APHRODISIAC AND REPRODUCTIVE STUDIES ON METHANOL ROOT BARK EXTRACT OF *SECURINEGA VIROSA* (ROXB. EX. WILLD.) BAILL IN MALE WISTAR RATS

By

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DECLARATION

I, TENDE Yohanna Adamu with matric number: M.Sc/Pharm-Sci/35433/2012-2013, declare that the work in this dissertation entitled: **"Aphrodisiac and Reproductive Studies of Methanol Root Bark Extract of *Securinega virosa* (Roxb Ex. Wild) Baill. in Male Wistar Rats"** has been carried out by me in the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under the joint supervision of Dr. M.G. Magaji and Prof. A.U. Zezi

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

_____ _____

TENDE Yohanna Adamu Date

CERTIFICATION

This dissertation entitled **"Aphrodisiac and Reproductive Studies of Methanol Root Bark Extract of *Securinega virosa* (Roxb Ex. Wild) Baill in Male Wistar Rats"** by TENDE Yohanna Adamu, meets the regulations governing the award of the degree of Master of Science degree (M.Sc) in Pharmacology, Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to all those having one form of sexual dysfunction

ABSTRACT

Securinega virosa (SV) is a widely used plant in traditional medicine. The root is used as an aphrodisiac in north western Nigeria. The aim of the study was to provide pharmacological rationale for the ethnomedical use of the root of *Securinega virosa* as aphrodisiac as well as to establish its general male reproductive effects. A total of twenty four male wistar rats were randomly divided into four groups of six animals each (n=6), and used to assess the aphrodisiac potential of the extract which includes; mounting, intromission, ejaculation frequencies and latencies. Female Wistar rats used for pairing were brought to estrus phase of their reproductive cycle using oral suspension of ethinyl oestradiol (100 μ/ml/ animal), 48 hours prior to the pairing plus progesterone at a dose of I mg/animal injected subcutaneously, 6 hour prior to the experiment. For the assessment of male reproductive functions, a total of sixty (60) male Wistar rats were randomly divided into four groups (A, B, C, D) each consisting of fifteen (n=15) rats. Groups B, C and D were administered with the extract of *Securinega virosa* once daily at 24 hours interval at different graded doses of (31.25 mg/kg, 62.5mg/kg, and 125 mg/kg) respectively for 21 days. The rats in group A served as control and received 1ml/kg of distilled water for the same number of days. The extract significantly ($P< 0.05$) improved mating behavior, increased intromission frequency (IF) at 125 mg/kg, and significantly decreased ($P< 0.05$) ejaculatory latency (EL) at 31.20 mg/kg. Serum testosterone (ng/ml) level was significantly increased ($P< 0.05$) following first week of administration at doses of SV 62.50 mg/kg and 125 mg/kg; and also at third week of administration at dosage of SV 62.50 mg/kg. Serum LH (ng/ml) also increased at dose of 62.50 mg/kg following both first and the third week, while serum FSH (ng/ml) increased at 62.50mg/kg after the third week of administration. The extract caused a significant increase ($p<0.05$) in sperm motility, sperm count, sperm morphology and viability. There was a significant ($p< 0.05$) decrease in body weight at all the doses tested. The extract caused a significant increase ($p< 0.05$) in the weight of testes, epididymis and vas deferens weight (g). Histology of the rats treated with the highest dose showed normal seminiferous tubules with a markedly increased concentration of mature spermatozoa relative to the control. It is concluded that the methanol root bark extract of *Securinega virosa* possesses aphrodisiac activity with concurrent beneficial effects on sperm parameters.

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ABBREVIATIONS

ANOVA	Analysis of Variance
APA	American Psychological Association
CC	Corpus Carvenosa
CNS	Central Nervous System
CSBs	Compulsive Sexual Behaviour
ED	Erectile dysfunction
EL	Ejaculatory Latency
ELISA	Enzyme Linked Immunosorbent Assay
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
HSD	Hypoactive Sexual Desire
IF	Intromission Frequency
IL	Intromission Latency
LH	Luteinizing Hormone
MF	Mounting Frequency
ML	Mounting Latency
MSD	Male Sexual Dysfunction
Nacl	Sodium chloride
NO	Nitrogen Oxide
PEI	Post Ejaculatory Interval
PEPS	Psychogenic Post Ejaculatory Pain Syndrome

PRL	Prolactin
SD	Sexual Dysfunction
SEM	Standard Error of Mean
SV	Securinega Virosa
WHO	World Health Organization

CHAPTER 1

1.1 Introduction

The main aim of marriage is the procreation (reproduction) of the organism and for the sexual fulfillment of both partners. In order to prevent extinction of an organism, the organism must be able to reproduce itself before it dies. In human beings, reproduction is initiated when a male and female are involved in a sexual intercourse, which allows the fusion between the sperm from the male and egg from the female to form a zygote, which develops into a fetus (Fullick, 1994).

Normal sexual intercourse and fulfillment can only occur in males when the male sexual organs (the copulatory organ, the penis) and factors relating to erection are functioning normally. Inability to perform this function effectively is known as sexual dysfunction, and it is a problem of the reproductive process (Guay *et al.,* 2003). The increasing incidence of male sexual dysfunction has necessitated an increase in the patronage of aphrodisiacs; this has invariably resulted in an increase research into the beneficial effects of these medicinal plants, as well as their general effects on male reproductive function. Some of the medicinal plants that have been proven to have aphrodisiac potentials in male rats includes: *Terminalia catappa* seeds (almond fruit), *Syzygium aromaticum* flower bud (clove), *Fadogia agrestis* stem (Black aphrodisiac) (Ratnasooriya *et al.*, 2000; Yakubu *et al.*, 2005).

Erectile dysfunction is best defined as persistent failure to generate sufficient penile body pressure to achieve vaginal penetration and/or the inability to maintain penile rigidity until ejaculation. Many commonly prescribed pharmacological agents can adversely influence sexual function of the male. Mechanisms by which some medications can induce erectile dysfunction may include central and/or peripheral neurological blockade or stimulation of prolactin (PRL)

secretion. Hyperprolactinemia may reduce testosterone concentration and action through a variety of mechanisms including disruption of the anatomic integrity of the hypothalamic-pituitary axis, decreased GnRH expression, interference with GnRH action on the pituitary, inhibition of gonadotropin secretion, and reduction of testosterone conversion to the more active metabolite dihydrotestosterone. Hypogonadism has recently been shown to be associated with decreased nitrogen oxide (NO) formation and action in the penis, thus reducing erectile capacity (Lugg *et al*., 1995; Fouad *et al*., 2001).

1.2 Statement of Research Problem

Male sexual dysfunction is one of the most common health problems affecting men and is more common with increasing age. It can be caused by physical or psychological problems. Sexual dysfunction can lead to infertility, which is the inability of a sexually active non-contracepting couple to achieve pregnancy in one year of unprotected, regular intercourse(WHO, 2000). This has led to the end of several marriages. About 15% of couples do not achieve pregnancy within 1year; almost 50% of them do so spontaneously in the second year of unprotected intercourse, and another 14% in the third year. Ultimately, less than 5% remain childless (Te Velde and Pearson, 2002). Throughout all ages, men and women have incessantly pursued every means to increase, preserve and recapture their sexual capacity, or to stimulate the sexual desire of selected members of the opposite or same sex. One of the most recurrent methods has been the use of aphrodisiacs. Herbal medicines are a major source of aphrodisiacs and have been used worldwide for thousands of years by different cultures and civilizations. However, a deeper understanding of phytochemistry, pharmacognosy and ethnopharmacology is fairly available to support the production of new and safe pharmacologically active compounds with minimal undesired toxic effects. In infertile couple, there is often a coincidence of male and female

factors. With routine medical investigations for infertility, no cause can be found in about 10-15% of infertile couple. In the cases of infertility recorded, the male factor contributes 45-50% of cases (WHO, 2000).

1.3 Justification of Study

Sexual dysfunction is a major medical and social issue that affects the general populace. It has led to the end of several homes and increasing challenges to the nation at large. Several orthodox medications such as Sildenafil citrate (Viagra), Vardenafil (Levitra), Tadalafil (Cialis), Apomorphine (Uprima) have been used in the treatment of sexual dysfunctions. However, with increasing side effects, high cost of obtaining synthetic drugs and failure of treatment, there has been renewed vigorous interest in the medicinal herbs with folkloric use for sexual dysfunction.

Medicinal plants are extensively used as aphrodisiac to manage sexual dysfunction (Yakubu *et al.,* 2007). One of such plants claimed to have aphrodisiac potentials is *Securinega virosa;* however, there is paucity of data on its effectiveness in sexual dysfunction as well as it reproductive safety. This study is therefore directed at the investigation of the methanol root bark extract of *Securinega virosa* for aphrodisiac properties and its effects on male reproductive functions.

1.4 Aim and Objectives

1.4.1 Aim of study

The aim of the study was to provide pharmacological rationale for the ethnomedical use of the root of Securinega *virosa* as aphrodisiac as well as its general effects on male reproductive function.

1.4.2 Specific objectives.

The objectives of the study were to:

i. To establish the acute toxicity profile of the methanol root bark extract of *Securinega virosa.*
ii. To establish the preliminary phytochemical profile of the methanol root bark extract of *Securinega virosa.*
iii. Establish the effect of the methanol root bark extract of *Securinega virosa* on male reproductive functions viz; serum Testosterone, Luteinizing hormone, Follicle stimulating hormone, sperm motility, count, viability, morphology and testicular histology.
iv. Establish the aphrodisiac potential of the methanol root bark extract of *Securinega virosa* on male Wistar rats

1.5 Research Hypothesis

Methanol root bark extract of *Securinega virosa* possesses significant aphrodisiac activity and significantly enhances serum testosterone, FSH, LH and sperm parameters

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Male Reproductive System

2.1.1 Anatomy of the penis

The human penis is a pendulous organ suspended from the anterior and lateral aspect of the pubic arch containing the distal part of the urethra. It is composed of three erectile bodies running in parallel; the corpus spongiosum, encompassing the urethra and terminating in the glans penis; and the two corpus cavernosa (CC) which function as blood filled capacitors providing the structure to the erect organ (Wagner, 1981). The cavernosal tissue is composed of a complex meshwork of interconnected cavernosal spaces, or sinusoids, lined by vascular endothelium. Each sinusoid is separated by trabeculae consisting of fibrous tissue, elastic fibres and plain muscular fibre. The fibro-elastic network fills with blood during erection (Wagner, 1981)

2.1.2 Blood supply

2.1.2.1 Arterial blood supply

There are three main arteries of the penis; cavernosal, dorsal and bulbourethral. All three arise from a shared branch of the internal pudendal artery and provide an extensive vascular network. The artery of the bulb (bulbourethral artery) passes through the deep penile (buck) fascia to enter and supply the bulb of the penis and penile (spongy) urethra. The dorsal artery travels along the dorsum of the penis between the dorsal nerve and deep dorsal vein and gives off circumflex branches that accompany the circumflex veins; the terminal branches are in the glans penis. The deep penile (cavenosal) artery is usually a singe artery that arises on each side and

enters the corpus cavernosum at the crus and runs the length of the penile shaft, giving off the helicine arteries, which are an integral component of the erectile process (Wessells *et al*., 1996).

2.1.2.2 Venous drainage

The penis is drained by 3 venous systems consisting of the superficial, intermediate and deep veins. The superficial veins are contained in the dartos fascia on the dorsolateral surface of the penis and coalesce at the base to form a single superficial dorsal vein, which usually drains into the great saphenous veins via the superficial external pudendal veins. The intermediate system contains the deep dorsal and circumflex veins, lying within and beneath the deep penile (buck) fascia. Emissary veins begin with the erectile tissue of the corpora cavernosa and course through the tunica albuginea and drain into the circumflex or deep dorsal veins. The circumflex veins arise from the spongiosum, ventrum of the penis and often, the emissary veins drain into them. The circumflex veins course laterally around the cavernosa, passing beneath the dorsal arteries and nerves and drain into the deep dorsal vein. The deep dorsal vein lies in the midline groove between the 2 corpora cavernosa and is formed from 5-8 veins emerging from the glans penis, forming the retrocoronal plexus. It receives blood from the emissary and circumflex veins and passes underneath the symphysis pubis at the level of the suspensory ligament, leaving the shaft of the penis at the crus and draining into the prostatic plexus (Wessells *et al*., 1996).

