reagent was added to each well at the same interval and mixed gently for 5 – 10 seconds. The result was read at spectrophotometric absorbance of 450 nm within 20 minutes.

3.7.3.*6DPPH assay*

The antioxidant activity of the plant extract and that ofthe standard (ascorbic acid) were assessed based on the free radical scavenging effect of 2, 2-dipheny-1-picryl-hydrazyl radical (DPPH) as described by Mensor *et al.*, (2001).

The solution of the extract were prepared in methanol; and compared with the standard 0.002% of DPPH methanol solution was prepared in methanol and 1ml of this solution was added to 2ml of the methanolic extract of *Cissus populnea*root at concentration of 25, 50, 100 and 200µg/ml and the standard solution, separately. The mixture was left at room temperature in the dark for 30 minutes. The absorbance was measured at 517nm.

The DPPH solution in methanol was used as the blank; the percentage inhibition was calculated using the optical density of the blank (A), that of the sampled solution (B) as:

% scavenging capacity =

$$\left[\frac{(Abcontrol - Absample)}{Abcontrol}\right] \text{x } 100$$

Where,

$Ab_{control}$ – Absorbance of DPPH solution

Ab_{sample} – Absorbance of DPPH solution with test sample.

3.7.3.7*Liver and kidney function tests*

The blood sample obtained from the rats was sent to the Clinical Chemistry laboratory for assay of liver and kidney monitoring parameters (alkaline phosphatase, aspartate amino transferase, alanine amino transferase, total and conjugated bilirubin levels, creatinine, urea, sodium, potassium, chloride and bicarbonate levels) function parameters based on the method described by (Afia and Momoh, 2006).

3.7.3 Psychological method of assessing plants with aphrodisiac and fertility effect

3.7.3.1 *Anxiety study*

The anxiety test was carried out based on a method by Pellow and File, (1986).Forty rats were randomly allotted into five groups of eight rats each. The negative control group was given distilled water, the standard group received sildenafil, the third, fourth and fifth groups were given different doses of the methanol root extract of *Cissus populnea*. On the 27th day the rats were subjected to anxiety test and were observed for 5 minutes to ascertain how much

time was spent in the open and closed arm.Six rats from each group were subjected to the EPM by placing it at the center of the maze and observed for five minutes; the EPM was swapped clean with ethanol and another rat was observed. The open and close arm entries were recorded; percentage entries into the closed arm and percentage into the open arm and the time spent were recorded and were used to measure anxiety indices. The close arm entries was used to deduce locomotors activities.

3.7.3.2 *Learning and memory studies*

This was based on a modified method described by Singh and Parle (2003).This test measures the effect of the extract on memory and cognition. The control group was given distilled water, the standard group received sildenafil. The other groups were given different doses of the methanol root extract of *Cissus populnea*. The rats were subjected to the elevated plus maze test (EPM), on the 7^{th}, 14^{th} and 21^{st} day for 90 seconds; the time spent by the rats in the open arm was recorded.

3.8 Statistical analysis

All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Newman Keuls post- hoc. $P \leq 0.05$, ≤ 0.01 and ≤ 0.001 were considered significant.

CHAPTER FOUR

4.0 RESULT

4.1 Extract Preparation and Yield

The solid extract of *Cissus populnea* was brown in colour with a yield of 9.6%w/w.

4.2Phytochemical Screening

Preliminary phytochemical test of the methanolic root extract of *Cissus populnea* showed that it contains saponins, flavonoids, tannins and terpernoids (Table 4.1).

Table 4.1. Phytochemical constituents of the root of *Cissus populnea*

PHYTOCHEMICAL	**Type of Test**	**Inference**
Alkaloids	Dragendroffs	-
Saponins	Froth test	+
Phenols	Ferric Chloride Test	-
Tannins	Gelatin Test	+
Flavonoids	Alkaline reagent test	+
Cyanoglycosides	Guignard test	-
Terpernoids	Lead acetate test	+
Steroids	Keller-kiliani test	-

(–) ABSENT, (+) PREESNT

N = 6

4.3 Acute Toxicity Study

4.3.1 Acute toxicity studies

The LD_{50} was taken to be $\geq$ 5,000mg/kg since no mortality was recorded at this dose. The test dose was thus determined to be 500mg/kg which is $1/10^{th}$ of the LD_{50}, the other doses used were 1000mg/kg and 250mg/kg. No toxic sign was observed during the acute toxicity test.

4.4 Effect of*Cissus populnea* on Physical Sexual Behaviour

4.4.1 Mounting frequency test

There was a significant (P≤0.05) increase in mounting frequency in the treated groups compared to the control group. At 1000mg/kg the extract had the same effect as the standard drug (sildenafil citrate).The highest percentage of mounting frequency was observed in the group that received 500mg/kg of the extract (P≤0.01).(Figure 4.1).

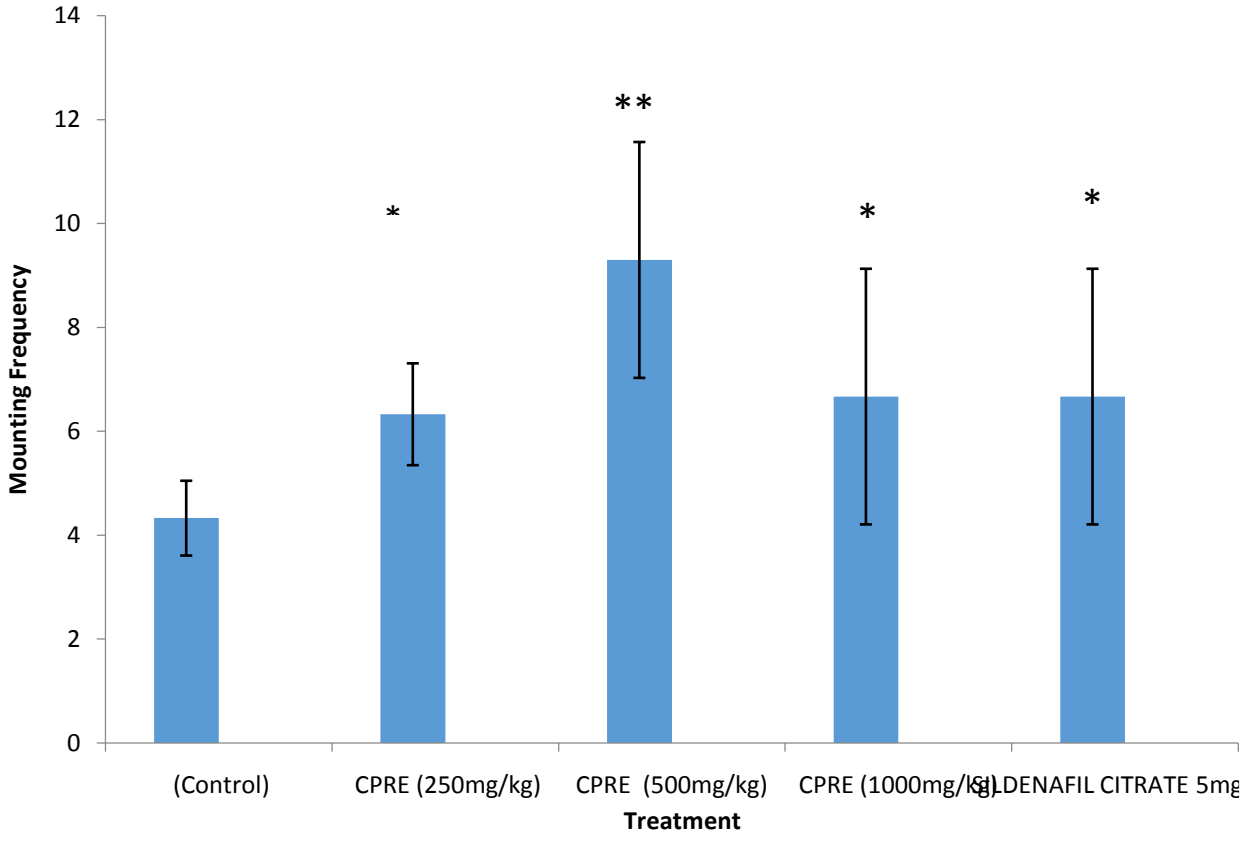


Figure 4.1: Effect of methanolic extract of*Cissus populnea* on male mounting frequency in male rats. *P≤ 0.05, **P≤0.01, compared to the control. CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.4.2 Mating frequency test (sexual episode)

There was significant (P≤0.01) increase in mating frequency in rats treated with the extract at 250mg/kg and 500 mg/kg and sildenafil compared to the untreated control. However, there was no significant (P≥0.05) effect at the extract dose of 1000mg/kg. (Figure 4.2).

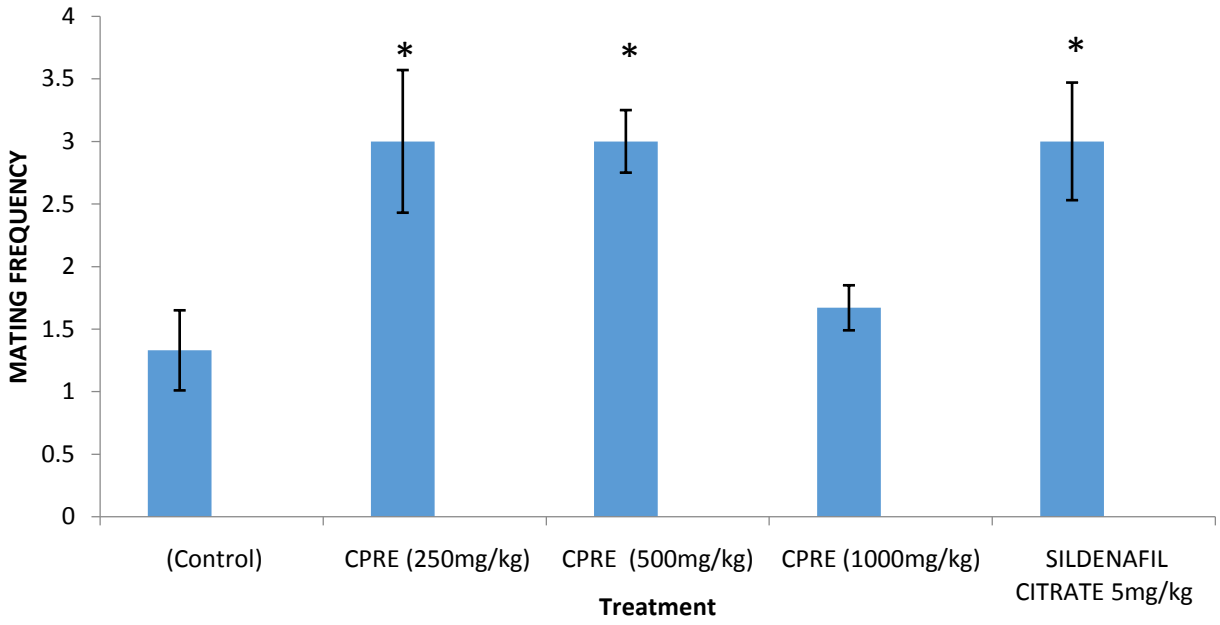


Figure 4.2: Effect of methanolic extract of*Cissus populnea*on male mating frequency in male rats. *P≤ 0.01, compared to the control CPRE = *Cissus populnea* root extract

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5 Effect of *Cissus Populnea* Root Extract on Biochemical Parameters

4.5.1Effect of extract on sperm count

The extract caused significant (P≤0.05, P≤0.01) dose dependent decrease in sperm count in all the extract treated rats compared to the control. Sildenafil citrate also caused a comparable effect in sperm count in the rats compared to the control. (Figure 4.3).