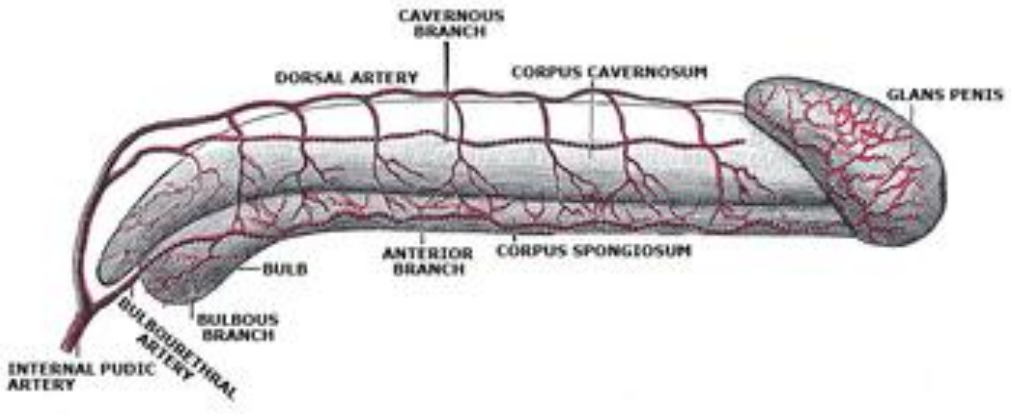


Figure 2.1: Blood supply to penis (Wessells *et al.*, 1996)

2.1.3 Nerve supply

The innervations of the penis is both autonomic (sympathetic and parasympathetic) and somatic (sensory and motor). From the neurons in the spinal cord and peripheral ganglia, the sympathetic and parasympathetic nerves merge to form the cavernous nerves, which enter into corpus carvenosum and corpus spongiosum to affect neurovascular events during the tumescence and detumescence (Dean and Lue, 2005)

2.1.4 Physiology of erection

An erection is a hemodynamic balance between inflow and outflow of blood within two chambers, called the corpora cavernosa. These cavities, which run the length of the organ, are filled with spongy tissue, and surrounded by a membrane, called the tunica albuginea. The spongy tissue contains smooth muscles, fibrous tissues, spaces, veins, and arteries. Erection begins with sensory and mental stimulation. Impulses from the brain and local nerves cause the muscles of the corpora cavernosa to relax, allowing blood to flow in and fill the open spaces. The blood creates pressure in the corpora cavernosa, making the penis expand. The tunica albuginea

helps to trap the blood in the corpora cavernosa, thereby sustaining erection. Erection is reversed when muscles in the penis contract, stopping the inflow of blood and opening outflow channels. The relaxation of the muscles of the corpora and penile arterial vessels is mediated by nitric oxide (NO), which is synthesized in the nerve terminals innervating these muscles. Any problem in blood inflow and outflow can be the origin of erectile dysfunction (Colpo, 1998).

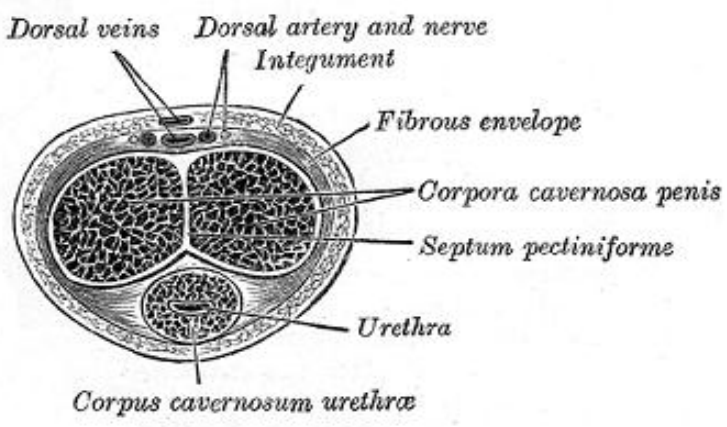


Figure 2.2: Transverse section of the penis (Colpo, 1998)

2.2 Physiology of Penile Erection

2.2.1 The interactions between autonomic and somatic innervations in the control of penile erection

The sensory input from the genital tract is carried by the pudendal nerve to the S2–S4 segment of the spinal cord. Ascending sensory fibers synapse in the corticomedullary junction and the thalamus, and then terminate in the contralateral primary sensory area deep in the interhemispheric tissue. The somatic motor fibers originate from the sacral segments S2–S4 and supply the pelvic floor muscles and the external anal sphincter. The higher centers for the erectile

function are located in the cortex, inter-hemispheric area, and limbic system. The descending parasympathetic innervation exits the spinal cord at the S2–S4 level and reaches the penis via nervi erigentes. It is responsible for the corporeal vasodilatation and corporeal smooth muscle relaxation, and hence the penile transformation from the flaccid to the erect state. Penile tactile stimuli reaching the spinal cord via the pudendal nerve generates additional reflex arcs to help initiate and/or maintain the erection. The sympathetic innervation exits the spinal cord at T11–L2 level and reaches the penis via the inferior mesenteric, hypogastric, and pelvic plexuses. It is responsible for the emission and ejaculation through coordinated contractions of the vas deferens, ampulla, seminal vesicles, prostate, and the bladder neck. Somatic innervation-mediated contraction of the pelvic floor muscles aids in achieving the maximum penile rigidity and in discharging the ejaculatory fluid. Sympathetic innervation mediates corporeal vasoconstriction and corporeal smooth muscle contraction, and hence it causes penile detumescence after the orgasmic relief. It also maintains the flaccid state in the absence of sexual arousal. Activation of each division of the autonomic nervous system appears to occurs in a reciprocal manner (i.e., activation of one division is associated with inhibition of the other) (Colpo, 1998).

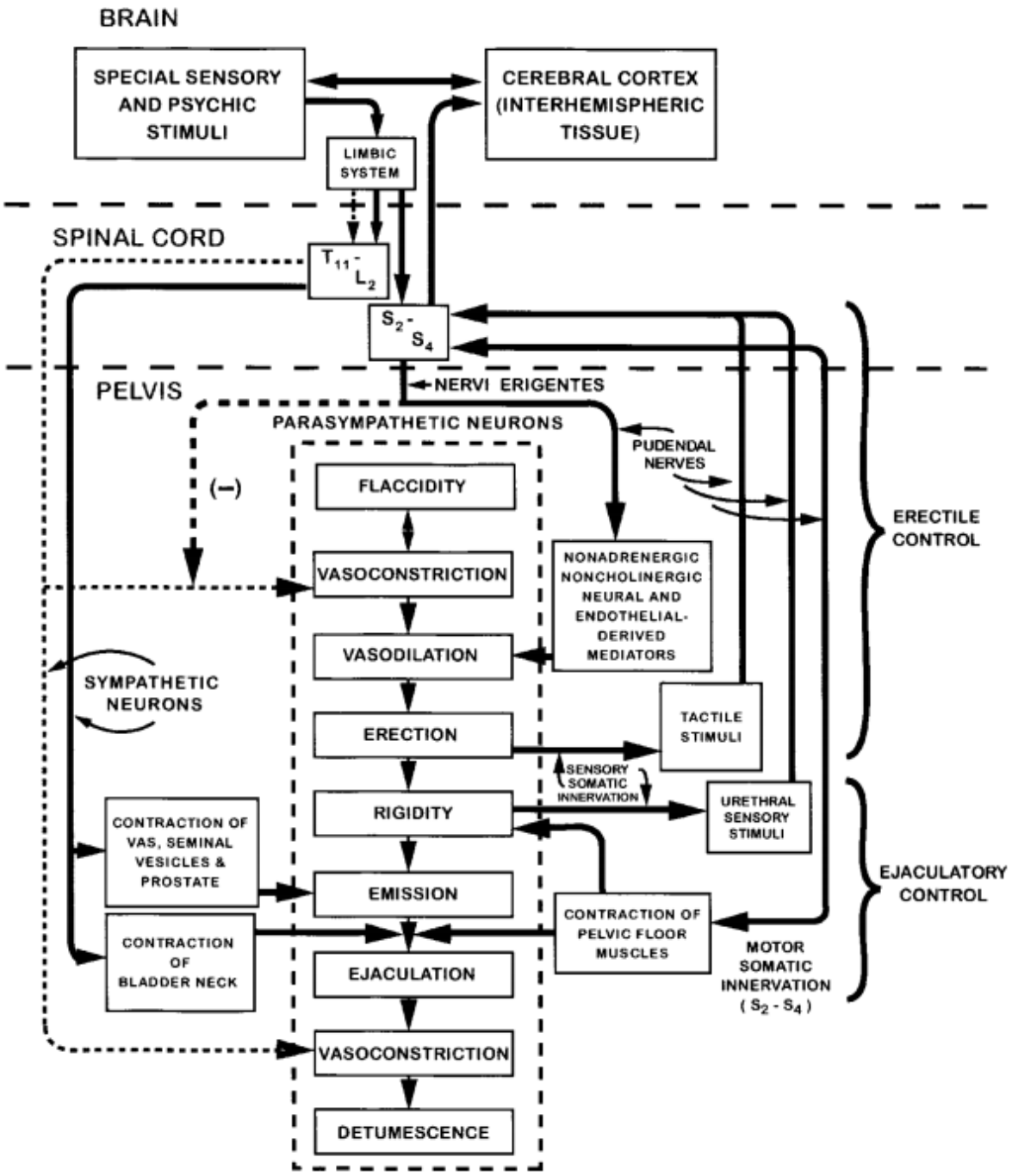


Figure 2.3: Neuroendocrine control of penile erection (Adapted from: Fouad *et al*., 2001)

2.2.2 Libido or sexual desire

Libido is defined as the biological need for sexual activity (the sex drive) and frequently is expressed as sex-seeking behavior (Hull *et al*., 1997). Its intensity is variable between individuals as well as within an individual over a given time. Little is known about the physiological basis of libido. However, previous and recent sexual activity, psychosocial background, brain and spinal cord dopaminergic receptor activation, and gonadal hormones are among the factors that are believed to participate in regulation of male sexual desire. Several lines of evidence in animal and human males support a role for central dopaminergic neurotransmission in mediating sexual behavior and erection. Further, testosterone promotion of copulation appears to be mediated by an increase in dopamine release in the medial preoptic area, possibly via up-regulation of nitrogen oxide (NO) synthesis (Hull *et al*., 1997).

A role for dopaminergic activation in stimulation of sexual behavior in the human is supported by the following observations: administration of the dopamine agonists apomorphine, bromocriptine, and pergolide mesylate frequently elicits spontaneous penile erection; use of the dopamine precursor levodopa is associated with increased libido, return of spontaneous erection, or onset of nocturnal emissions in 20 –30% of patients with Parkinson's disease who are treated with this agent. Higher serum testosterone appears to be associated with greater sexual activity in healthy older but not younger men (Yakubu *et al*., 2005). Further more, higher testosterone levels may also shorten the latency of erection stimulated by the exposure to erotic material. Testosterone replacement in hypogonadal males restores sexual interest, shortens latency, and increases frequency and magnitude of nocturnal penile tumescence (NPT) (Lange *et al*., 1980; Fouad *et al*., 2001). Conversely, withdrawal of androgen therapy in hypogonadal males leads to a decline of libido in 3 – 4 weeks, and unreplaced hypogonadal men have impairment in

spontaneity of erection. Despite these androgen deficiency-related abnormalities, hypogonadism does not appear to compromise the ability to achieve erection in response to viewing of erotic films (Mills *et al*., 1992; Fouad *et al*., 2001).

2.2.3 Erection

An erection is the stiffening and rising of the penis, which occurs during sexual arousal, though it can also happen in non-sexual situations. Spontaneous erections frequently occur during adolescence due to friction with clothing, a full bladder or large intestine, hormone fluctuations, nervousness, and undressing in a nonsexual situation. It is also normal for erections to occur during sleep and upon waking. The primary physiological mechanism that brings about erection is the autonomic dilation of arteries supplying blood to the penis, which allows more blood to fill the three spongy erectile tissue chambers in the penis, causing it to lengthen and stiffen. The now-engorged erectile tissue presses against and constricts the veins that carry blood away from the penis. More blood enters than leaves the penis until an equilibrium is reached where an equal volume of blood flows into the dilated arteries and out of the constricted veins; a constant erectile size is achieved at this equilibrium. The scrotum will usually tighten during erection (Fouad *et al*., 2001).