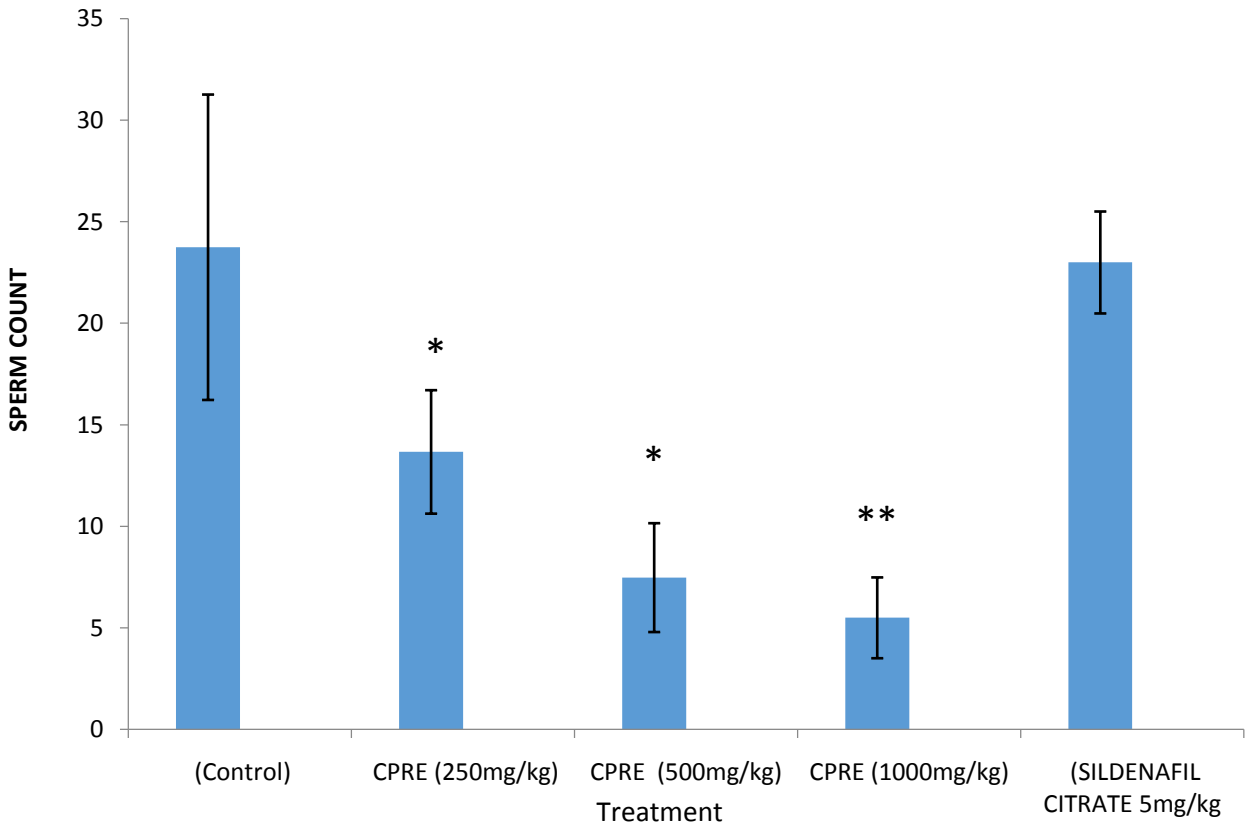


Figure 4.3: Effect of methanolic extract of*Cissus populnea* on sperm count in male rats.*P≤ 0.05, **P≤0.01 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.1.1 *Effect of extract on sperm morphology and motility*

There was significant (P≤0.01) increase in percentage of normal sperm cells in the rats treated with 250 mg/kg extract dose and 5 mg/kg sildenafil citrate compared to the control.

The percentage of active sperm cells significantly (P≤0.01) increased in rats treated with 250 mg/kg extract dose and (P≤ 0.05) in the sildenafil treated compared control.

There was significant (P≤0.05) increase in percentage of abnormal sperm cells in the rats treated with 250 mg/kg extract dose and 5 mg/kg sildenafil citrate compared to the control.

There was no significant (P≥0.05) effect in sperm morphology and motility in the 500 and 1000 mg/kg extract treated rats compared to the control. (Figure 4.4).

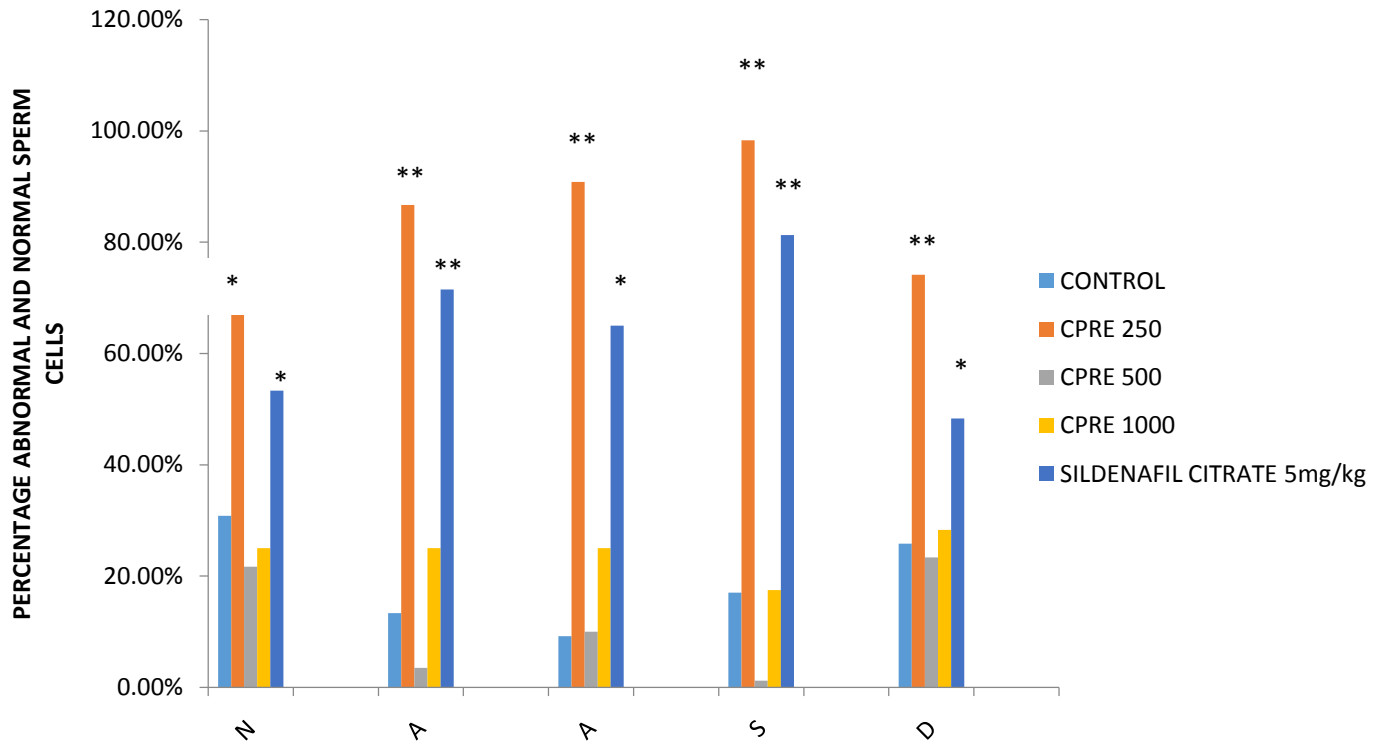


Figure 4.4: Effect of methanolic extract of *Cissus populnea* on sperm morphology and motility in male rats.*P≤0.05, **P≤0.01 sperm cells compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.2 Effect of the extract on testosterone level

The extract produced significant (P≤0.001) dose-dependent increase in serum level of testosterone in treated rats compared to rats in the control group. The effect of sildenafil citrate treated group is comparable to that of 1000mg extract/kg group. (Figure: 4.5).

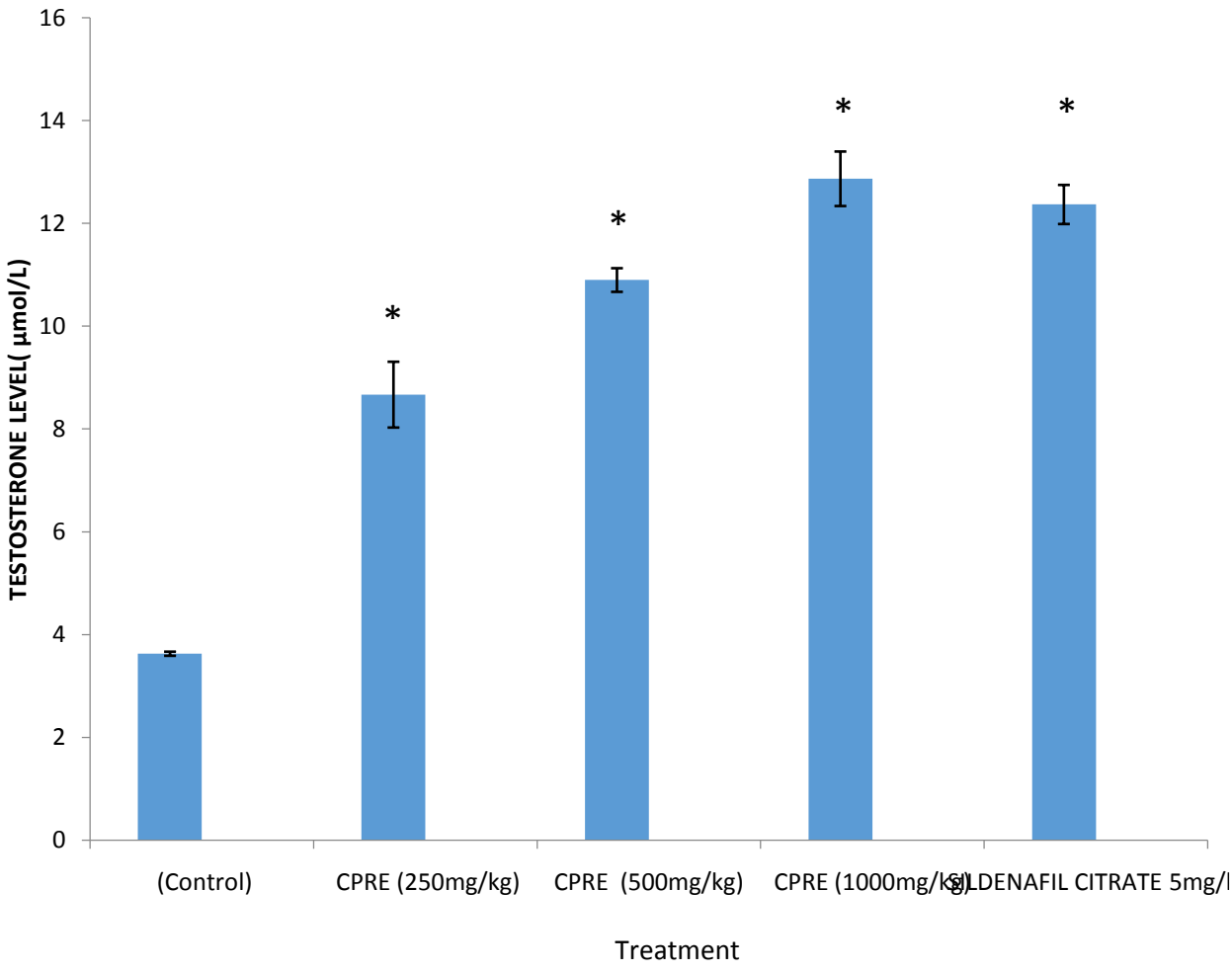


Figure 4.5: Effect of methanolic extract of *Cissus populnea* on the serum level of testosterone in male rats. *P≤ 0.001 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.3. Effect of the extract on cortisol level

There was significant (P≤0.01) increase in cortisol level at extract doses of 250mg/kg, 500mg/kg and sildenafil 5mg/kg compared to the control group. (Figure: 4.6).