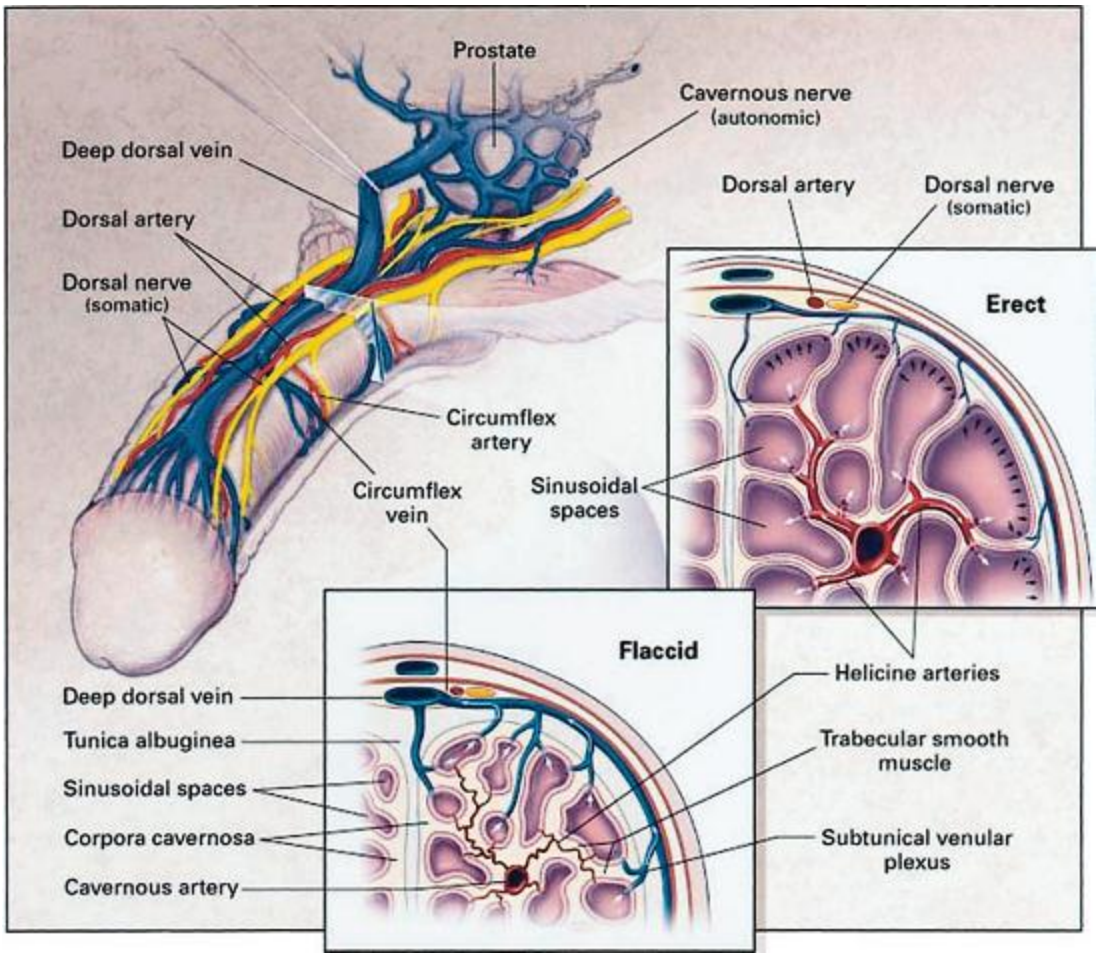


Figure 2.4: Anatomy and mechanism of penile erection (Fouad *et al*., 2001)

2.2.4 Ejaculation

Ejaculation is the ejecting of semen from the penis, and is usually accompanied by orgasm. A series of muscular contractions delivers semen, containing male gametes known as sperm cells or spermatozoa, from the penis. It is usually the result of sexual stimulation, which may include prostate stimulation. Rarely, it is due to prostatic disease. Ejaculation may occur spontaneously during sleep (known as a nocturnal emission or wet dream). Anejaculation is the condition of being unable to ejaculate. Ejaculation has two phases: emission and ejaculation proper. The emission phase of the ejaculatory reflex is under control of the sympathetic nervous system,

while the ejaculatory phase is under the control of a spinal reflex at the level of the spinal nerves S2-S4 via the pudendal nerve. A refractory period succeeds the ejaculation, and sexual stimulation precedes it (Carlson, 2013).

2.2.5 Orgasm

Both physiological and psychogenic elements contribute to genesis of the orgasmic phase (Walsh and Wilson, 1987). Afferent stimuli that transmit via the pudendal nerve induce the following physiological events: smooth muscle contraction of the accessory sex organs; build up and release of pressure in the posterior urethra; sensation of the ejaculatory inevitability; contraction of the urethral bulb and perineum; rhythmic contractions of the pelvic floor muscles; semen emission and ejaculation; and finally, the reversal of the generalized physiological changes and sexual tension. Sensory cortical neurons perceive these events as pleasurable. Factors that influence the subjective sensation of orgasmic pleasure include the degree of sexual excitement, recency of sexual activity, and the orientation of the individual. It is possible for orgasm to occur without being preceded by the previous two phases of erection and ejaculation. Conversely, contractions of pelvic musculature and ejaculation could occur in the absence of orgasmic sensations (Masters and Johnson, 1966; Fouad *et al*., 2001).

2.2.6 Detumescence

During this phase the penis returns to the flaccid state. Vasoconstriction of the arterioles and reversal of events within the contractile corporeal units divert the blood away from the cavernous sinuses and allow an increase in the venous drainage of their contents. Initially, the rate of blood outflow increases by about 10-fold, followed by a progressively decreasing rate until it reaches the pretumescence level and a period of inhibition to resumption of erectile and ejaculatory

functions. The length of this refractory phase is dependent upon many variables including age, physical state, and psychological environment. However, the traditional view that assumes male orgasm is instantly followed by detumescence and refractoriness has recently been challenged by reported observations in which some men were multi-orgasmic, and the phenomenon of repeated orgasms without intervening detumescence and refractoriness was actively learned by some males (Fouad *et al*., 2001).

2.3 Male Sexual Dysfunction

For most couples, procreating is a natural part of life that involves neither special planning or intervention (Trussell, 2013). Unfortunately, when trying to conceive, 15% to 25% of the couples struggle and, consequently, seek medical advice on how to improve their chances of fertilization and successful pregnancy (WHO, 2010). An estimated six percent of adult males are thought to be infertile. Infertility is defined by most authorities as the inability to achieve a pregnancy after one year of unprotected regular intercourse (Purvis and Christiansen, 1992). Sexual dysfunction in men refers to repeated inability to achieve normal sexual intercourse. It can also be viewed as disorders that interfere with a full sexual response cycle. These disorders make it difficult for a person to enjoy or to have sexual intercourse. While, sexual dysfunction rarely threatens physical health, it can take a heavy psychological toll, bringing on depression, anxiety, and debilitating feelings of inadequacy. Unfortunately, it is a problem often neglected by the health care team who strive more with the technical and more medically manageable aspects of the patient's illness. Sexual dysfunction is more prevalent in males than in females and thus, it is conventional to focus more on male sexual difficulties. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems (Guay *et al.,* 2003). Generally, a prevalence of about 10% occurs

across all ages. Since sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age (Rendell *et al.,* 1999).

As men age, the absolute number of Leydig cells decreases by about 40%, and the vigour of pulsatile lutenizing hormone release is dampened. In association with these events, free testosterone level also declines by approximately 1.2% per year. These have contributed in no small measure to prevalence of sexual dysfunction in the aged (Guya *et al.,* 2003). Male sexual dysfunction (MSD) could be caused by various factors. These include: psychological disorders (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure); androgen deficiencies (testosterone deficiency, hyperprolactinemia); chronic medical conditions (diabetes, hypertension, vascular insufficiency: atherosclerosis and venous leakage; penile disease (priapism, phimosis, smooth muscle dysfunction); pelvic surgery (to correct arterial or inflow disorder); neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzhemier's disease, spinal cord or nerve injury); drugs side effects (anti-hypertensives, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens); life style (chronic alcohol abuse, cigarette smoking); aging (decrease in hormonal level with age); and systemic diseases (cardiac, hepatic, renal pulmonary, cancer, metabolic, post-organ transplant) (Feldman *et al.,* 1994; Kandeel *et al.,* 2001; Guay *et al.,* 2003)

2.4 Classification of Male Sex Disorders

Sex disorders of the male are classified into disorders of sexual function, sexual orientation, and sexual behaviour. In general, several factors must work in harmony to maintain normal sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide

system and androgens (Guay *et al.,* 2003). Thus, malfunctioning of at least one of these could lead to sexual dysfunction of any kind.

2.4.1 Disorders of desire

Disorders of desire can involve either a deficient or compulsive desire for sexual activity. Dysfunctions that can occur during the desire phase include:

2.4.1.1 Hypoactive sexual desire (HSD)

It is defined as persistently or recurrently deficient (or absent) sexual fantasy and desire for sexual activity leading to marked distress or interpersonal difficulty. It results in a complete or almost complete lack of desire to have any type of sexual relation (APA, 1994).

2.4.1.2 Compulsive sexual behaviours (CSBs)

Constitute a wide range of complex sexual behaviours that have strikingly repetitive, compelling, or driven qualities. They usually manifest as obsessive-compulsive sexuality (e.g. excessive masturbation and promiscuity), excessive sex-seeking in association with affective disorders (e.g. major depression or mood disorders), addictive sexuality (e.g. attachment to another person, object, or sensation for sexual gratification to the exclusion of everything else), and sexual impulsivity (failure to resist an impulse or temptation for sexual behaviour that is harmful to self or others such as exhibitionism, rape, or child molestation) (Kaplan, 1996).

2.4.2 Erectile dysfunction (ED)

This is a problem with sexual arousal. ED is the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration, at least 50% of the time, for a period of six months. Erectile dysfunction is also defined as the persistent inability to obtain and maintain an

erection sufficient for naturally satisfactory intercourse. It results in significant psychological, social and physical morbidity, and annihilates the essence of masculinity (Laumann *et al.*,1999; Kandeel *et al.,* 2001). Many people believe that impotence is a disorder associated with modern civilization. However, preoccupation with potency has been present through the ages (Shah, 2002). The term "impotence" is derived from the Latin word *impotentia*, which when literally translated means lack of power. It has traditionally been used to signify the inability of the male to attain and maintain erection of the penis sufficiently long to permit satisfactory sexual intercourse. This term, together with its pejorative implications, has often generated confusion in both clinical and basic science investigations; so it was recently replaced by the more precise term "erectile dysfunction" (NIH, 1993). Erectile dysfunction is adversely affected by diabetes mellitus, antihypertensive, antipsychotic, antidepressant therapeutic drugs. Organic causes of ED include hypogonadism, hyperprolactinaemia, and neurological disorders (Sharma *et al.,* 2012). For years, psychological factors were implicated as the main cause of impotence, but during the last decade there has been an important change in the management of sexual dysfunction. This revolution was essentially due to the improved understanding of erectile physiology and to the development of new and effective medical therapies. Even though the loss of erectile function is not an inevitable consequence of normal aging, it becomes more frequent with age (Sharma *et al.,* 2012).

2.4.3 Disorders of ejaculation

There exists a spectrum of disorders of ejaculation ranging from mild premature to severely retarded or absent ejaculation. Normally, by age 17 or 18 years, 75% of men are able to control their ejaculation. Premature ejaculation is the most common male sexual dysfunction (Sharma *et al.,* 2012). Several surveys among different populations estimate its prevalence at 29%, with a

range between 1% and 75% depending on the population and criteria used to define the condition. The diagnostic criteria for premature ejaculation as follows (Sharma *et al.,* 2012):

(i) Persistent or recurrent ejaculation with minimum sexual stimulation that occurs before, upon, or shortly after penetration and before the person wishes it.

(ii) Marked distress or interpersonal difficulty

(iii) The condition does not arise as a direct effect of substance abuse, i.e., opiate withdrawal (Sharma *et al.,* 2012).

Premature ejaculation and sexual desire disorders were the frequent reported problems in young adult males with adverse familial relationship to attachment figures. Premature ejaculation was also found to be associated with anxiety. Several classifications for premature ejaculation have been reported. In one, premature ejaculation was classified into primary and secondary disorders. Primary premature ejaculation describes persons who, since the beginning of sexual experience, have never been able to control the ejaculatory function, whereas secondary premature ejaculation describes individuals who develop the condition after years of satisfactory sexual activity (Fouad *et al*., 2001). Painful ejaculation has been reported as a side effect of tricyclic antidepressants in at least two patients. Psychogenic post ejaculatory pain syndrome (PEPS) is a rare sexual disorder of male dyspareunia that was first described in 1979 as a persistent and recurrent pain in the genital organs during ejaculation or immediately afterward (Fouad *et al*., 2001). Ejaculatory pain in the testicular region may result from epididymal congestion after vasectomy or from duct obstruction and/or infection, testicular torsion, mass lesion, or prostatitis. In some cases, specific etiological factors other than psychological stress cannot be identified (Courtois *et al*., 1993; Betts *et al*., 1994; Fouad *et al*., 2001).

2.4.4 Disorders of orgasm

Male orgasmic disorder is defined as a persistent or recurrent delay in or absence of orgasm after a normal sexual excitement phase during sexual activity (APA, 1994). The most common causes of orgasmic disorders include:

(i) Drugs like (selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, substance abuse)

(ii) CNS disease: (multiple sclerosis, Parkinson's, Huntington's chorea, lumbar sympathectomy)

(iii) Psychogenic: (performance anxiety, conditioning factors, fear of impregnation, hypoactive sexual desire) (Fouad, 2001)

2.4.5 Failure of detumescence

It is a prolonged erection usually lasting for between 4 h or more. It is painful and always unaccompanied by sexual desire despite the fact that it is often preceded by usual sexual stimuli. The condition is self perpetuating and is characterized by diminished perfusion of the corporeal bodies. When chronically present, corporeal fibrosis and erectile dysfunction occur. At least two classifications of priapism have been described. The first is etiologically based and classifies the condition into primary (idiopathic) and secondary priapism. The second classification is patho physiologically based and depends on measurement of penile blood gases and pressures. It classifies priapism into low-blood flow (ischemic) and high-blood flow (non ischemic) conditions. In the majority of ischemic priapism cases, erection probably starts with a normal or high-blood flow state, particularly in cases induced with intra-penile drug (Courtois *et al*., 1993; Betts *et al*., 1994; Fouad *et al*., 2001).

2.4.6 Management of male sexual dysfunction

As more and more information is gained on the mechanism underlying penile erection, more drugs are being developed to treat erectile dysfunction. The most promising of these synthetic medications are the phosphodiesterase inhibitors which are effective in treating both organic and psychological impotence.

2.4.7 Some drugs used to manage male sexual dysfunction

As information is progressively gained on how the relaxation process takes place in the penis, more drugs are being developed to treat erectile dysfunction. The phosphodiesterase type 5 (PDE5) inhibitors are new drugs which affect local regulation of erectile function by potentiating the effects of nitric oxide (NO). The first developed and consequently the most famous is sildenafil, the active principle of the oral pill Viagra®. This active compound is effective in treating both organic and psychological impotence (Boolell*et al*., 1996).