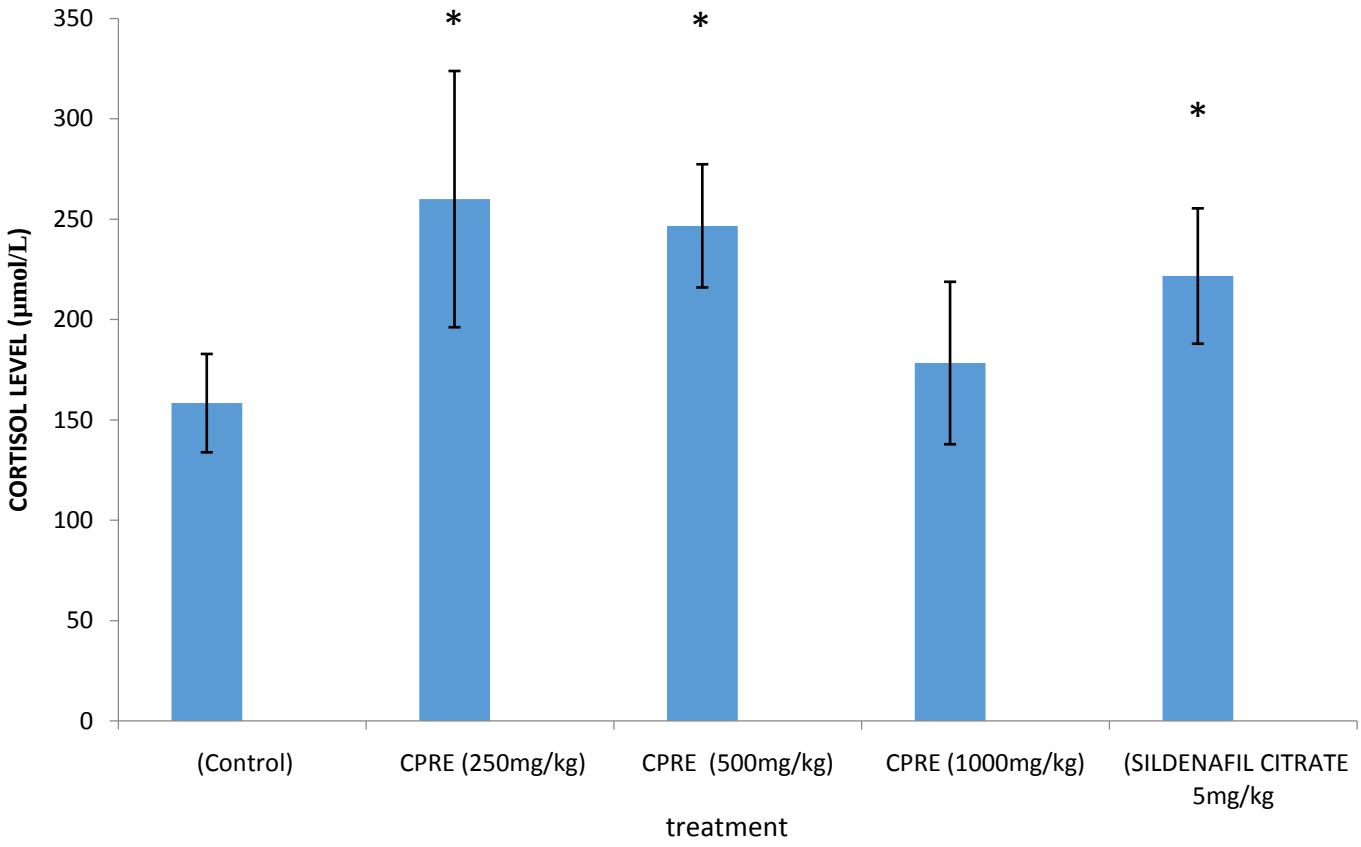


Figure 4.6: Effect of methanolic extract of *Cissus populnea* on the serum level of cortisol in male rats.*P≤ 0.01 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.4 Effect of the extract on prostate specific antigen (PSA) level

The extract of *Cissus populnea* at all the extract doses used did not produced significant effect on the PSA levels. However, sildenafil citrate significantly (P≤0.05) increase the level of PSA when compared to the control group. (Fig: 4.7).

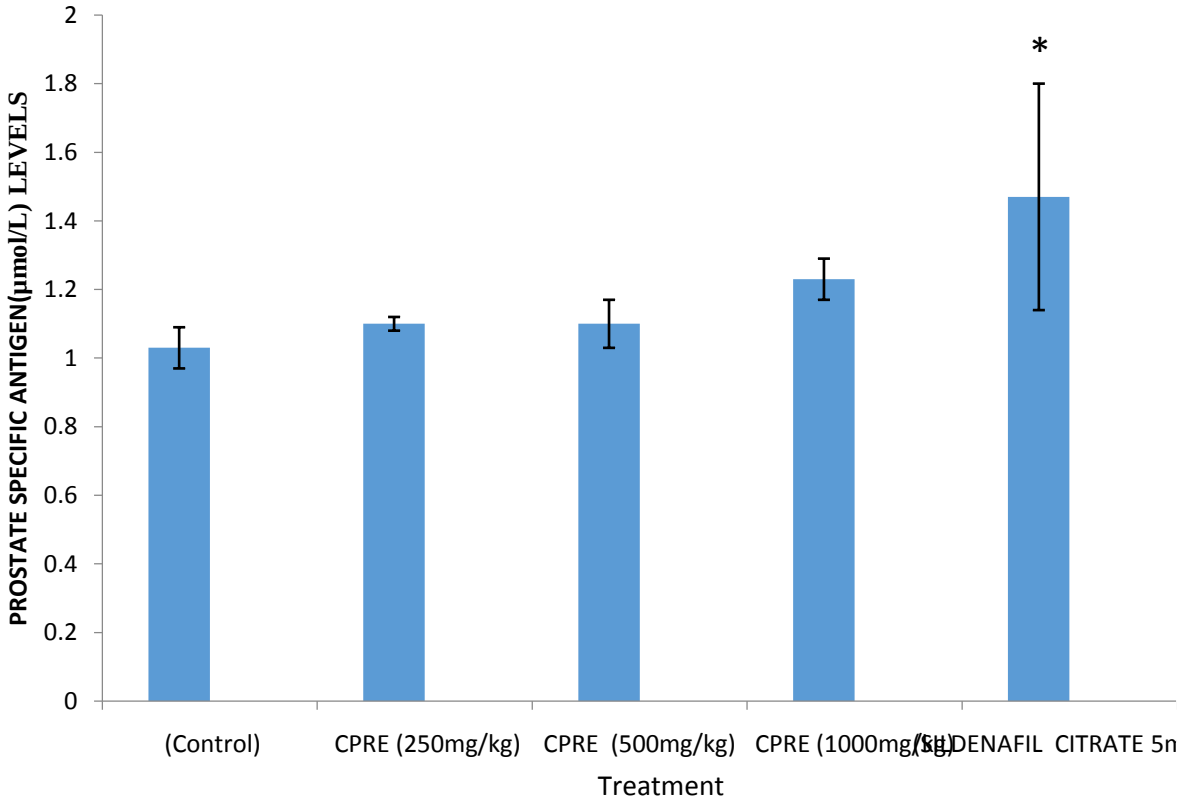


Figure 4.7: Effect of methanolic extract of *Cissus populnea* on the serum level of PSA in male rats.*P≤ 0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.5. Antioxidant effect of *Cissus populnea* root.

The methanolic root extract of *Cissus populnea*produced non-significant (P≥0.05) antioxidant scavenging effecton 2, 2 diphenyl-1- picrylhydrazyl (DPPH) radical compared to the ascorbic acid. (Figure: 4.8; Appendix IX).

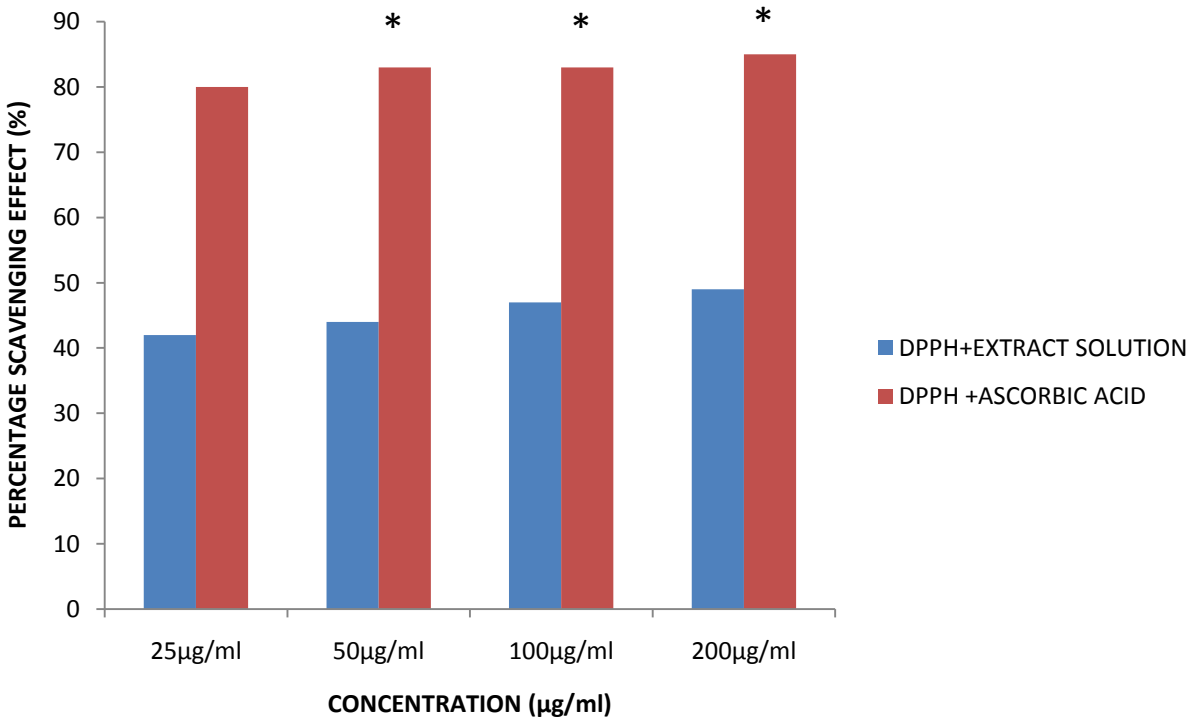


Figure 4.8: In vitro DPPH scavenging activity of the methanolic root extract of *Cissus populnea* and ascorbic acid. *P≤0.05.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.5.6 Effect of *Cissus populnea* on serum liver function monitoring parameters

There was significant ($P \leq 0.05$) decrease in the alkaline phosphatase (ALP) level in the 500mg/kg extract treated rats compared to the control. The extract at 250mg/kg and 1000mg/kg and sildenafil (5mg/kg) showed no significant effect on serum ALP levels when compared to the control (Figure 4.9). The serum level aspartate aminotransferase (AST) significantly ($P \leq 0.05$) increased in the extract and sildenafil treated rats compared to the control (Figure 4.10) while the alanine aminotransferase serum level significantly ($P \leq 0.05$) decreased at extract dose of 500mg/kg (Figure 4.11).