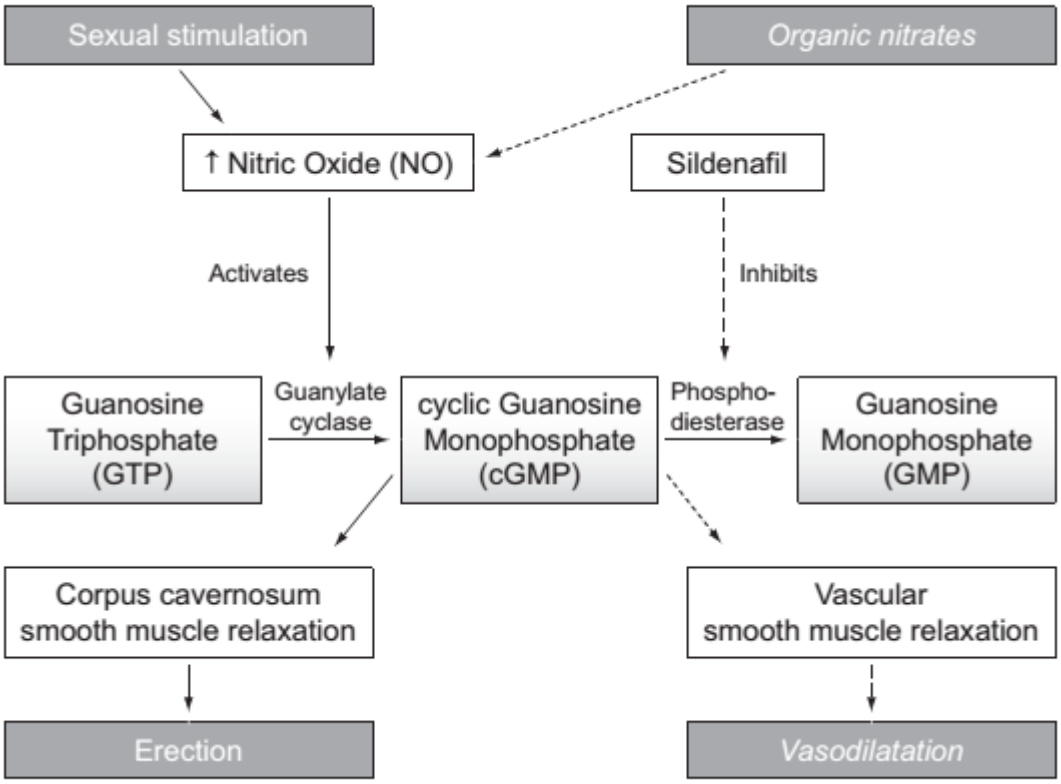


Figure 2.5 Action of sildenafil (Viagra®) in erectile dysfunction (Drewes *et al*., 2003)

2.5 Aphrodisiacs

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces veneral desire and increases pleasure and performance (Yakubu *et al.,* 2007). There are two main types of aphrodisiacs; psychophysiological stimuli (visual, tactile, olfactory and aural) preparations and internal preparations (food, alcoholic drinks and love portion) (Rosen and Ashton, 1993). Aphrodisiac was named after Aphrodite, the Greek goddess of sexual love, beauty and fruitfulness identified in Roman Mythology with the goddess Venus, who was the daughter of Zeus and Dione. However, the Greek word 'aphros' means 'foam' and according to the tradition recounted by Hesoid, Aphrodite arose from the foam generated when the severed genitals of Uranius personification of Heavens were thrown into the sea. Several ancient authorities agreed that she was the wife of the lame blacksmith, Hephaestus (Rosen and Ashton, 1993).

Sexual potency is part of the male ego, and the anxiety and the humiliation that are associated with a declining sexual ability are common to all cultures. That is why the popularity of aphrodisiacs has not shown any sign of decline throughout history. In ancient as in current times, in oriental and western cultures, virility has always been one of the central and most desirous characteristics of the human male (Yakubu *et al.,* 2007). Plants, since ancient times, have been used globally across varied cultures throughout civilizations as a valuable and safe natural source of medicines and as agents of therapeutic, industrial and environmental utilities (Yakubu *et al.,* 2007). The medical historians have recorded plants that could be used as aphrodisiacs.

2.5.1 Some mechanisms involved in aphrodisiac potentials

Aphrodisiacs function through several mechanisms such as:

(i) Some aphrodisiac simply provide a burst of nutritional value improving the immediate health or well being of the consumer and consequently improving sexual performance and libido. This simple improvement in general health can lead to a burst of energy and translate into an increased sexual appetite (Barry *et al.,* 1996). For example, in Chinese tradition, the use of rhinoceros horn as an aphrodisiac could be based on the fact that rhino horn consists of fibrous tissue with large proportions of elements like calcium and phosphorus; beyond the fact that rhino horn resembles an erect penis. Deficiency in these elements could lead to muscle weakness and general fatigue while large doses of these elements could lead to general increased vigour and stamina (Davis, 1985)

(ii) There are those which include: aphrodisiacs that have more specific physiological effects, but not psychologically active. They may affect blood flow; increase duration of sexual activity by numbing the genital area. Other physiologically active drugs used to sustain erection help to limit the influence of sympathetic nervous system. e.g. Sildenafil citrate (viagra) and yohimbine from *Pausinystalia yohimbe* (Taberner, 1985)

(iii) Some other aphrodisiacs are made up of compounds that are psychopharmacological, i.e. they actually cross the blood brain barrier and stimulates some area in sexual arousal. This category includes a wide range of neurotransmitters, hormones, pheromones, and drugs that interfere with the normal function of these molecules (Alok *et al.,* 2013).

2.5.2 Some Medicinal Plants with Aphrodisiac Potentials

A number of medicinal plants have been reportedly used to improve or enhance sexual behaviours in males which include; *Abelmoschus manihot L.*(Malvaceae) which showed pronounced anabolic and spermatogenic effect; *Anacyclus pyrethrum* (Compositae), shown to improve sperm count and fructose levels in seminal vesicles with increased mounting and intromission frequency(Sharma *et al.,* 2012); and *Asparagus racemosus* Willd. (Liliaceae), which improves sexual behaviour by reducing mounting latency, ejaculation latency and intromission latency. Others include *Asteracanta longifolia* (Acanthaceae) (Javeed *et al.,* 2011) and *Blepharis edulis*Linn. (Acanthaceae) (Sharma *et al.,* 2012).

2.6 *Securinega virosa*

Securinega virosa is a commonly used medicinal plant in Africa described as a true "cure all" of which all parts are used as remedies particularly the root and is used for the management of several ailments including male sexual dysfunction (Neuwinger, 1996).

It is a deciduous shrub or a small tree, usually with many erect branches, and grows up to 6 m high but commonly 2 m to 3 m. The branches usually arise from the base and are arranged upwards spirally. The plant can be deciduous in seasonal climates but ever green with regular supply of moisture. It grows in tropical climate. It is commonly spared when clearing land or weeding operations and widely distributed throughout tropical Africa (Dalziel, 1986).

Some common names of the plant are: **English:** Sweet warmwood, White Berry Bush, Snowberry Tree; **Hausa:** *tsuwaawun karee, gussu, tsa, gwiiwar karee;* **Yoruba:** *iranje,* **Igbo:** *njisi nta;* **Kanuri:** *shimshim;* **Fulani:** *carnal cambe, came;* **Arabic-Shuwa:** *dabalab;* and **Idoma:** *okpla* (Neuwinger, 1996).



Plate 2.1: *Securinega virosa* plant in its natural habitat (Snapped by candidate)

- Family: Euphorbiaceae
- Order: Geraniales
- Genus: *Securinega*
- Specie: *virosa*

2.6.1. Distribution

Securinega virosa is widely distributed throughout tropical Africa, Arabian Peninsula, Indian subcontinent, China, Southeast Asia to northern Australia (Dalziel, 1986). It is commonly found in forest edges and associated bush land; generally in higher rainfall areas, bush lands and thicket, extending into dry areas along water courses, sometimes on termite moulds; and

common in disturbed places, from sea level to 2,300 meters (Ruffo and Birnie, 2002). The fruit is edible when mature enough to fall from bush. It is juicy and sweet, with a slightly bitter flavor (Burkil *et al*., 2004).

2.6.2. Ethnomedical uses

Securinega virosa is a widely used plant in traditional medicine. It has been employed in the treatment of many medical conditions. The plant is used for the treatment of epilepsy and mental illness in children (Magaji *et al*., 2007). It is seen as analgesic, astringent, ophthalmic, purgative, sedative and soporific. It is also used alone or in conjunction with other plants, often to provide synergistic effect for liver, bile, kidney and urogenital complaints, diuresis, renal stones, shistosomiasis, rheumatisim, veneral diseases, orchitis, dysmenorrhea, sterility, arthritis e.t.c. (Neuwinger, 1996).The roots boiled in water are also used to treat dysmenorrhea, shistosomiasis, stomach aches, and also given to nursing mothers whose baby is sickly or have still-born. When combined with *Anacardium occidentale*, it is seen as an aphrodisiac and elixir of longevity (Neuwinger,1996). The powdered charcoal is used as a cicatrizant on wounds. The leaves also have some aphrodisiac and laxative effects. They are used to treat fevers, veneral diseases and constipation. It also used in the treatment of epilepsy, and combined with other plants, is used as a tranquilliser in cases of insanity. The leaf is also mashed into a paste with tobacco and applied externally to destroy worms in sores (Burkil *et al*., 2004). The fruit mixed with pulses, is eaten to relieve digestive disorders. Women also take the fruits to promote fertility (Burkil *et al*., 2004).

There are some known toxicities observed from the use of the plant. Alkaloid virosecurinine, isolated from the plant have been reported to be mildly toxic with LD_{50} of 73mg/kg producing

tonic-clonic convulsion with paralysis similar to that observed in strychnine poisoning (Burkil *et al*., 2004).

2.6.3. Ethnobotanical uses

Black dye is obtained from the bark and is used for dye matting. A red dye is also obtained from the fruit and is used as red ink. Twiggs are cut and used as tooth brushes. A gum is obtained from the stems which has been used for sealing envelops, while the pounded leaves are used as insect repellent. The tough vigrate stems are usually used to make beds, fishing stakes, wicker-traps, and part of roof structures e.t.c. They are woven into 'sheves' and also split for use in basketry. The wood is a good fuel and also used to make charcoal.

2.6.4. Previous pharmacological studies

Previous study showed that the crude methanol root bark extract of *Securinega virosa* possesses anticonvulsant and sedative activities (Magaji *et al.,* 2007). In other studies, the plant was observed to have some bioactive constituents with analgesics and anti-inflammatory activities (Magaji *et al*., 2008).

The leaf extracts have very good antioxidant activities which may be due to the presence of phenols and flavonoids in the extracts (Danlami *et al.,* 2013). Methanol extract of *Securinega virosa* leaves possess anti-diabetic properties (Tanko *et al.,* 2008).

The aqueous extract of the roots exhibited hypoglycaemic effect (Moshi *et al*., 2000). Alcoholic leaf extract of the plant have also been reported to have cytotoxic properties on tumour cells (Tatematsu, 1991).

Other studies show that the alcoholic extract of *Securinega virosa* exhibited antibacterial and antifungal activities (Khan *et al*., 1980; Sawhney, 1978).The plant also exhibited significant antimalarial activity against *Plasmodium falciparum, in vitro*, comparable to quinine used as standard drug (Gbeassor *et al*., 1989)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and apparatus

Plastic animal cages, Conical flask, Cotton wool, Desiccators, Distilled water, Glass cylinder, Microscope, Pestle and mortar, Pasteur pipettes, Rat pellets, Stop clock, Sucrose solution, Syringes, Tally counters, Tap water, and Sony video camcorder.

3.1.2 Animals

One hundred and twenty (120) Wistar rats of both sexes with body weight ranging from 200 g-250 g were obtained from Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria.

The animals were kept in a well-ventilated room in the Animal House under ambient laboratory temperature and light. They were fed on standard laboratory animal feeds (Vital feed pellets) and water *ad libitum*. All experiments performed on the laboratory animals in this study was based on the "Principles of laboratory animal care" (NIH Publication, 1996) and Ahmadu Bello University Research Policy, (2010).

3.1.3. Drugs, Chemicals and Extract

Anesthetic ether (Narsons Pharma), Ethinyly oestradiol (Lyronal tablets, Organon Pharma) Progesterone (Dubaget tablets, Glenmark Pharma), and Methanol root bark extract of *Securinega virosa.*

3.2 Plant Materials

3.2.1 Collection and Identification of Plant Material

The whole plant was collected from Area BZ, Ahmadu Bello University, Main Campus Samaru Kaduna State on 28th April, 2015 and was taken to the Herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria Kaduna State, Nigeria for identification. The plant was then identified and authenticated by Mall. Musa and Mall.Umar Galla in the herbarium unit. A voucher specimen (No. 918) was obtained and deposited for future reference

3.2.2 Preparation of Extract

The root of the plant was cleansed and the bark removed. The root bark of the plant was air dried under shade at room temperature until constant weight was obtained. It was then reduced into coarse powder using pestle and mortar. The powder root bark (652g) was extracted with unsealed aqueous methanol using cold maceration with occasional shaking after every 48 hours for 7 days. The solvent was allowed to evaporate to dryness at room temperature. The extract was then stored in a desiccator until needed for the study. Fresh solutions of the extract were prepared in distilled water for each study.