There was significant ($P \leq 0.01$) decrease in the serum level of total bilirubin level at 500mg/kg extract dose compared to the control. However, the extract at 250 mg/kg and 1000 mg/kg and sildenafil (5mg/kg) showed no significant effect on serum total bilirubin levels when compared to the control (Figure 4.12).

The conjugated bilirubin serum levels at extract doses of 250 and 500mg/kg decreased significantly ($P \leq 0.05$) compared to the control. The extract at 1000 mg/kg and sildenafil 5 mg/kg showed no significant effect serum conjugated bilirubin levels compared to the control (Figure 4.13).

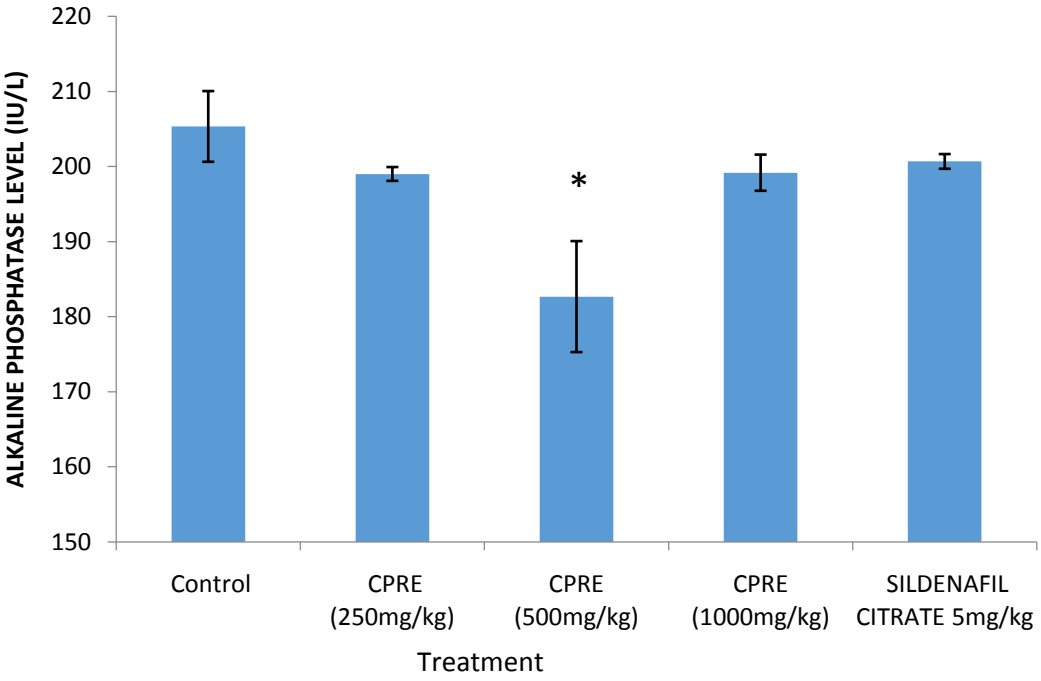


Figure 4.9: Effect of methanolic extract of *Cissus populnea* on serum alkaline phosphatase in male wistar rats.*P≤ 0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

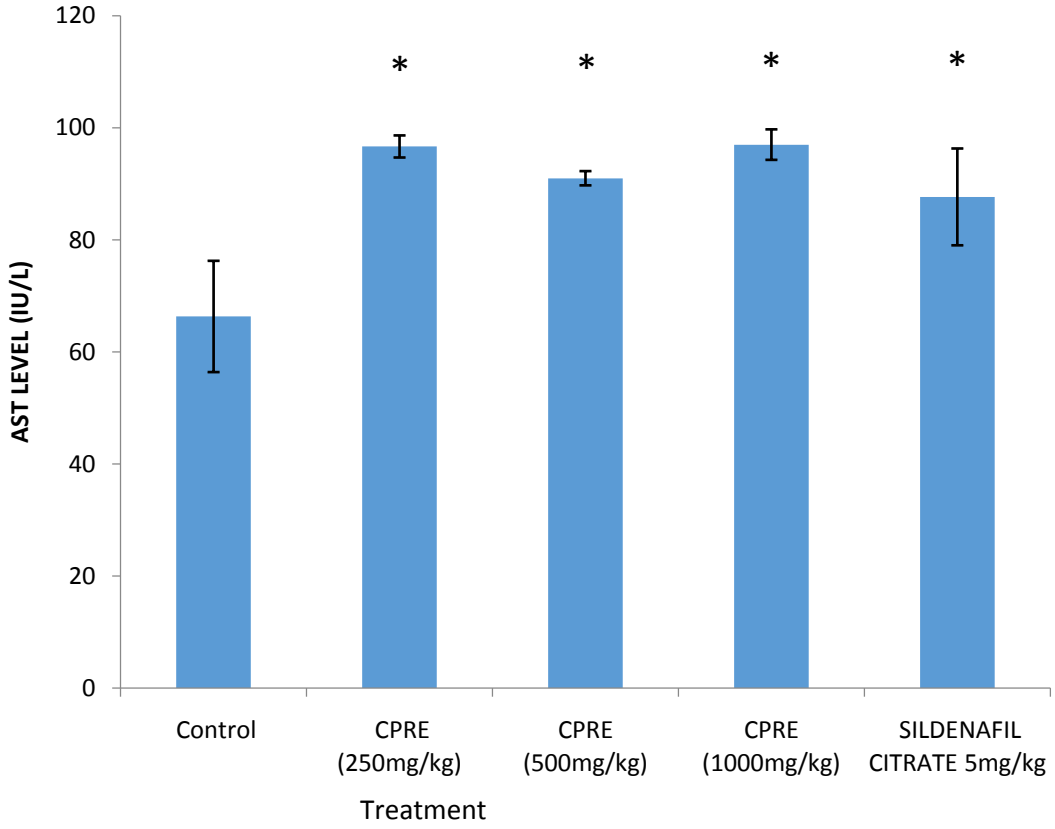


Figure 4.10 Effect of methanolic extract of *Cissus populnea* on serum aspartate aminotransferase in male wistar rats.*P≤ 0.05 compared to the control.CPRE = *Cissus populnea* root extract

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

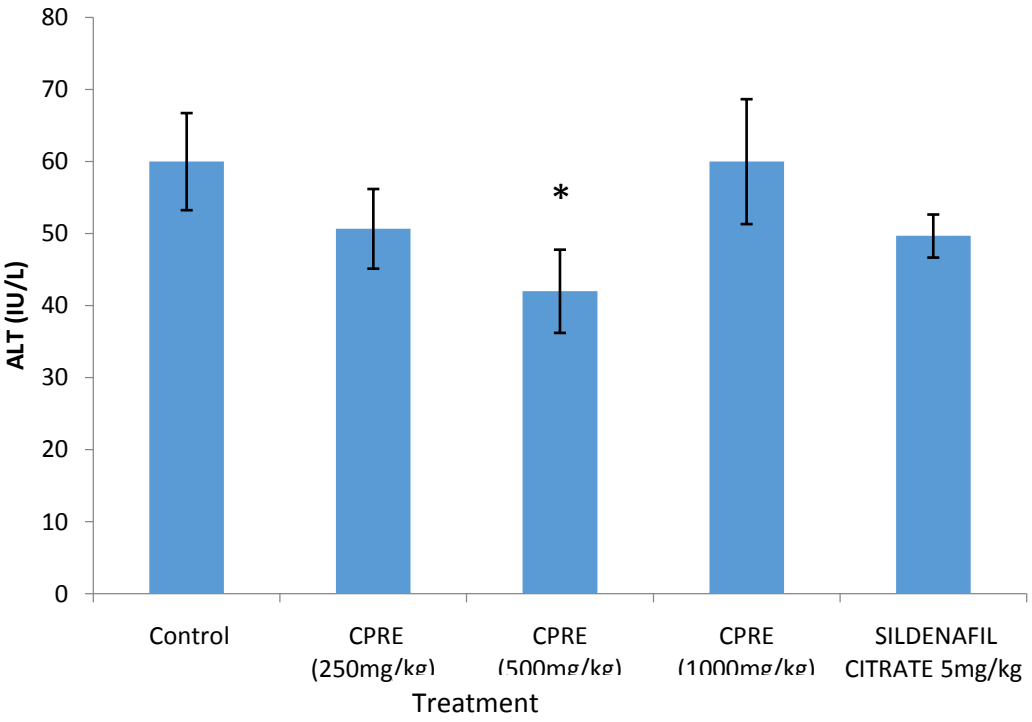


Figure 4.11 Effect of methanolic extract of *Cissus populnea* on serum alanine aminotransferase level in male wistar rats.*P≤ 0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

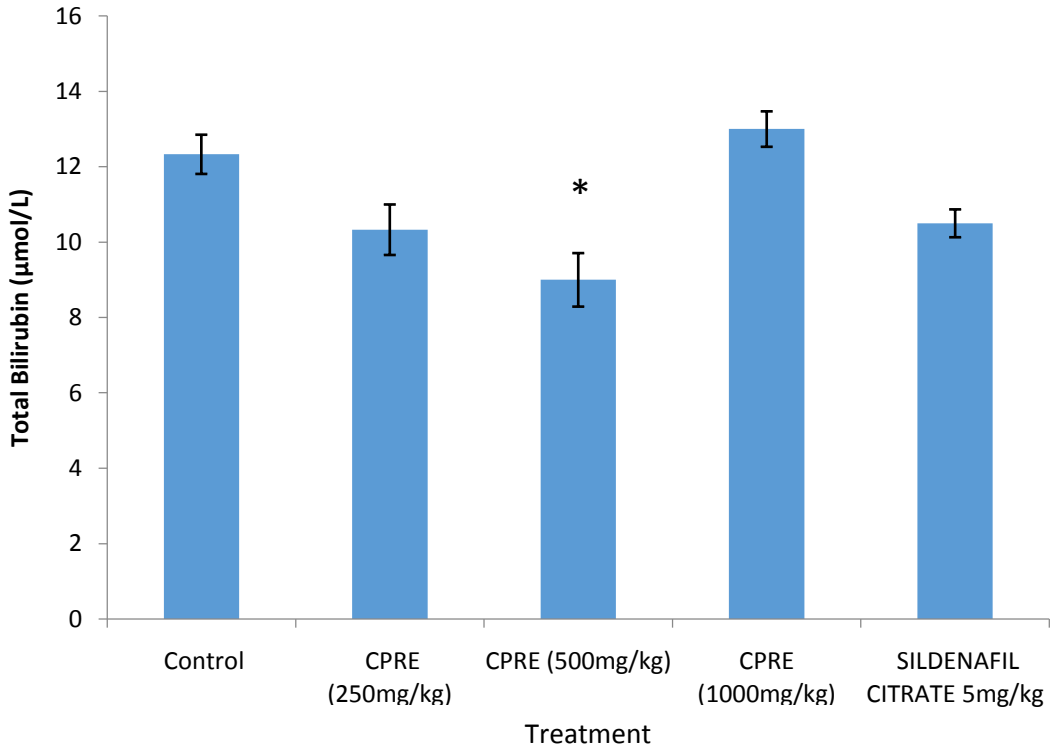


Figure 4.12 Effect of methanolic extract of *Cissus populnea* on serum total bilirubin level in male wistar rats.*P≤ 0.01 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