3.3 Phytochemical study

3.3.1 Test for carbohydrates

3.3.1.1 Molisch test

Half gram (0.5g) of the extract was dissolved in water in a test tube. Three drops of 1% α-naphthol in 80% ethanol was added followed by 5 drops of concentrated sulphuric acid without mixing. Appearance of a purple ring at the interphase as a result of the reaction between α-napthol and furfural and 5-hydroxymethylfurfural aldehydes produced by the dehydration of saccharides indicated the presence of carbohydrates (Silva *et al.,* 1998).

3.3.2 Test for Flavonoids

3.3.2.1 Shinoda reduction test (cyaniding test)

Three milliliter (3ml) of the alcoholic solution of the extract was evaporated to dryness. The residue was dissolved in 2ml of 50% methanol with heat. Then 4 drops of concentrated hydrochloric acid was added followed by some chips of magnesium metal. Immediate appearance of orange colour denoted presence of flavones; red-crimpson colour denoted flavonols and pink-magenta colour denoted flavonones (Mahran *et al.,* 1980).

3.3.2.2 Concentrated sulphuric acid

About 0.2 mL of methanolic extract was dissolved in concentrated sulphuric acid. The appearance of a deep yellow solution indicated the presence of flavones and flavonols.

3.3.3 Test for Cardiac glycosides

3.3.3.1 Keller kiliani test

One milliliter (1 ml) of the methanolic extract was diluted with 20 ml of water and 1ml of strong lead sub acetate solution was added to precipitate the pigments, which was filtered off. The filtrate obtained was shaken with equal volume of choloroform and allowed to separate into two layers in a small separating funnel. The chloroform layer was removed and evaporated to dryness on a water bath. The residue was dissolved in 3ml of ferric chloride in glacial acetic acid, which was left for 1min and then transferred into a dry test tube. Six drops of concentrated sulphuric acid was added by the walls of the test tube. On standing, the appearance of a brown colour at the interface (due to deoxy sugars) and a pale green colour at the upper layer (due to the steroidal nucleus) indicate the presence of cardenolides (Brain and Turner, 1975)

3.3.4 Test for Cyanogenetic glycosides

3.3.4.1 Guinard test

Half a gram (0.5g) of the powdered drug was mixed with 0.1ml of water in a test tube. A prepared damp sodium picrate paper was suspended at the mouth of the test- tube by means of a cork. The tube was placed in a water bath and allowed for an hour. A brick-red colour on the picrate paper indicated the presence of cyanogenetic glycoside (Silva *et al.*, 1998)

3.3.5 Test for Saponins

3.3.5.1 Froth test

Two milliliter (2ml) of the methanolic extract was diluted with twice its volume of water and shaken in a test tube for 15 minutes. The occurrence of foam column, of about 1cm in height persisting for about 15 minutes indicated the presence of saponins (Silva *et al.,* 1998).

3.3.6 Test for Tannin

3.3.6.1 Ferric chloride test

One milliliter (1ml) of the methanolic extract was diluted with 2 ml of water. Then a dilute solution of ferric chloride (3drops) was added. The occurrence of black-blue indicate the presence of tannins (Evans, 1996).

3.3.6.2 Lead Subacetate test

One milliliter 1ml of the methanolic extract was diluted with 2 ml of water. The dilute solution was mixed with lead subacetate solution. The appearance of a light brown precipitate indicate the presence of tannins (Evans, 1996).

3.3.7 Test for Steroids and Terpenoids

3.3.7.1 Liebermann-Burchard's test

Half a gram (0.5g) of the powdered plant material was extracted with 5 ml of methanol and then filtered. The filtrate was evaporated to dryness on water bath. The residue was shaken with chloroform and filtered into clean and dry test tube. 2ml of acetic acid anhydride was added to the filtrate and shaken. 1mL of concentrated sulphuric acid was then added carefully down the

side of the test tube to form a lower layer. The appearance of a brownish-red ring at the zone of contact of the two liquids and the upper layer turning green denoted the presence of sterols and terpenes (Cuilei, 1990)

3.3.8 Test for Alkaloids

One milliliter (1ml) of the methanolic extract of *Securinega virosa* was treated with strong ammonia to make alkaline (pH 8) and then extracted twice with 10 ml portions of chloroforms. The chloroform extracts was combined and concentrated *in vacuo* to about 5ml. The concentrate was then tested using the following reagents: Dragendoff, Meyer, Picric acid and Wagner. The presence of precipitate in the entire reagent tested indicated the presence of alkaloids (Evans, 1989), that is:

(i) Light brown to brownish precipitate –Wagner's reagent.

(ii) White or creamy white precipitate-Mayer's reagent

(iii) Orange or orange-yellow precipitate-Dragendoff's reagent

(iv) Yellowish precipitate-Picric acid reagent.

3.3.9 Test for Anthraquinones and their derivatives

Two grams (2.0g) of the plant material was extracted with 10ml of benzene and filtered. 5mL of 10% ammonia solution was added to the filtrate and shaken. Absence of a pink, red or violet colour in the ammoniacal (lower layer) phase indicated the absence of free hydroxyl anthraquinone. For the anthraquinone glycosides, 2.0 ng of the powdered plant material was boiled with 10 mL of dilute sulphuric acid and filtered, while hot. The filtrate was shaken with 5 ml benzene. The benzene layer was separated, and then 3 ml of 10% ammonia solution was

added and the mixture shaken. Absence of a pink, red or violet colouration in the lower layer indicated the absence of anthraquinone glycosides (Sofowora 1993).

3.4 Acute Toxicity Study

3.4.1 Acute toxicity study (Estimation of mean lethal dose, LD_{50})

Oral median lethal dose of the methanol root bark extract of *Securinega virosa* was estimated in rats using the method of Lorke (1983). The method was divided into two phases. In the initial phase, 3 groups each containing three rats were treated with the methanolic root bark extract of the plant at widely different doses of 10, 100 and 1000 mg/kg body weight, orally and observed for signs of toxicity and death for 24 hours. In the second phase, 3 groups each containing one rat was administered with 4 more specific doses of the extract. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

3.5 Evaluation of Aphrodisiac potentials of methanolic root bark extract of *Securinega virosa*

3.5.1. Mating performance test and mounting behaviour test (Test for libido)

The test was carried out by the methods of Dewsbury and Davis Jr (1970) and Szechtman *et al.,* (1981) modified by Amin *et al.* (1996). Healthy and sexually active male Wistar rats (200-250g) that showed brisk sexual activity were selected for the study. Four groups (1, 2, 3, and 4) of six males each were used. They were kept singly in separate cages during the experiment. Group 1 represented the control group, and they each received 1ml/kg of distilled water orally daily for 21 days. Animals in Groups 2, 3, and 4 received methanol root bark extract of *Securinega virosa*

orally at three different graded doses (31.25mg/kg, 62.50mg/kg and 125mg/kg) respectively, daily for 21 days at 18:00 hour. The male animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment to make them familiar with the environment.

The female animals were artificially brought into oestrus (heat) by the method previously described by Szechtman *et al.* (1981) since female rats allow mating only during the oestrus phase. Oral suspension of ethinyl oestradiol (Lynoral tablets, Organon Pharma) at dose of 100 µ/animal, 48 hours prior to the pairing plus progesterone at a dose of 1mg/animal, 6 hour prior to the experiment was injected subcutaneously (Amin *et al.*, 1996; Yakubu, 2006).

The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control or experimental animals. Receptive females adopted a copulation stance and beat their ears. The most receptive females were selected for the study.

Treatment:

The experiment was carried out on the 21st day after commencement of the treatment of the male animals at 20:00 hour in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals at ratio of 1 female to 1 male. The observation for the mating behaviour commenced immediately and continued for first two mating series. The test was terminated when the male failed to evince sexual interest. When the female did not show receptivity she was replaced with another. The occurrence of events and phases of mating were recorded with video cassette (Sony Camcorder) as soon as they appeared. Their disappearance was also recorded. Later, the frequencies and sexual behaviour phases were determined from the video replay:

(i). Number of mounts before ejaculation or Mounting Frequency (MF)

(ii). Number of intromission before ejaculation or Intromission Frequency (IF)

(iii). Time from the introduction of female into the cage of the male up to the first mount or Mounting Latency (ML)

(iv). Time from the introduction of the female up to the first intromission by the male or Intromission Latency(IL)

(v). Time from the first intromission of a series up to the ejaculation or Ejaculatory Latency (EL)

(vi). Time from ejaculation and the first intromission of the following series or Post-ejaculatory interval.

Using the above parameters of sexual behaviour, the following parameters of male sexual behavior were calculated:

$$i.\ \%\ Mounted = \frac{Number\ Mounted}{NumberPaired} x100$$

$$ii.\ \%\ Intromitted = \frac{Number\ of\ rats\ that\ intromitted}{Number\ Paired}\ x100$$

$$iii. Intromission\ ratio = \frac{Number\ of\ Intromissions}{Numberof\ mounts + Number\ of\ Intromissions}$$

$$iv. \%\ Ejaculated = \frac{Number\ of\ rats\ that\ ejaculated}{Number\ Paired} x100$$

$$v. Copulatory\ Efficiency = \frac{Number\ of\ Intromissions}{Number\ of\ Mounts}\ x100$$

$vi. Intercopulatory\ Efficiency = Average\ between\ intromissions$

$vii. \%\ Index\ of\ Libido = \frac{Number\ Mated}{Number\ Paired} x100$ (Yakubu *et al*., 2006; Ratnasooriya *et al*., 2000)

3.5.2 Effect on sexual and vital organ weight

Twelve hours after the mating behaviour analysis (Day 22), at 8 am, all the control and experimental groups of male rats were weighed, completely anaesthetized with anesthetic ether (Narsons Pharma), and sacrificed by cervical decapacitation. Subsequently the testis, seminal vesicles, epididymis, vas-deferens, penis and prostate glands alongside vital organs like liver, kidney, adrenal gland, and spleen were carefully removed and weighed using a weighing scale (Salter, HoMedics Group Ltd. Production no. IB-1066-1011-03) (Thakur and Dixit, 2006; Amini and Kamkar, 2005).

3.5.3 Histopathological examination

The method of Hematoxylin and Eosin staining technique was used. Briefly, the technique involved hydrating the tissue sections in descending grades of alcohol from 100%, 95%, 90% and 70% (Laporta *et al*., 2013). Each of these steps lasted for three (3) minutes and the tissues were washed in running tap water and stained with haematoxylin for twenty five (25) minutes, washed with water and then differentiated in acid alcohol. The tissues were counter stained with eosin and blued in Scott water. The tissues were hydrated in ascending grades of alcohol and cleared in xylene for three (3) changes in five (5) minutes each. A thin glass-covered slip was placed on the covering-mounting medium, and the underlying tissue sections were allowed to dry. The slides were then observed later using the Leitz, DIALUX research microscope at x200 and photomicrographs taken in bright field at x 200 magnification.

3.6 Evaluation of effects of methanolic root extract of *Securinega virosa* on male sex hormones and sperm parameters

A total of 60 male rats were randomly divided into four groups (A, B, C, D) of 15 rats each after being allowed to acclimatize for 2 weeks. Rats in groups B, C and D were administered with the graded doses of methanol root bark extract of *Securinega virosa* once daily at 24hour interval at different graded doses (31.25 mg/kg, 62.5mg/kg, and 125 mg/kg) respectively for 21 days. The extract of methanol root bark extract of *Securinega virosa* was administered orally using plastic syringes attached to metal oropharyngeal cannula. The rats in group A received 1ml/kg of distilled water each for the same number of days. They served as the control. All the rats were given free access to their rat pellets and tap water before and after their daily doses of the extract/ distilled water. Five rats each from groups A, B, C, D were sacrificed 24h after 7, 14 and 21 daily doses.

3.6.1 Effects of *Securinega virosa* on male sex hormones

3.6.1.1 Animal Sacrifice and blood collection

The procedure described by Yakubu *et al.* (2005) was employed. The 60 male rats were weighed individually before the sacrifice. They were then anaesthetized in a jar containing cotton wool saturated with diethyl ether. Under anaesthesia, the neck areas were quickly cleared of fur and skin to expose the jugular veins. The jugular veins was slightly displaced from the neck region to prevent contamination of the blood with interstitial fluid, and then cut with a sharp sterile blade. The rats were held downwards and allowed to bleed into clean, dry centrifuge tubes, which was left at room temperature for an hour. The tubes were then centrifuged for 15mins using laboratory Centrifuge (bench Centrifuge (Hawksley RegNo: 891481 Ser. No. 07.4.26). The sera

obtained were aspirated with Pasteur pipettes into clean, dry, sample bottles and then frozen overnight. The rats were then quickly dissected and the testes excised from the animals. The testes were cleaned properly of superficial fatty layer, weighed and then transferred into 0.25M sucrose solution. Then they were blotted with tissue paper, cut very thinly with sterile scalpel blade and homogenized in ice-cold 0.25 M sucrose solution (1:5, w/v). The homogenates were further centrifuged for 15 min and supernatant obtained, which was aspirated with Pasteur pipette into sample bottle, stored overnight at 40^0C before being used for the biochemical assays.

3.6.1.2 Testosterone determination

Testosterone concentration was assayed according to the method described in the Manufacturer's Protocol Version (2001) using Seroenzyme I Serono (Diagnostics Freiburg, Germany). The assay procedure consisted of three main stages of reaction of antibody with serum testosterone and testosterone enzyme label, magnetic solid phase separation step and colour development step based on a direct assay of a limited (competitive) type following the general antibody-antigen reaction based on the enzyme linked immunosorbent assay (ELISA) principle as described by Tietz (1995). The serum testosterone concentration was obtained by correlating the absorbance of the test sample at 550 nm with the corresponding absorbance on the standard curve.

3.6.1.3 Determination of Luteinizing Hormone Concentration

The concentration of Luteinizing Hormone (LH) in the serum was determined based on a solid phase enzyme –linked immunoabsorbent assay as described by Uotila *et al*. (1981). The assay system utilized a mouse monoclonal anti-LH antibody for solid phase (microliter wells)

immobilization and another monoclonal anti-LH antibody in the antibody enzyme (horseradish peroxidase)-conjugated solution. Sixty microliters of standard, test and control were pipetted into appropriate wells after which 100µl of enzyme conjugated reagent was taken and then added into each of the well, and then mixed thoroughly for 30secs and then incubated at room temperature for 45 min. The incubation mixture was removed by flickering plate contents into a waste container. The microliter was rinsed five times with distilled water after which the wells were shaken sharply onto absorbent paper to remove all residual water droplets. Hundred microliters of tetramethyl benzidine (TMB) reagent was pipette into each well and gently mixed for 5 secs. This was then incubated in the dark for 20 mins. 100 µl of the stop solution was added to each well to stop the reaction. This was then observed for 30 secs for colour development from blue to yellow. The absorbance at 450 nm was read with microtitre plate reader within 15 min. The absorbance of the test sample was correlated with that of the standard curve to give the concentration of the LH in the serum.

3.6.1.4 Determination of Follicle Stimulating (FSH) Hormone Concentration

The Follicle Stimulating Hormone (FSH) concentration was determined based on the principle of solid phase enzyme-linked immunoabsorbent assay similar to that described by Uotiola *et al.* (1981). The assay system utilized a mouse monoclonal anti-FSH antibody for solid phase (microliter wells) immobilization and another monoclonal anti-FSH antibody in the antibody enzyme (horseradish peroxidase)-conjugated solution. Sixty microliters of standard, test and control were pipetted into appropriate wells after which 100 µl of enzyme conjugated reagent was taken and then added into each of the well, and then mixed thoroughly for 30 s and then incubated at room temperature for 45 min. The incubation mixture was removed by flickering plate contents into a waste container. The microliter was rinsed five times with distilled water

after which the wells were shaken sharply onto absorbent paper to remove all residual water droplets. Hundred microliters of TMB reagent was pipette into each well and gently mixed for 5 secs. This was then incubated in the dark for 20 mins. 100 µl of the stop solution was added to each well to stop the reaction. This was then observed for 30 secs for colour development from blue to yellow. The absorbance at 450 nm was read with microtitre plate reader within 15min. The absorbance of the test sample was correlated with that of the standard curve to give the concentration of the FSH in the serum.

3.6.2 Evaluation of sperm motility and morphology

The animals were completely anaesthetized with anaesthetic ether sacrificed by cervical decapacitation at the end of 7, 14 and 21 days administration. The testis and epididymis were excised and weighed. The right causda epididymis was used for sperm count, while the left one was used for sperm motility and morphology analysis.

The epididymis was excised, three cuts was made in the mid-to- distal region of cauda epididymis with a scapel blade in a pre-warmed Petri dish containing 0.2 ml calcium and magnesium free Hank's solution at 37^0C for 15 minutes, prior to determination of the sperm motility. The suspension was stirred, then a drop was placed on a warmed microscope slide, and a 22 mm x 22 mm cover slip was added. At least 10 microscopic fields were observed at 400 x magnification using a standard optical microscope, and the percentage of motile sperm was determined (Linder *et al.,* 1995; Wyrobeck *et al.,* 1975; Farag *et al.,* 2000; Farag *et al.,* 2007).

To determine the sperm morphology, the cover slip was removed and the spermatozoa suspension was allowed to air dry. The sample was stained with 1% eosin Y/5% nigrosin and examined at 400 x magnification by the light microscope for sperm morphologic abnormalities. Three hundred spermatozoa from different fields were examined for each sample (Linder *et al.,*

1995; Wyrobeck *et al.,* 1975; Farag *et al.,* 2000; Farag *et al.,* 2007), and classified for morphological abnormalities according to the description by Wyrobeck *et al.* (1975).

3.6.3 Assessment of sperm parameters

The right testis and epididymis were frozen immediately after weighing until evaluation. After thawing at room temperature, the whole epididymis and the testicular parenchyma was homogenized in 0.5 ml of a solution of 0.9% NaCl containing 0.01ml Triton X-100. Ten strokes of a manual homogenizer were used for each sample. The testis and epididymis homogenates were diluted with 1.5 ml of the same solution and spermatozoa and spermatid were counted at 400x magnifications by light microscopy in Neubauer hemocytometer. The average of three counts per sample was done (Freund *et al.,* 1964; Farag *et al.,* 2000; Farag *et al.,* 2007; Lobet *et al.,* 1995).

3.7 Statistical Analysis

Data obtained from hormonal analysis and sperm analysis were analysed by repeated measure ANOVA followed by Bonferroni post hoc test and expressed as mean ± S.E.M. Whereas data obtained from sexual behavioural studies, vital organ and reproductive organ weight were analysed by one way ANOVA followed by Tukey's post hoc test for multiple comparisons using SPSS version 20 Values of $p< 0.05$ were considered statistically significant.

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CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening revealed the presence of tannins, alkaloid, flavonoids, cardiac glycosides, resins, steroids (terpenoids) and carbohydrate (Table 4.1)

Table 4.1 Phytochemical constituents present in the methanolic root bark extract of *Securinega virosa*

Constituents	Remarks
Tannins	Present
Saponins	present
Flavonoids	present
Alkaloids	present
Cardiac glycosides	present
Cyanogenic glycosides	present
Resins	present
Steroids/Terpenoids	present
Carbohydrates	present
Anthraquinone	Absent

4.2 Acute Toxicity Studies

The oral acute toxicity of Methanolic root bark extract of *Securinega virosa* was found to be greater than 5000 mg/kg body weight (Table 4.2)

Phase I

GROUPS	**LD_{50} Value (mg/kg)**
Group I	> 10
Group II	> 100
Group III	> 1000

Phase II

GROUPS	**LD_{50} Value (mg/kg)**
Group I	> 1200
Group II	> 1600
Group III	> 2900

Phase III

Table 4.2 Median lethal dose (LD_{50}) value of the methanolic root back extract of *Securinega virosa*

Route of Administration	**LD_{50} Value (mg/kg)**
Oral	> 5000 mg/kg

4.3 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Mating Behaviour in Adult Male Wistar Rats Treated for Twenty-one (21) days

There was no statistically significant ($P > 0.05$) difference in mounting frequency (MF), mounting latency (ML), and Intromission latency (IL) in all the treated groups when compared to the control (Table 4.3). There was however a statistically significant ($P < 0.05$) increase in intromission latency of SV 125 mg/kg treated group; (6.00±1.15 vs 3.25±0.25) compared to control. There was also a statistically significant increase ($P < 0.05$) in ejaculation frequency in SV 125 mg/kg treated group when compared to control; (3.00 ± 0.71 vs 0.75±0.48).

Table 4.3: Effect of methanolic root bark extract of *Securinega virosa* (SV) on mating behaviour of adult male wistar rats treated for twenty one (21) days

Treatment Groups	Mount frequency (MF)	Mount Latency (Sec)	Intromission frequency (IF)	Intromission Latency (IL) (Sec)	Ejaculation Frequency (EF)	Ejaculation Latency (Sec)
Control	3.25 ± 0.25	228.50 ± 31.16	3.25 ± 0.25	244.25 ±54.68	0.75 ± 0.48	143.50 ± 48.28
SV 31.25 mg/kg	3.50 ± 0.29	229.00 ± 30.83	3.00 ± 0.41[d]	228.50 ± 50.42	1.00 ± 0.58	94.50 ± 54.49[a]
SV 62.50 mg/kg	4.50 ± 0.50	210.50 ±32.63	2.75 ± 0.25[d]	138.25 ± 22.36	0.75 ± 0.25[d]	125.50 ± 44.58
SV 125 mg/kg	5.50 ± 1.50	133.50 ± 15.52	6.00 ± 1.15[a,b,c]	207.25 ± 19.23	3.00 ± 0.71[a,c]	184.50 ± 20.98

Superscripts a,b,c,d indicate significant difference ($P < 0.05$) when compared to groups; Control, SV 31.25 mg/kg, 62.50 mg/kg and 125 mg/kg

4.4 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Computed Mating Behaviour in Adult Male Wistar Rats Treated for 21 days

Following three weeks treatment with SV, there was 100% recorded index of libido in all the treated groups (Table 4.4). Percentage mounting was 100% in SV 62.50 and 125 mg/kg treated groups as compared to the 75% in the SV 31.25 mg/kg and the control. There was also a 100% Intromission in all the SV treated groups compared to the control, which recorded a 75% intromission rate. The intromission ratio was increased uniformly (0.25) in all the SV treated groups compared to the control (0.24). Copulatory efficiency was 100% in all the treated groups compared to the control. From the result, there was a statistically significant ($P< 0.05$) decrease in the intercopulatory interval of SV 62.50 mg/kg and 125 mg.kg treated groups, when compared with the control; (233.68 ± 7.86 vs 523.209 ± 9.48 and 154.04 ±11.40 vs 523.20 ±9.48) respectively

Table 4.4: Effect of methanolic root bark extract of *Securinega virosa* (SV) on computed sexual behaviour of adult male wistar

Treatment Groups	% index of libido	% Mounted	% Intromitted	Intromission ratio	% Ejaculated	% Copulatory Efficiency	Intercopulatory interval (Sec)
Control	75	100	75	0.24	50	75	523.20 ± 9.48
SV 31.25 mg/kg	75	100	100	0.25	50	100	407.18 ± 10.02
SV 62.50 mg/kg	100	100	100	0.25	75	100	233.68 ± 7.68*
SV 125 mg/kg	100	100	100	0.25	100	100	154.04 ± 11.40*

Asterisk (*) indicate significant difference (P< 0.05) when compared to groups; Control, SV 31.25 mg/kg, 62.50 mg/kg and 125 mg/kg

4.5 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Sexual Orientation Activity in Adult Male Wistar Rats Treated for 21 days

Table 4.5 shows a statistically significant increase (P< 0.05) in the licking activity of SV 125 mg/kg treated group when compared to the control (29.25 ± 2.14 vs 12.25 ± 0.85). There was also a statistically significant (*P*< 0.05) increase in the anogenital smelling activity of the male towards the female in the SV 125 mg/kg treated group when compared to the control; (17.00 ± 3.32 vs 7.50 ± 0.50). A statistically significant (*P*< 0.05) increase in the non genital grooming activity was observed in SV (62.50 and 125) mg/kg treated groups when compared to the control (11.25 ± 1.03 vs 7.00 ± 0.41 and 12.00±1.22 vs 7.00 ± 0.41) respectively. However, frequency of genital grooming activity was significantly higher (*P*< 0.05) in all the SV treated groups compared to the control: SV 31.25 mg/kg (5.50 ± 0.96 vs 2.50 ± 0.50), SV 62.50 mg/kg (15.50 ± 1.71 vs 2.50 ± 0.50) and SV 125 mg/kg (15.00 ± 1.29 vs 2.50±0.50).

Table 4.5: Effect of methanolic root bark extract of *Securinega virosa* (SV) on sexual orientation activity of adult male wistar rats treated for twenty one (21) days

Treatment Groups	Doses (mg/kg)	Mean Activity score towards Female		Mean Activity score towards self		Mean activity score towards Environment		
		Licking	Anogenital Smelling	Non-Genital grooming	Genital grooming	Exploration	Rearing	Climbing
Control		12.25±0.85	7.50±0.50	7.00±0.41	2.50±0.50	21.23±1.50	24.12±1.38	1.01±1.18
SV 31.25 mg/kg		14.25±0.75	7.50±0.96	7.75±0.25	5.50±0.96$^{\#}$	29.80±2.00	12.00±1.12$^{\#}$	Nil
SV 62.50 mg/kg		16.50±2.22	10.50±1.71	11.25±1.03$^{\#}$	15.50±1.71$^{\#}$	34.34±2.02$^{\#}$	19.09±2.23$^{\#}$	Nil
SV 125 mg/kg		29.25±2.14$^{\#}$	17.00±3.32$^{\#}$	12.00±1.22$^{\#}$	15.00±1.29$^{\#}$	37.32±1.98$^{\#}$	21.56±1.08$^{\#}$	3.43±1.60$^{\#}$

Superscripts a,b,c,d indicate significant difference (P< 0.05) when compared to groups; Control, SV31.25, 62.50 and 125 mg/kg

4.6 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Serum Testosterone level in Adult Male Wistar Rats Treated for 21 days.