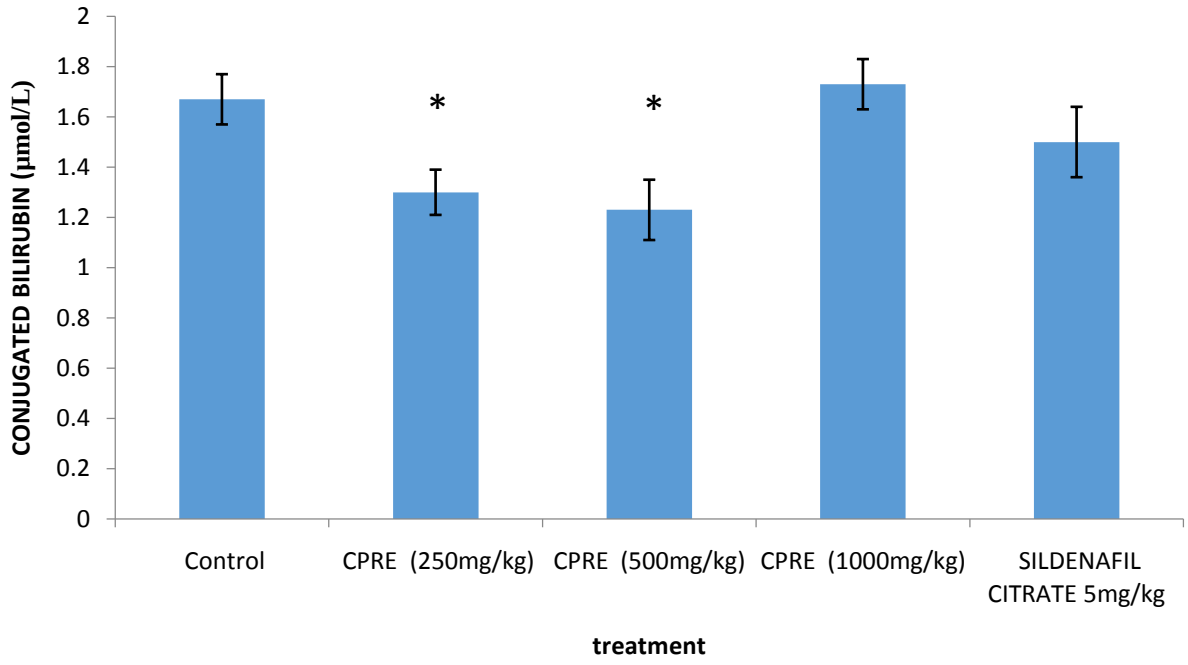


Figure 4.13 Effect of methanolic extract of *Cissus populnea* on serum conjugated bilirubin level in male wistar rats.*P≤0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.7.2 Effect of *Cissus populnea* on serum kidney function monitoring parameters

There was significant (P≤0.05) decrease in the serum creatinine and urealevels in the extract treated rats compared to the control. Similarly, there was a highly significant (P≤0.01) decrease in the creatinine level in the sildenafil treated compared to the control (Figures 4.14 and 4.15).

The extract at all tested doses and sildenafil did not produce significant changes in the levels of sodium when compared to the control (Figure. 4.16).

There was no significant effect on the potassium level in the extract treated rats compared to the control. However, significant (≤0.05) decreased was observed in the sildenafil treated rats (Figure 4.17).

The extract at 500 and 1000 mg/kg and sildenafil 5 mg/kg produced highly significant (P≤0.01) decreased in the serum of chloride levels in the treated rats compared to the control (Figure 4.18). Similarly, there was significant (P≤0.05) decrease in bicarbonate levels at 500 and 1000mg/kg extract doses and highly significantly (P≤0.01) decrease in the sildenafil treated rats compared to the control. At 250mg/kg of the extract there was no significant effect (Figure 4.19).

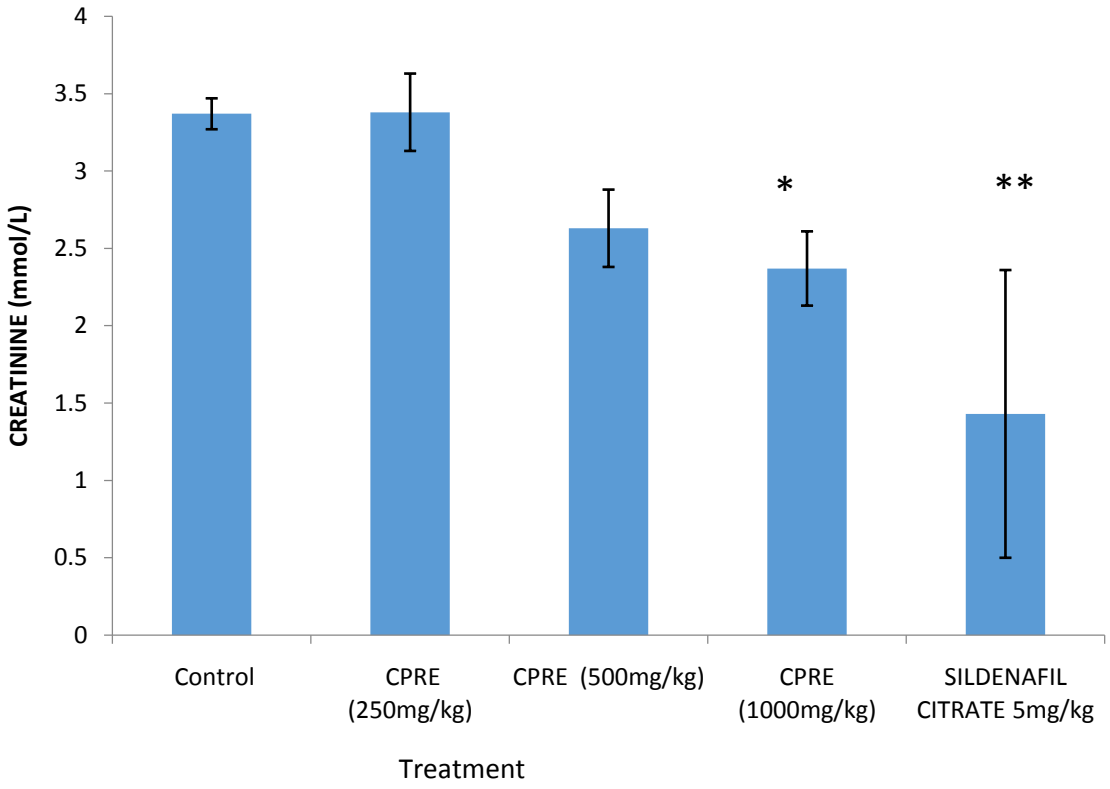


Figure 4.14: Effect of methanolic extract of *Cissus populnea* on serum creatinine level in male wistar rats.*P≤ 0.05, **P≤0.01 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

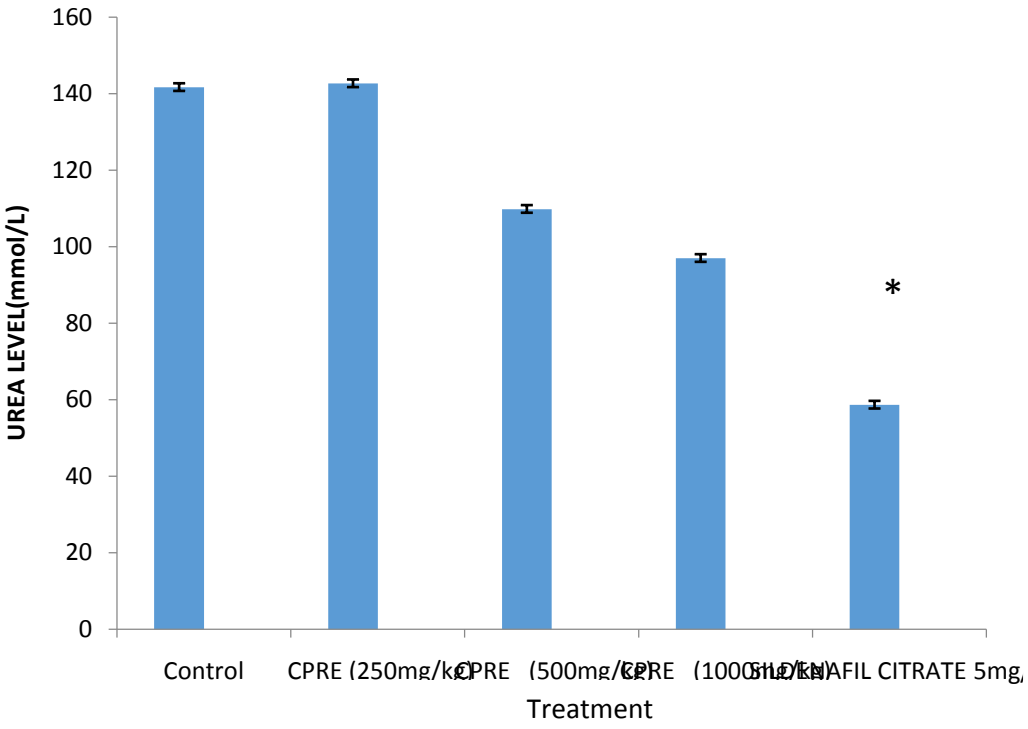


Figure 4.15 Effect of methanolic extract of *Cissus populnea* on serum urea level in male wistar rats.*P≤ 0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

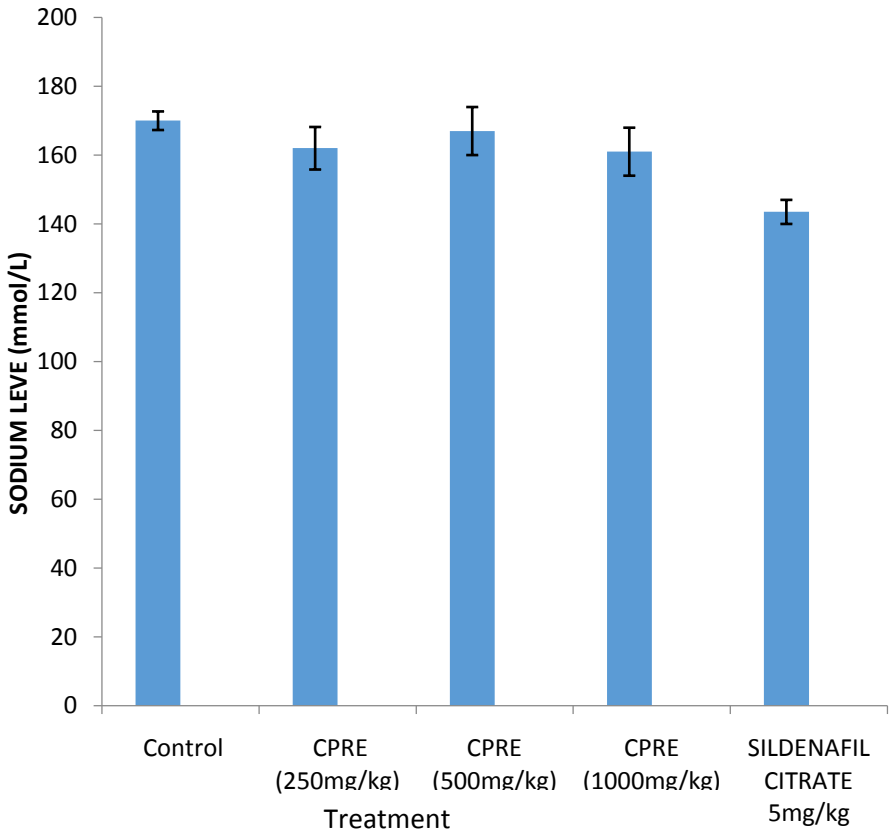


Figure 4.16: Effect of methanolic extract of *Cissus populnea* on serum sodium level in male wistar rats compared to the control. CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

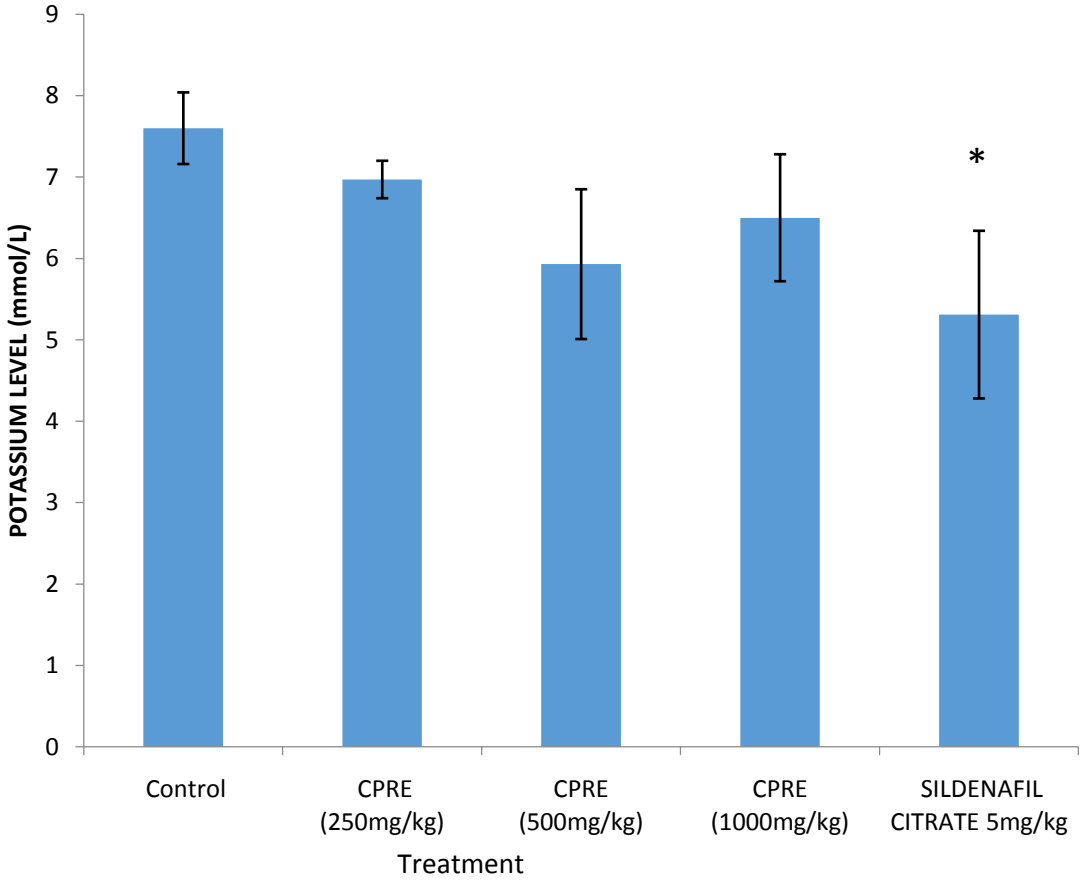


Figure 4.17 Effect of methanolic extract of *Cissus populnea* on serum potassium level in male wistar rats.*P≤0.05 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

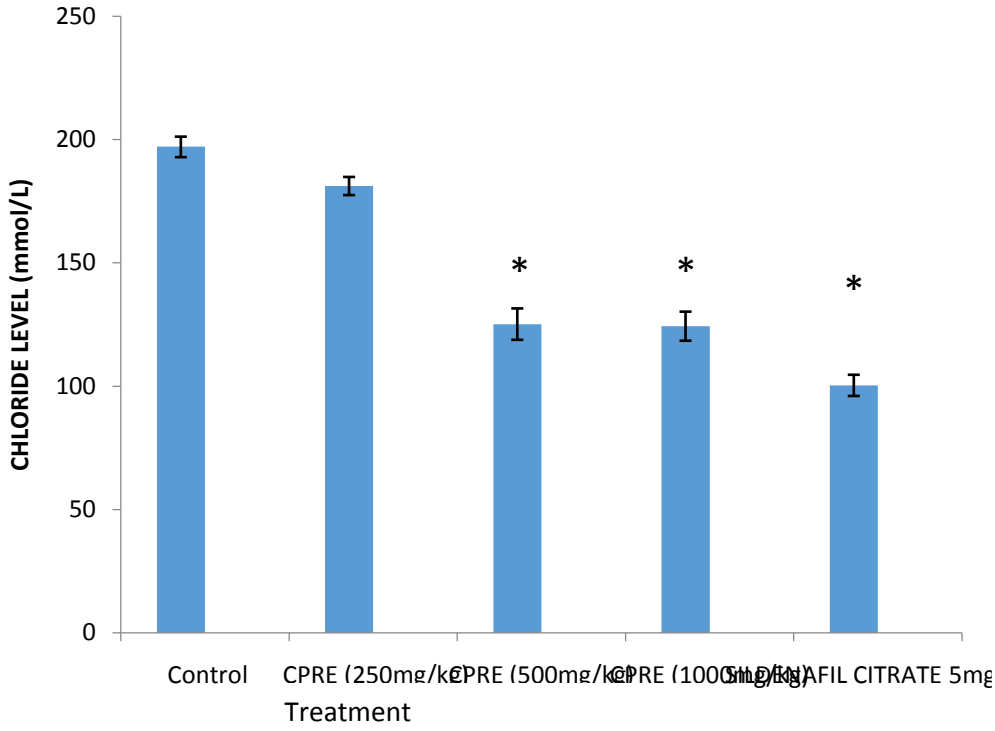


Figure 4.18 Effect of methanolic extract of *Cissus populnea* on serum chloride level in male wistar rats.*P≤0.01 compared to the control.CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

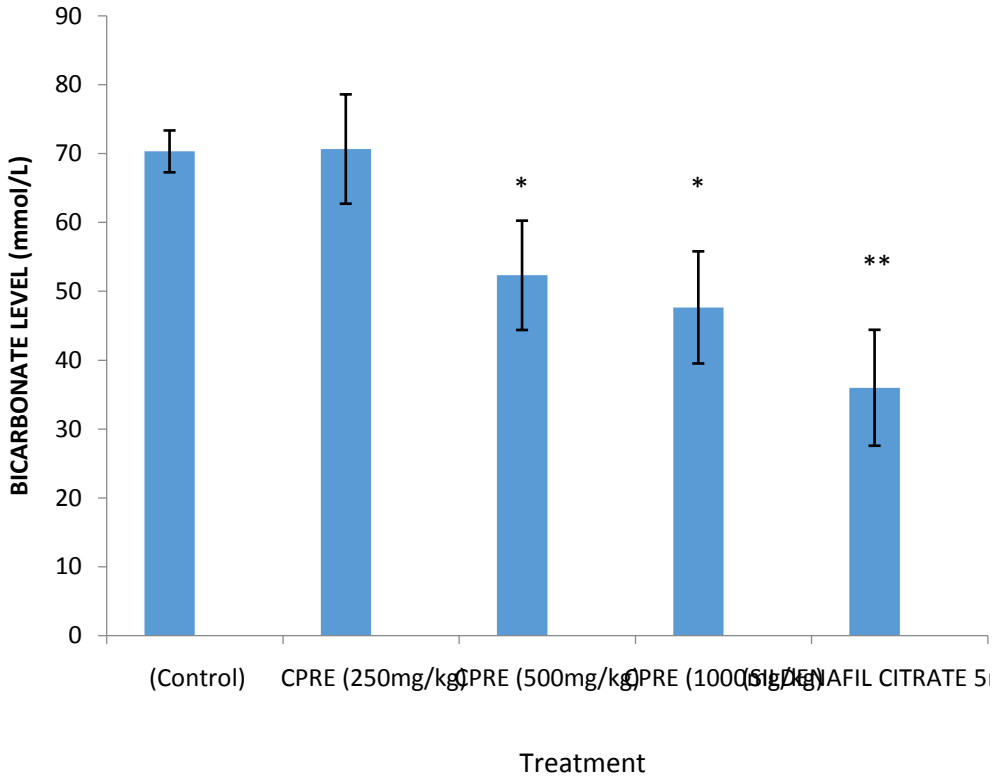


Figure 4.19 Effect of methanolic extract of *Cissus populnea* on serum bicarbonate level in male wistar rats.*P≤ 0.05, **P≤0.01 compared to the control. CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.6 Psychological Effect of *Cissus populnea Root Extract*

4.6.1 Effect of the extract on anxiety

The extract treated rats showed significant (P≤0.0.1) increase in time spent in the open arm at 500mg/kg and 1000mg/kg doses. There was corresponding significant decrease (P≤0.01) in time spent in the closed arm of the maze in rats treated with the extract at both dose levels (500 and 1000 mg/kg compared to the control rats. Similarly, the sildenafil citrate treated rats showed significant (P≤0.0.1) increase in time spent in the open arm.

The extract at 250mg/kg showed no significant effect in the time spent in both the open and closed arms of the maze. (Figure 4.20).