In figure 4.6: there was a significant difference in serum testosterone concentration across the extract treated groups after the first week of administration when compared to the control; SV 62.50 mg/kg (2.38±0.06 vs 1.75±0.18) and SV 125 mg/kg (2.32±0.08 vs 1.75±0.18). Although there were differences observed following the second week of administration, it was however significant ($P< 0.05$) only in the SV 62.50 mg/kg treated group when compared to the control; (2.33±0.07). Following third week of administration, there was a significant increase ($P< 0.05$) in the SV 62.50 mg/kg treated group compared to the control; (2.91±0.08 vs 2.63±0.03).

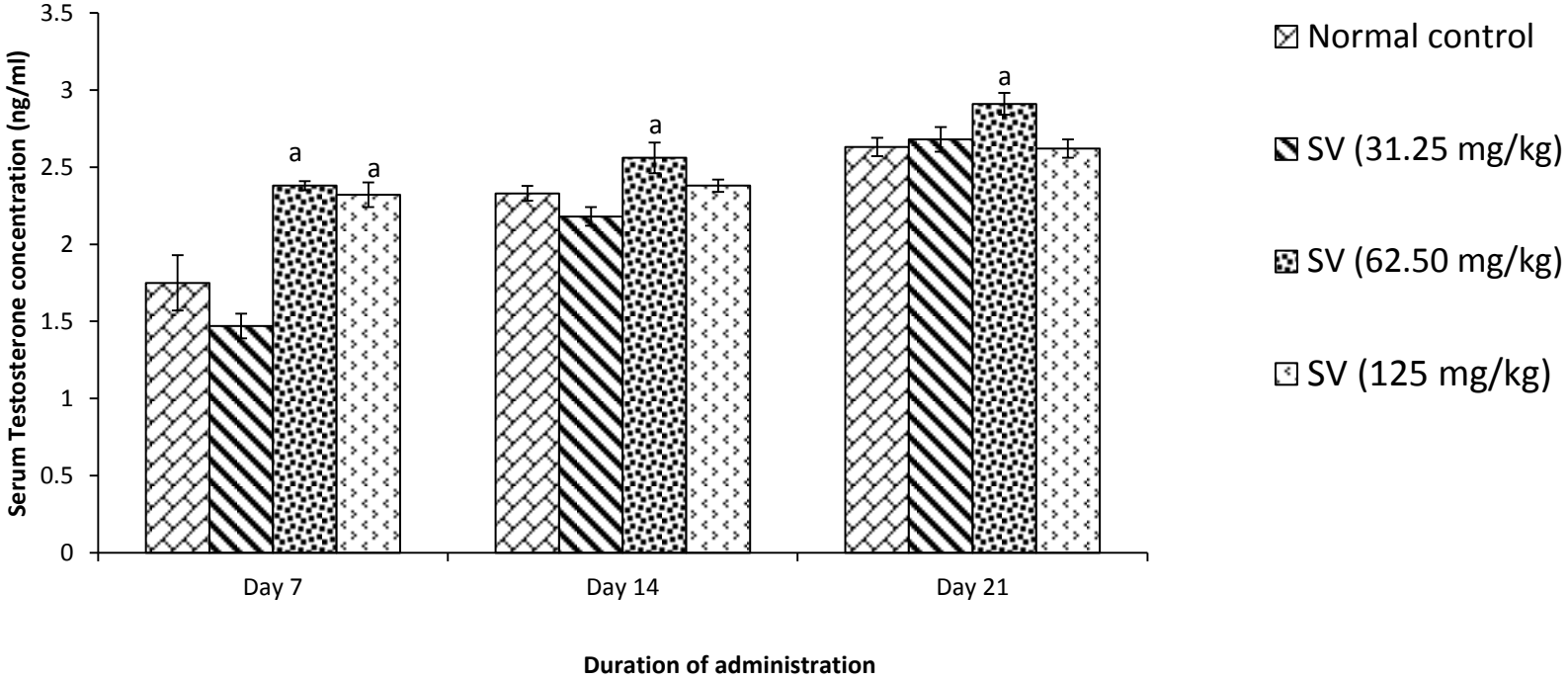


Figure 4.6: Serum testosterone levels in adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa* (31.25, 62.50 and 125 mg/kg), SEM with superscript (a) indicate statistically significant difference ($P< 0.05$) compared to Normal control.

4.7 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Serum Luteinizing Hormone (LH) Level in Adult Male Wistar Rats Treated for 21 days.

In figure 4.7: following first week of administration, the serum LH showed a significant increase ($P< 0.05$) in the SV 62.50 mg/kg treated group compared to control; (0.07±0.01 vs 0.06±0.01). At second week of administration, although there were differences between the treated groups compared to control, however, none was statistically significant. Serum LH level following third week of administration was significantly higher ($P< 0.05$) in SV 62.50 mg/kg treated group when compared to control (0.12± 0.012 vs 0.09±0.01)

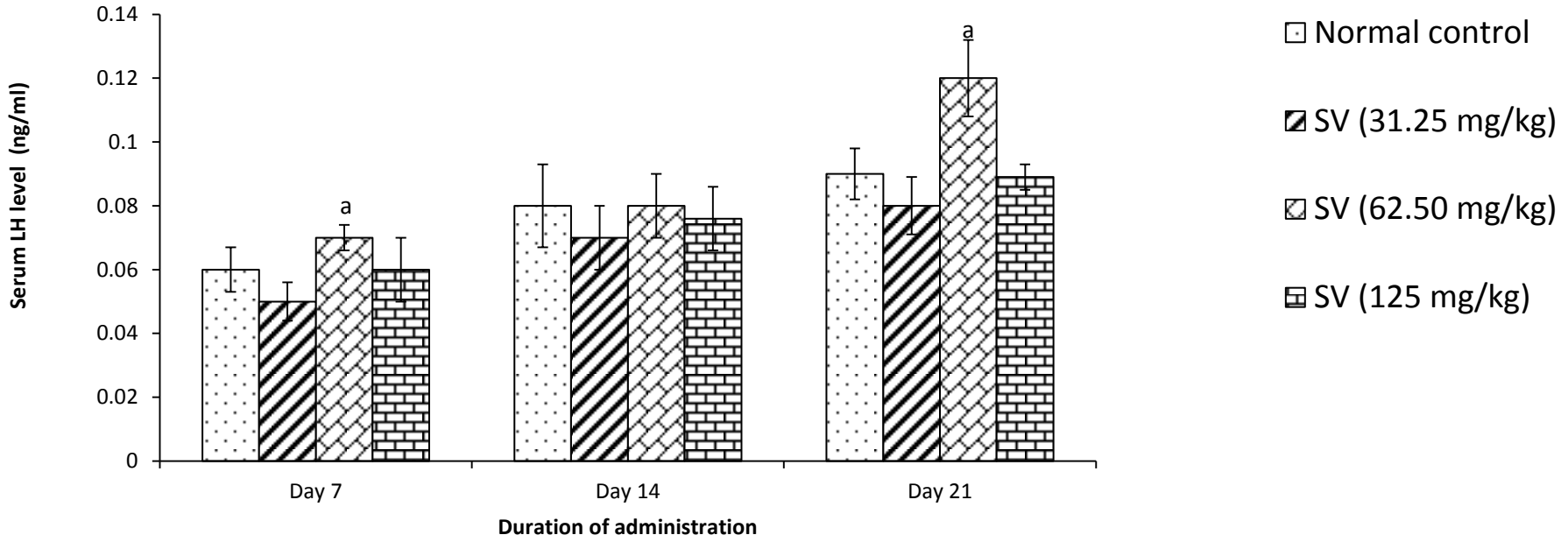


Figure 4.7: Serum LH levels in adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa* (31.25, 62.50 and 125 mg/kg), SEM with superscript (a) indicate statistically significant difference (P< 0.05) compared to Normal control.

4.8 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Serum levels of Follicle Stimulating hormone in Adult Male Wistar Rats Treated for 21 days.

In figure 4.8, methanol root bark extract of *S. virosa* induced a significant ($P< 0.05$) increase in the serum level of FSH compared with the control only in the third week of administration; (0.16±0.02 vs 0.07±0.01). None was statistically significant compared to control following first and second week of administration ($P> 0.05$).

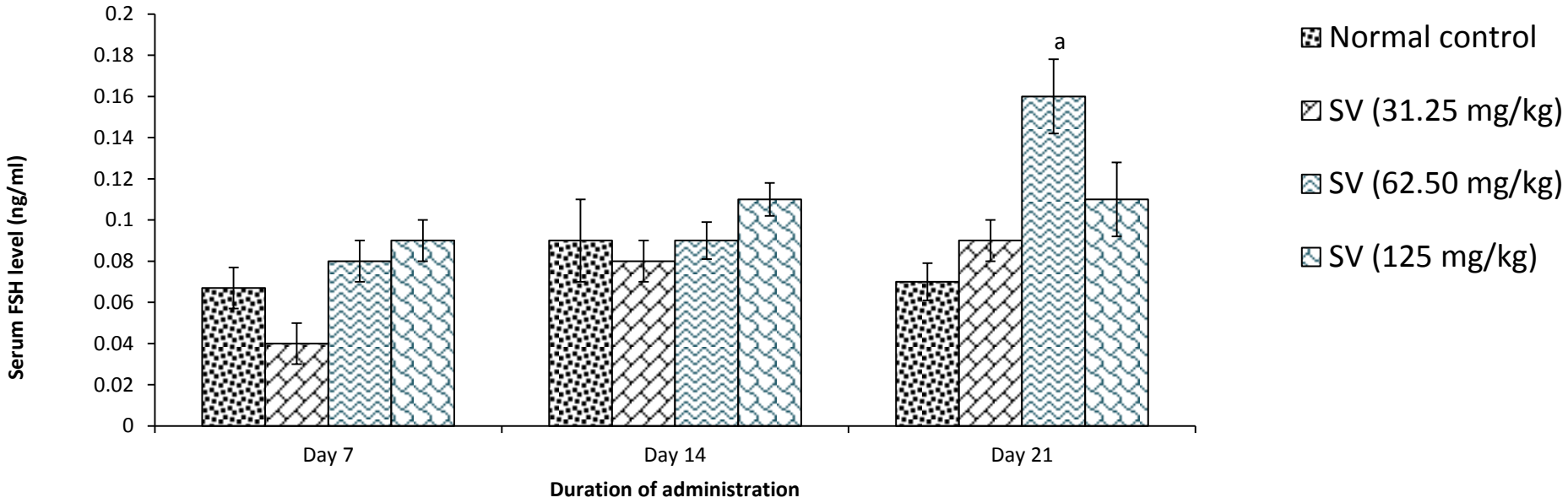


Figure 4.8: Serum FSH levels in adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa* (31.25, 62.50 and 125 mg/kg), SEM with superscript (a) indicate statistically significant difference (P< 0.05) compared to Normal control.

4.9 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Sperm Motility in Adult Male Wistar Rats Treated for 21 days.

Figure 4.9 shows the result of sperm motility in adult male wistar rats treated with SV for 21 days. Following treatment for one week treatment there was a significant increase ($P< 0.05$) in sperm motility in all the SV treated groups when compared to control; SV 31.25 mg/kg (64.07 ± 0.25 vs 62.99 ± 0.18), SV 62.50 mg/kg (62.17 ± 0.11 vs 62.99 ± 0.18) and SV 125 mg/kg (65.80 ± 0.01 vs 62.99 ± 0.18).

Following two weeks of treatment, there was also a significant increase in sperm motility in all the treated groups when compared to control; SV 31.25 mg/kg (63.98 ± 0.29 vs 62.96 ± 0.08), SV 62.50 mg/kg (65.43 ± 0.02 vs 62.96±0.08) and SV 125 mg/kg (66.18±0.23 vs 62.96±0.08).

Similarly, at third week of treatment, there was a significant ($P< 0.05$) increase in sperm motility in all the treated groups when compared to the control; SV 31.25 mg/kg (70.52 ± 0.37 vs 67.41 ± 0.31), SV 62.50 mg/kg (71.48 ± 0.21 vs 67.41 ± 031) and SV 125 mg/kg (71.50 ± 0.08 vs 67.41 ± 0.31). The results following the third week of treatment showed increased ($P< 0.05$) activity of the extract on sperm motility relative to the first and second week.