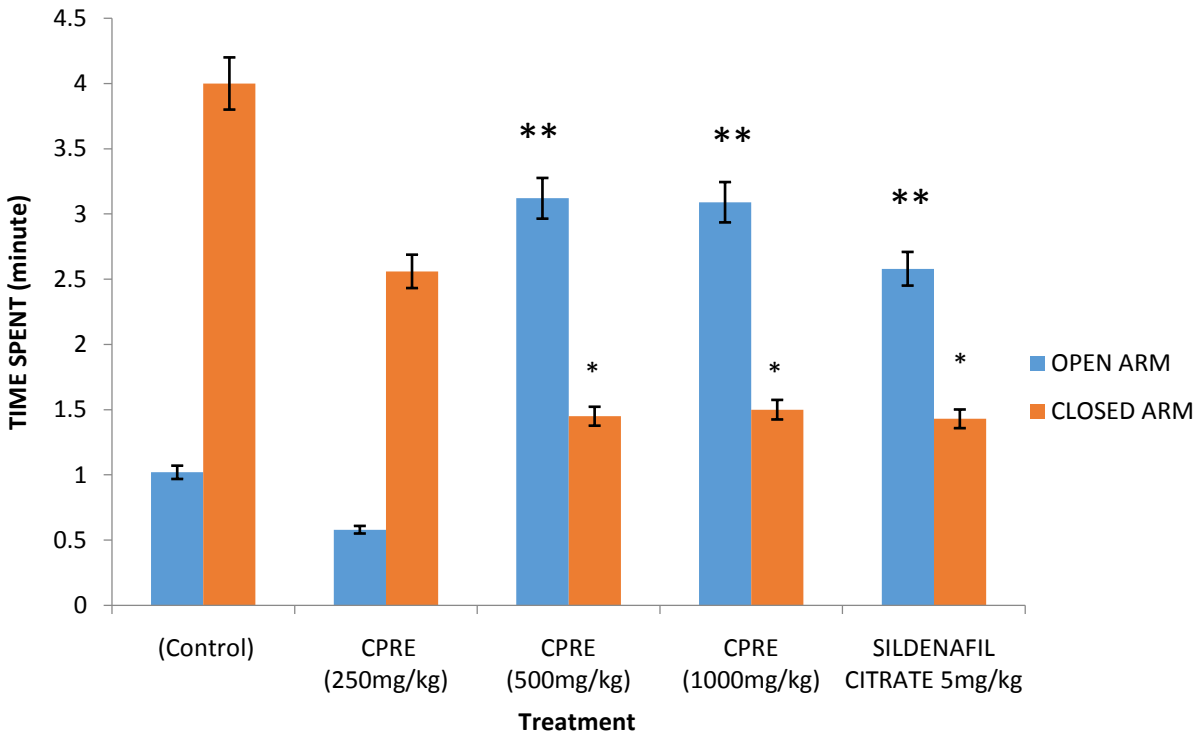


Figure 4.20: Effect of methanolic extract of *Cissus populnea* on the time spent in the open and closed arm of the elevated plus maze. **P≤0.01, *P≤0.05 compared to the control. CPRE = *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

4.6.2 Effect of the extract of *Cissus populnea* on cognition

Thetime spent on the open arm by rats treated with 250 and 500 mg/kg extract dose and sildenafil (5 mg/kg) increase significantly (P≤0.01) from the 7th day to the 14th. Similarly, the 1000 mg/kg extract treated rats showed significant (P≤0.05) inthe time spent in the open arm from the 7th to the 14th day compared to the control.(Figure 4.21).

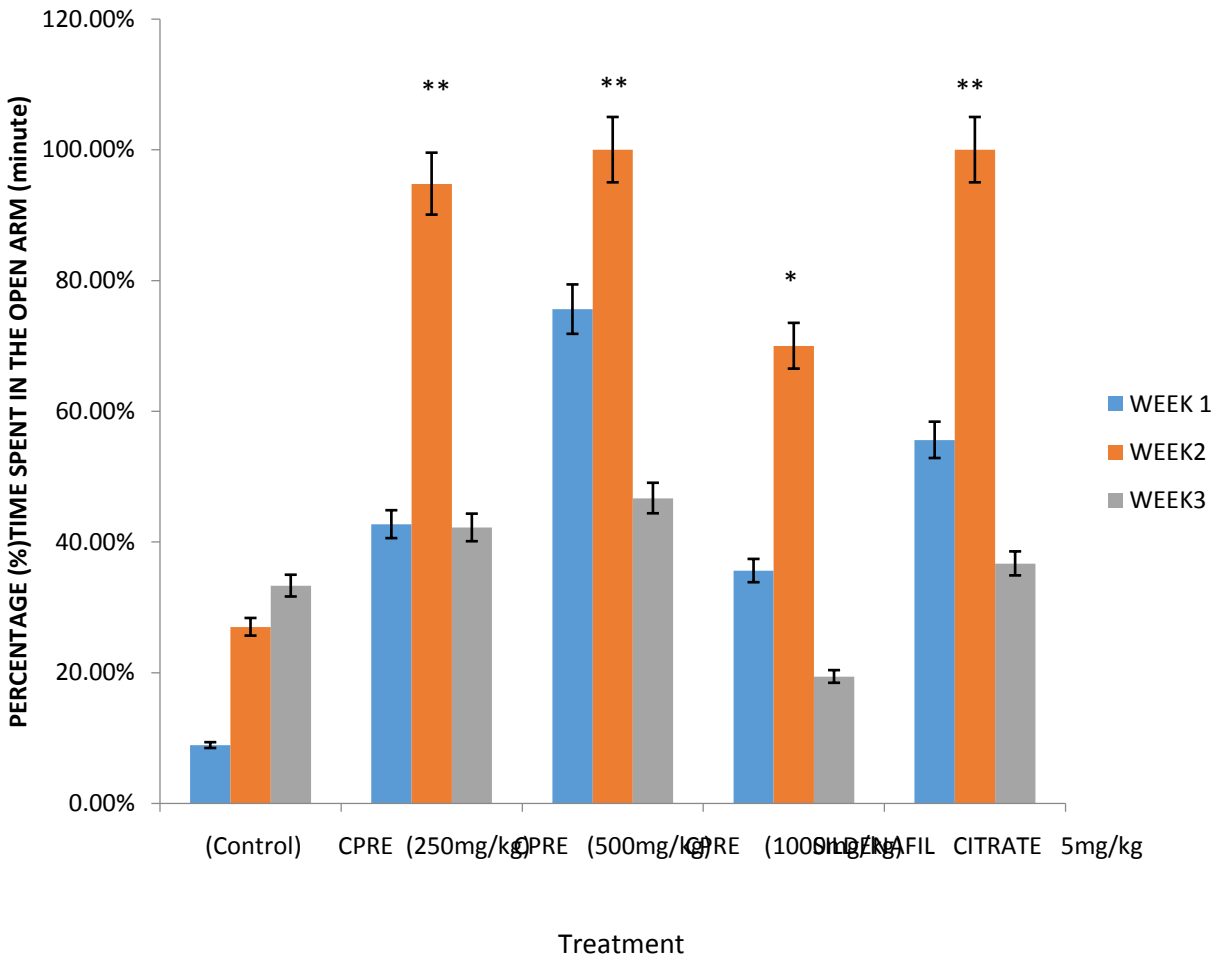


Figure 4.21.Effect of the methanolic root extract of *Cissus populnea* on memory/cognition in male wistarrats.*P≤0.05, **P≤0.01 compared to the control. CPRE= *Cissus populnea* root extract.

N = 6, SEM: standard error of mean, ANOVA and Newman-Keuls Posthoc test

CHAPTER FIVE

5.0 DISCUSSION

The preliminary phytochemical screening of the methanolic root extract of *Cissus populnea* revealed the presence of tannins, saponins, terpernoids and flavonoids. Soladoye and Chukwuma (2012), reported that, *Cissus populnea* root and stems contain alkaloids, saponins, flavonoids, polyphenols and glycosides. Saponins found in *Tribulis terrestris* is used as aphrodisiac in India (Singh *et al*., 2011), while flavonoids found in *Moringa. oleifera* were shown to increase androgen level leading to increased libido and sexual performance (Padashetty *et al*., 2007). Tannins have also been reported to elicit antioxidant properties (Okwu and Okwu, 2004). These phytochemical constituents observed to be present in *Cissus populnea* root extract may be responsible for the observed male sexual behaviour.

In the present study toxicity symptoms like respiratory distress, weight loss, salivation, changes in fur and skin colour, paw licking and mortality were not observed. The high oral LD_{50}value of more than 5000mg/kg implies that *Cissus populnea* root extract is relatively safe.

Aphrodisiacs can be any kind of food, drug, scent or device that can arouse or increase sexual drive or libido. They can therefore, be described as substances that enhance sexual drive or libido (Rosen and Ashton, 1993).

In males, increased mounting frequency is considered as an indication of sexual arousal and desire. Erection and ejaculation are considered as behavioral indication of sexual performance and facilitation (Neil *et al*., 1990). Disorder of sexual desire (libido) can involve either a deficient or compulsive desire for sexual activity and may include hypoactive sexual

desire (HSD), a persistent or recurrent deficient or absence of sexual fantasy and desire for sexual activity (American Psychiatric Association, 1994). The significant increase in mounting frequency in the extract- treated rats compared to the control group indicates arousability thus aphrodisiac activity.

Mating frequency is a behavioral indication of sexual performance. The inability of males to achieve and maintain an erection for sexual activity is a dysfunction. Erectile dysfunction results in a psychological toll on sexual activity and male masculinity. Any medicinal plant with aphrodisiac tendencies should produce statistically significant increase in mating frequencies (Ratnasoorinya *et al*., 2007, Tajuddin *et al*., 2004 and Yakubu *et al*., 2007). The significant increase in mating frequency produced by the extract implies that it has aphrodisiac properties specifically, arousal, motivation and vigor which enable penetration and consequently sexual intercourse at a maximum dose of 500mg/kg. The effect at this dose is comparable to that of sildenafil, the standard drug used in the studies.

Fertility abnormalities are associated with low sperm count and abnormal sperm cells (Jia *etal.*, 2003). Fertilization therefore, requires adequate and normal sperm count, morphology and motility to occur. The observed decrease in sperm count and increasedpercentage of abnormal sperm cells in the extract-treated rats compared to the control group indicates that the extract has no fertility - enhancing effect.

Testosterone, the main male hormone responsible for sexual character plays a major role in spermatogenesis. It has been reported to stimulate sexual desire and help maintain the tissues of the penis that enables erection (Aversa *et al*., 2000). Yakubu *et al*., (2007) reported that

male sexual dysfunction is associated with various factors like testosterone deficiencies and in old age the leydig cells decrease by about 40% with consequent decrease in thelevel of luteinizing hormone. Testosterone supplementation (hormone replacement therapy) has been shown to improve sexual function and libido. In addition, the intensity of orgasm and ejaculation are likely to improve. Any plant extract that causes increased level of testosterone is thus considered to be an aphrodisiac. The level of testosterone increased significantly in the extract-treated rats compared to the control indicating aphrodisiac properties. This may be a probable mechanism of aphrodisiac activity of the extract.

The glucocorticoid, cortisol is released from the adrenal cortex in response to oxidative stress (Vangelova *et al*., 2007). Feeling of stress may be accompanied by suppressed libido, production of gametes, and reduction in the frequency of sexual intercourse, fertilization, implantation and maintenance of pregnancy (Sanders and Bruce, 1997). Acute psychosocial stress has been reported to potentiate the secretion of cortisol (Biondi and Picardi, 1999). High level of cortisol has negative effects on sexual function by lowering sex drive (libido) and nocturnal penile erection. Stress may also have a negative influence on semen quality (Hell hammer *et al*., 1985, Harrison *et al*., 1987). The observed increase in cortisol levels in the extract-treated rats compared to the control group may explain the observed low sperm count.

Prostate specific antigen (PSA) is a biomarker of the integrity of the prostate. It is secreted into the semen to reduce its viscosity thus facilitating spermatozoa escape and ensuring fertilization (Overstreet *et al*., 1980). Hyperviscosity of semen has been associated with ejaculatory disorders (painful ejaculation, severe retarded or absent ejaculation) and low

sperm count (Stefan *et al*., 2013). Normal semen rarely prevents sperm movement; however, hyper viscous semen produces impaired trapping effect due to the visco-elasticity of the seminal plasma that inhibit normal sperm motility (Elzanaty *et al*., 2004). Very little PSA escapes from a healthy prostate into the blood thus increased PSA in blood is associated with erectile dysfunction. The extract at all doses used had no significant effect on PSA level compared to the control indicating that the extract does not adversely affect semen viscosity in the rats.

The dose-dependent increase in the time spent in the open arm of the EPM in the extract - treated rats compared to the control group rats indicates anxiolytic effect. This supports claims by Atmaca*et al*., (2004) that, drugs that increased open arm exploration on the EPM have anxiolytic effects. The psychological toll of sexual dysfunction has been associated with anxiety which is excessive worry about everyday life activities which may result in distress and significant impairment of normal activity. The diagnostic and statistical manual (DSM-IV-TR) defines anxiety as a feeling of persistent worry that hinders an individual ability to relax. It could also be pervasive persistent fear which due to its intensity and duration can lead to symptoms like headaches, sweating, trembling, muscle tension and urinary incontinence. Anxiety may be generalized anxiety disorder (GAD), panic disorders, obsessive compulsive disorders (OCD) and social phobia. Anxiety plays an important role in the pathogenesis and maintenance of sexual dysfunction. Neurobiological expression of anxiety, though complex, mainly involves a release of adrenergic substances (adrenaline and noradrenaline). Increased sympathetic activities has been shown to be negatively involved in arousal and orgasmic phases and may interfere with sexual arousal desire. Kaplan, (1988) reported that, any anomaly during any phase of the sexual cycle can be termed a disorder. These include desire disorder, arousal disorder, orgasm disorder and pain disorder. Theextract

may thus, relieve sexual phobia and enhance sexual activity which indirectly is an indication of an aphrodisiac effect.

Antioxidants scavenge free radicals from the system and protect germ and sertoli cells that are essential in spermatogenesis (Madhusudana *et al*., 1997). Kosuri and Ramakrishna (2013) reported that, oxidative stress produce free radicals which can lead to the development of neurodegenerative disease and memory impairment. Kalinin and Joaud (2011) also observed that, dietary antioxidants act on the central nervous system exhibiting cognitive, psychostimulating, anti -depressant and anti-anxiety properties. The observed mild antioxidant effect of the extract compared to ascorbic acid may explain the observed low sperm count, sperm cell morphology in the extract-treated rats.

Analysis of liver function parameters in rats is a relevant part of toxicity evaluation because changes in these parameters could be a valuable indicator of possible organ toxicity (Olson*et al*., 2000; Zimmerman, 1979). The aminotransferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are predominantly cytosolic liver enzymes (though some AST isoform can be found in the mitochondria) involved in transamination reactions during amino acid metabolism. Injury to the liver parenchyma following administration of drug/plant extract and metabolic activities can cause release or leakage of these hepatocellular enzymes into the bloodstream, thus elevating their serum levels (Pagana and Pagana, 1998). Alkaline phosphatase (ALP) level may be altered due to hepatitis, diseases affecting the thyroid and Paget disease in children. Haemolysis of erythrocytes produces bilirubin which is conjugated in the liver. When the rate of erythrocyte destruction is faster than normal it leads to increase level of indirect bilirubin. Cholestasis occurs when there is an obstruction to bile flow and there will be increase in direct or conjugated bilirubin. AST is release into the bloodstream following injury to hepatic or cardiac cells. Therefore, the serum level is used to evaluate myocardial injury and hepatic disease (Odutola, 1992). ALT is also

found in the same tissues as AST. However, ALT is a more sensitive clinical index in hepatocellular injury even in the presence of elevated aspartate aminotransferase (Odutola, 1992). The observed increase in AST level in the extract - and sildenafil - treated rats compared to the rats in the control group in the presence of decreased level of ALT and ALP levels implies that there was no hepatocellular injury. The observed decrease in the levels of conjugated and total bilirubin in the extract - and sildenafil - treated rats compared to the control group also indicate that the extract is not hepatotoxic.

Determination of serum electrolytes, urea and creatinine is an index of renal excretory function and can be used to diagnose impaired renal function (Pagana and Pagana, 1998). The decrease in serum levels of all the evaluated kidney parameters by the extract - and sildenafil - treated rats compared to the control group implies that the extract and sildenafil did not alter glomerular filtration and are therefore not nephrotoxic.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

The results showed that the methanolic extract of *Cissus populnea*:

i) is relatively safe.

ii) contains bioactive compounds that could be responsible for its enhanced sexual activity (arousal and performance) in male rats probably mediated by significant increase in testosterone level.

iii) does not have fertility-enhancing property as shown by the decrease in sperm count, motility and increase in percentage of abnormal sperm cell observed in the extract-treated rats.

6.2 Recommendation

1. The mechanism of action of *Cissus populnea* needs to be further investigated to evaluate its effect on other reproductive hormones.

2. The safety profile of *Cissus populnea* should be further investigated. However, the extract should be use with caution in those trying for babies.

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APPENDIX I

Table 4.1. Phytochemical constituents of the root of *Cissus populnea*

PHYTOCHEMICAL	Type of Test	Inference
Alkaloids	Dragendroffs	-
Saponins	Froth test	+
Phenols	Ferric Chloride Test	-
Tannins	Gelatin Test	+
Flavonoids	Alkaline reagent test	+
Cyanoglycosides	Guignard test	-
Terpernoids	Lead acerate test	+
Steroids	Keller-kiliani test	-

(–) ABSENT, (+) PREESNT

N = 6

APPENDIX II

Table 4.2 Effect of methanolic extract of *Cissus populnea* on mounting frequency.

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil Citrate 5mg/kg
MOUNTING FREQUENCY	4.33±0.72	6.33±0.98	9.30±2.27	6.67±2.46	6.67±2.46

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX III

Table 4.3 Effect of methanolic extract of *Cissus populnea* on mating frequency.

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5 mg/kg
MATING FREQUENCY	1.33 ± 0.32	3.00 ± 0.75	3.00 ± 1,25	1.67 ± 0.81	3.00 ± 0.47

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX IV

Table 4.4 Effect of methanolic extract of *Cissus populnea* on sperm cells count

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5mg/kg
SPERM COUNT	23.75±7.52x 10^6	13.67±3.04X10^6	7.48±2.68X10^6	5.5±1.99X 10^6	23.±2.51x10^6

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX V

Table 4.5: Effect of methanolic extract of *Cissus populnea* on sperm cells morphology and motility presented as the percentage mean

PERCENTAGE	MORPHOLOGY		MOTILITY OF SPERM CELL		
	NORMAL	ABNORMAL	ACTIVE	SLUGGISH	DEAD
CONTROL	30.83%	69.17%	21.67%	25%	53.33%
CPRE (250mg/kg)	9.20%	90.83%	10%	25%	65%
CPRE (500mg/kg)	13.33%	86.67%	3.5%	25%	71.5%
CPRE (1000mg/kg)	25.83%	74.17%	23.33%	28.33%	48.3%
Sildenafil citrate 5mg/kg	17%	98.33%	1.20%	17.5%	81.3%

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX VI

Table 4.6 Effect of methanolic extract of *Cissus populnea* on the serum testosterone level

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg	Sildenafil citrate 5mg/kg (s
TESTOSTERONE µmol/l	3.63±0.04	8.67±0.64	10.9±0.23	12.87±0.53	12.37±0.38

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX VII

Table 4.7 Effect of methanolic extract of *Cissus populnea* on serum cortisol level

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5mg/kg
CORTISOL (µmol/l)	158.33±24.56	260±63.84	246.67±30.70	178.33±40.48	221.67±33.73

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX VIII

Table 4.8 Effect of methanolic extract of *Cissus populnea* on the serum level of prostate specific antigen (PSA)

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5mg/kg
PROSTATE SPECIFIC ANTIGEN (µmol/L)	1.03 ±0.06	1.1 ± 0.02	1.1±0.07	1.23±0.06	1.47± 0.33

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX IX

Table 4.9: DPPH % scavenging effect of extract compared to ascorbic acid at various concentrations.

CONCENTRATION	25µg/ml	50µg/ml	100µg/ml	200µg/ml
DPPH+EXTRACT SOLUTION	42	44	47	49
ASCORBIC ACID	80	83	83	85

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX X

Table 4.10: Effect of methanolic extract of *Cissus populnea* on liver function monitoring parameters in male wistar rats

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5mg/kg
Total Bilirubin (µmol/L	12.33±0.52	10.33 ± 0.67	9.00 ±0.71	13.00 ± 0.47	10.50 ± 0.37
D. Bilirubin (µmol/L	101.67±0.10	1.30±0.09	1.23±0.12	1.50±0.14	1.73±0.
ALT (IU/L)	60.00±6.74	50.67±5.53	42.00±5.78	60.00±13.67	49.67±2.99
AST (IU/L)	66.33±9.93	96.67±1.97	91.00±1.27	97.00±2.72	87.67±8.64
ALK PO_4 (IU/L)	205.33.±4.71	199.±0.92	182.67±7.39	199.17±2.41	200.67.±0.98

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX XI

Table 4.11: Effect of methanolic extract of *Cissus populnea* on kidney function monitoring parameters in male wistar rats

Parameters	Control	CPRE (250mg/kg)	CPRE (500mg/kg)	CPRE (1000mg/kg)	Sildenafil citrate 5mg/kg
Sodium (mmol/L)	170.33±2.70	162±6.18	167.83±6.98	161.00±6.97	143.50±3.50
Potassium (mmol/L)	7.60±0.44	6.97±0.23	5.93±0.92	6.50±0.78	5.31±1.03
Bicarbonate (mmol/L)	70.33±3.04	70.67±7.94	52.33±7.94	47.67±8.14	36.00±8.42
Chloride (mmol/L)	197.17±4.03	181.17±3.68	125.17±6.37	124.33±5.90	100.33±4.30
Urea (mmol/L)	141.67±8.39	142.67±15.32	109.83±19.29	97. 00 ±15.06	58.67±22.57
Creatinine (mmol/L)	3.37±0.10	3.38±0.25	2.63±0.25	2.37±0.24	1.43±0.93

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX XII

Table 4.12: Effect of methanolic extract of *Cissus populnea* on anxiety in male rats

	TIME SPENT		NUMBER OF ENTIRES	
	OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM
CONTROL	1.02 ±0.41	4.00 ± 0.81	1.83 ± 0.55	1.83 ± 0.55
CPRE (250 mg/kg)	0.58 ± 0.32	2.58 ± 0.65	3.00 ± 0.71	2.67 ± 0.66
CPRE (500 mg/kg)	3.12 ± 0.72	1.45 ± 0.49	2.33 ± 0.62	2.00 ± 0.57
CPRE (1000 mg/kg)	3.09 ± 0.71	1.5 ± 0.50	2.67 ± 0.66	2.33 ± 0.62
Sildenafil citrate 5mg/kg	2.58 ± 0.65	1.43 ± 0.48	3.14 ± 0.72	2.43 ± 0.64

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test

APPENDIX XIII

Table 4.13: Effect of methanolic extract of *Cissus populnea* on rat cognition/ memory in the open arm of the EPM within 90 seconds interval.

	WEEK 1	WEEK 2	WEEK 3
CONTROL	8.90 ± 1.23	27.00 ± 2.12	33.30 ± 2.36
CPRE (250 mg/kg)	42.70 ± 2.67	94.80 ± 3.98	42.20 ± 2.66
CPRE (500 mg/kg)	75.60 ± 3.55	100.00 ± 4.09	46.70 ± 2.79
CPRE (1000 mg/kg)	35.60 ± 2.44	70.00 ± 3.41	19.40 ± 1.80
Sildenafil citrate 5mg/kg	55.60 ± 3.05	100.00 ± 4.08	36.70 ± 2.48

N = 6, SEM standard error of mean, ANOVA and Newman-Keuls Posthoc test