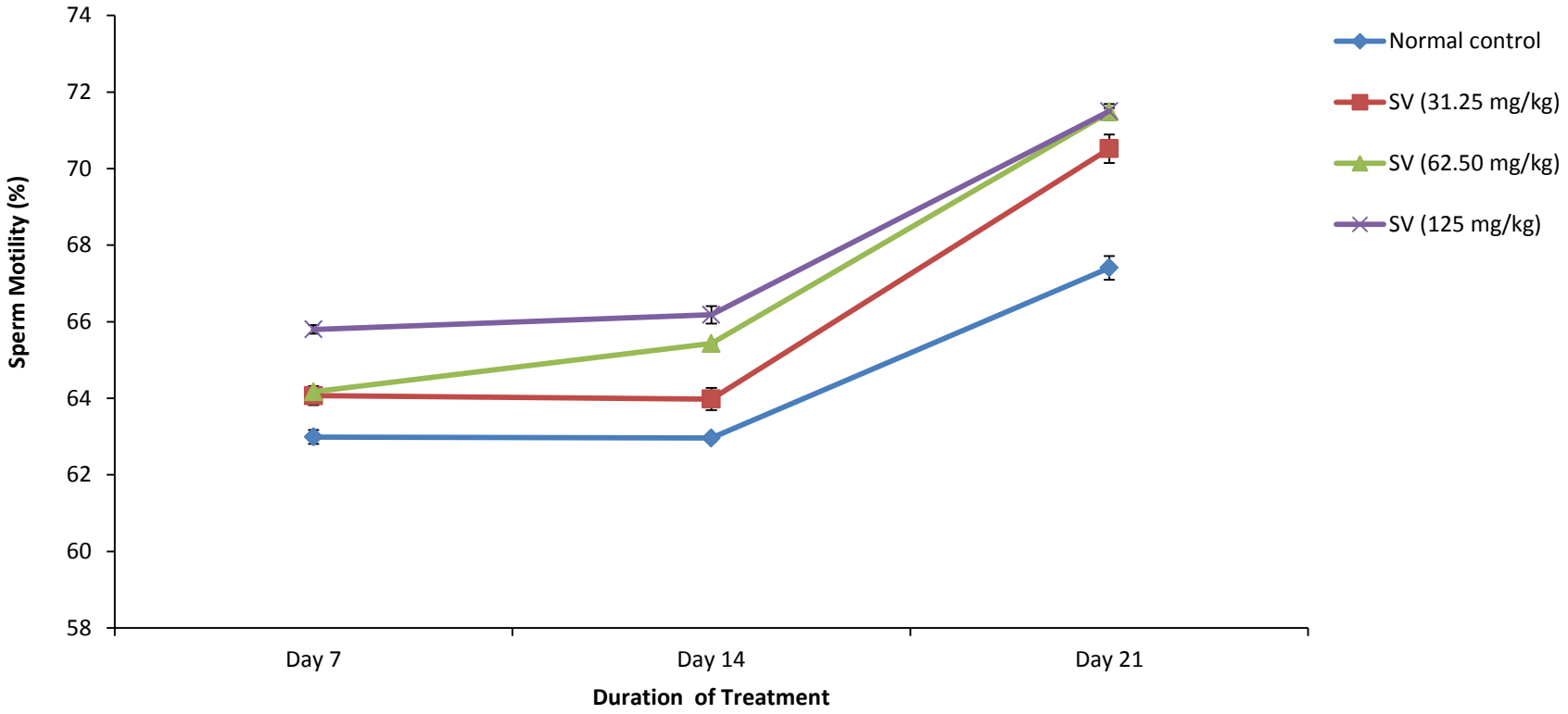


Figure 4.9: Sperm motility profile of adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa*

4.10 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Sperm Count in Adult Male Wistar Rats Treated for 21 days

Figure 4.10 shows the sperm cont profile of *S. virosa* treated and control rats. At one week treatment, there was a significant ($P< 0.05$) increase in sperm count in all SV treated groups when compared to the control; SV 31.25 mg/kg (88.49±0.37 vs 87.25±0.19), SV 62.50 mg/kg (89.63±0.07 vs 87.25±0.19) and SV 125 mg/kg (90.34±0.07 vs 87.25±0.19). There was also a significant ($P< 0.05$) difference between the treated groups when compared to each other. Following one week of treatment, the SV (125 mg/kg) showed the highest activity of the extract on sperm count relative to the other two SV treated groups.

Following two (2) weeks of treatment, there was also a statistically significant ($P< 0.05$) increase in sperm count in all the SV treated groups compared to the control with the highest activity observed in the SV 125 mg.kg treated groups; SV 31.25 mg/kg (89.10±0.22 vs 87.05±0.20), SV 62.50 mg/kg (89.48±0.09 vs 87.05±0.20) and SV 125 mg/kg (89.71±0.02 vs 87.05±0.20).

Sperm count following three weeks of treatment was significantly ($P< 0.05$) increased in all the treated groups when compared to the control; SV 31.25 mg/kg (91.50±0.08 vs 89.21±0.33), SV 62.50 mg/kg (91.83±0.11 vs 89.21±0.33) and SV 125 mg/kg (91.23±0.22 vs 89.21±0.33). The result of the sperm count also showed considerable increase in all the SV treated groups following the third week of treatment relative to that of the first and second week of treatment.

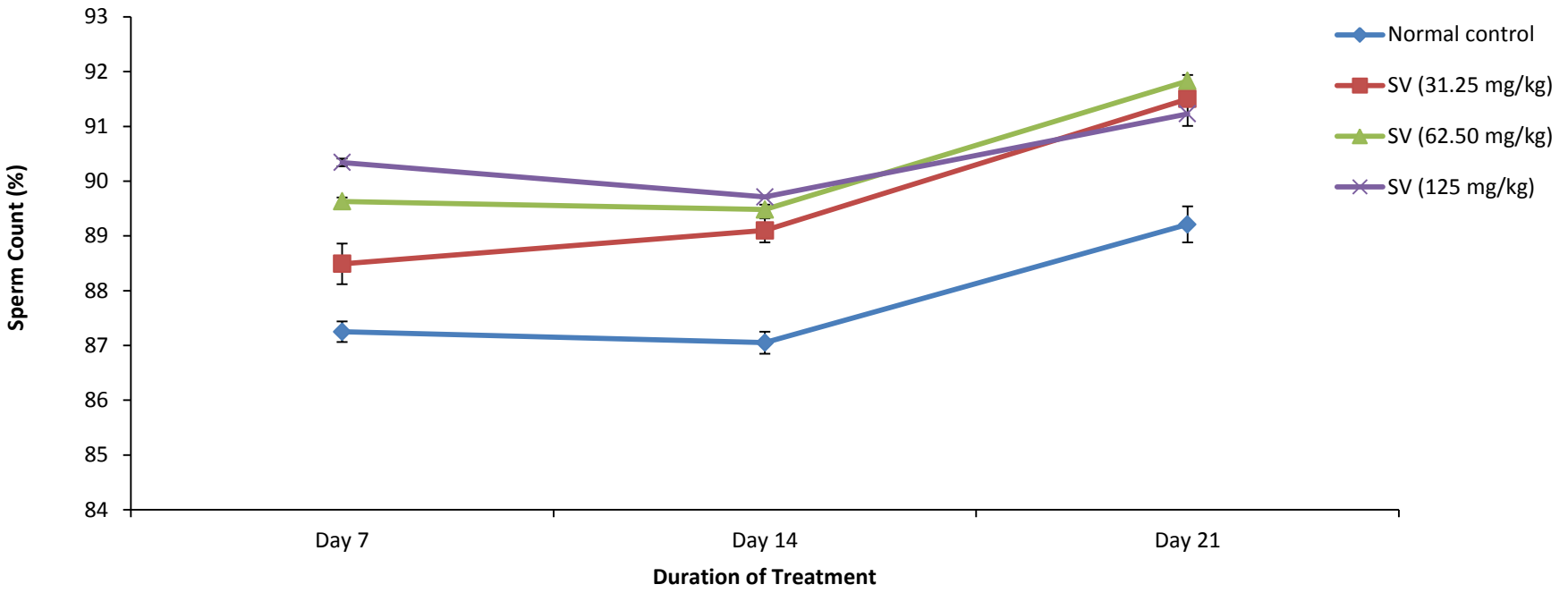


Figure 4.10: Sperm count profile of adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa*

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4.11 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Sperm Morphology in Adult Male Wistar Rats Treated for 21 days

Figure 4.11 shows the profile of sperm morphology in *S. virosa* treated and control groups for 21 days. Following one week treatment, there was a significant ($P< 0.05$) increase in sperm morphology (normal) in all SV treated groups when compared to the control; SV 31.25 mg/kg (97.99 ± 0.23 vs 94.09 ± 0.16), SV 62.50 mg/kg (98.59 ± 0.09 vs 94.09 ± 0.16) and SV 125 mg/kg (98.19 ± 0.02 vs 94.09 ± 0.16). The highest activity was observed in the SV (62.50 mg/kg) treated group. There was however no statistical difference observed in the treated groups when compared to each other ($P> 0.05$).

Sperm morphology was also significantly ($P< 0.05$) increased in all the SV treated groups compared to control, following two weeks treatment; SV 31.25 mg/kg (98.49 ± 0.05 vs 93.90 ± 0.11), SV 62.50 mg/kg (98.55 ± 0.02 vs 93.90 ± 0.11) and SV 125 mg/kg (99.03 ± 0.11 vs 93.90±0.11). The highest activity was observed in the SV 125 mg/kg treated group relative to the other treated groups, and this difference was statistically significant ($P< 0.05$).

Following three weeks treatment with SV, normal sperm morphology was also significantly higher in all the treated groups compared to the control (96.72 ± 0.12); SV 31.25 mg/kg (98.69 ± 0.17 vs 96.72 ± 0.12), SV 62.50 mg/kg (98.41 ± 0.16 vs 96.72 ± 0.12) and SV 125 mg/kg (98.25 ± 0.25 vs 96.72 ± 0.12).

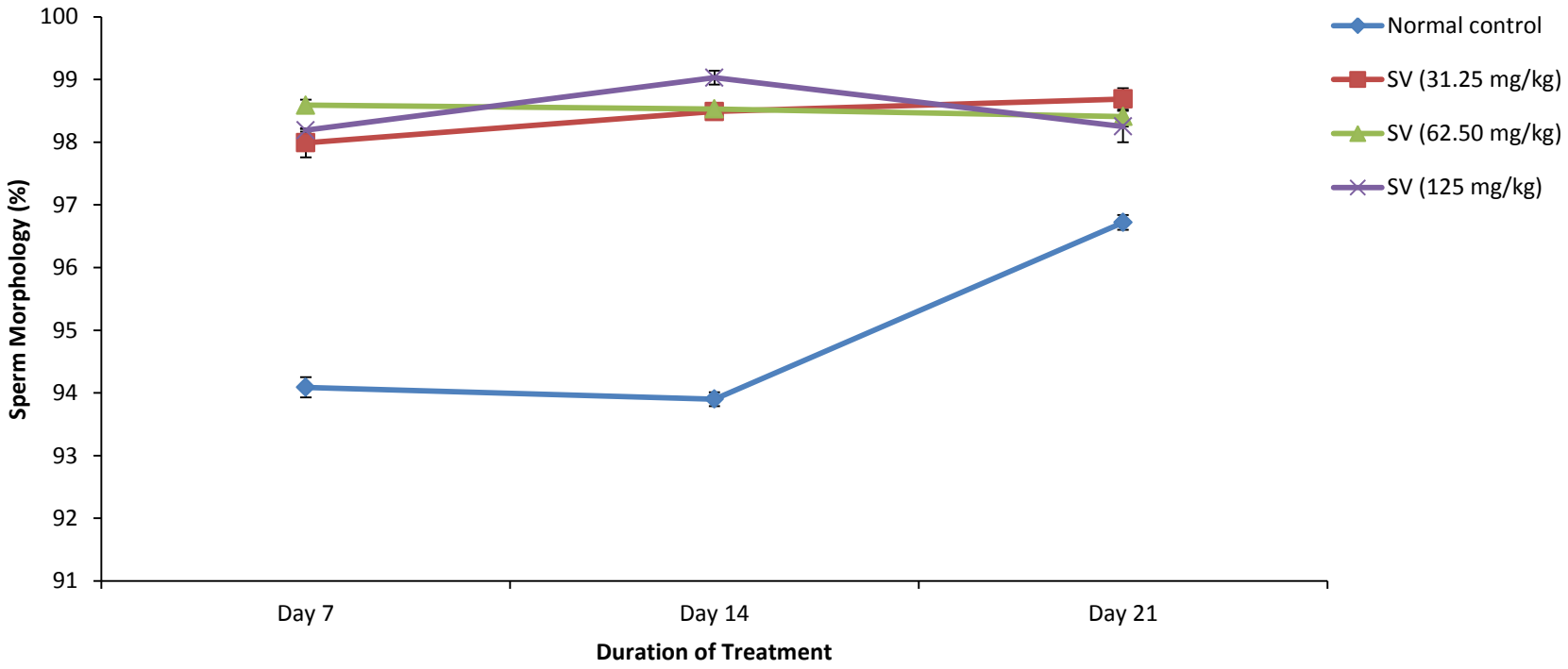


Figure 4.11: Profile of sperm morphology of adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa*.

4.12 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Sperm Viability in Adult Male Wistar Rats Treated for 21 days

Figure 4.12 shows changes in sperm viability in *S. virosa* treated and control rats. The methanolic root bark extract of *S. virosa* significant (P< 0.05) increased sperm viability after the 1st week, in SV 62.50 mg/kg and 125 mg/kg treated groups when compared to the control; SV 62.50 mg/kg (70.98 ± 0.24 vs 69.99 ± 0.18) and SV 125 mg/kg (71.64 ± 0.02 vs 69.99 ± 0.18). There was a significant (*P*< 0.05) increase in sperm viability in the 2nd week of treatment in the SV (62.50 and 125) mg/kg treated groups compared to the control; SV 62.50 mg/kg (70.78 ± 0.28 vs 70.00 ± 0.14) and SV 125 mg/kg (71.29 ± 0.18 vs 70.00 ± 0.14).

The extract of SV significantly (*P*< 0.05) increased sperm viability in all treated groups compared to control; SV 31.25 mg/kg (75.05 ± 0.17 vs 70.77 ± 0.13), SV 62.50 mg/kg (74.01 ± 0.26 vs 70.77 ± 0.13) and SV 125 mg/kg (74.63 ± 0.26 vs 70.77 ±0.13). The highest activity of the extract on sperm viability was observed in the SV (125 mg/kg) treated group relative to the other treated groups.

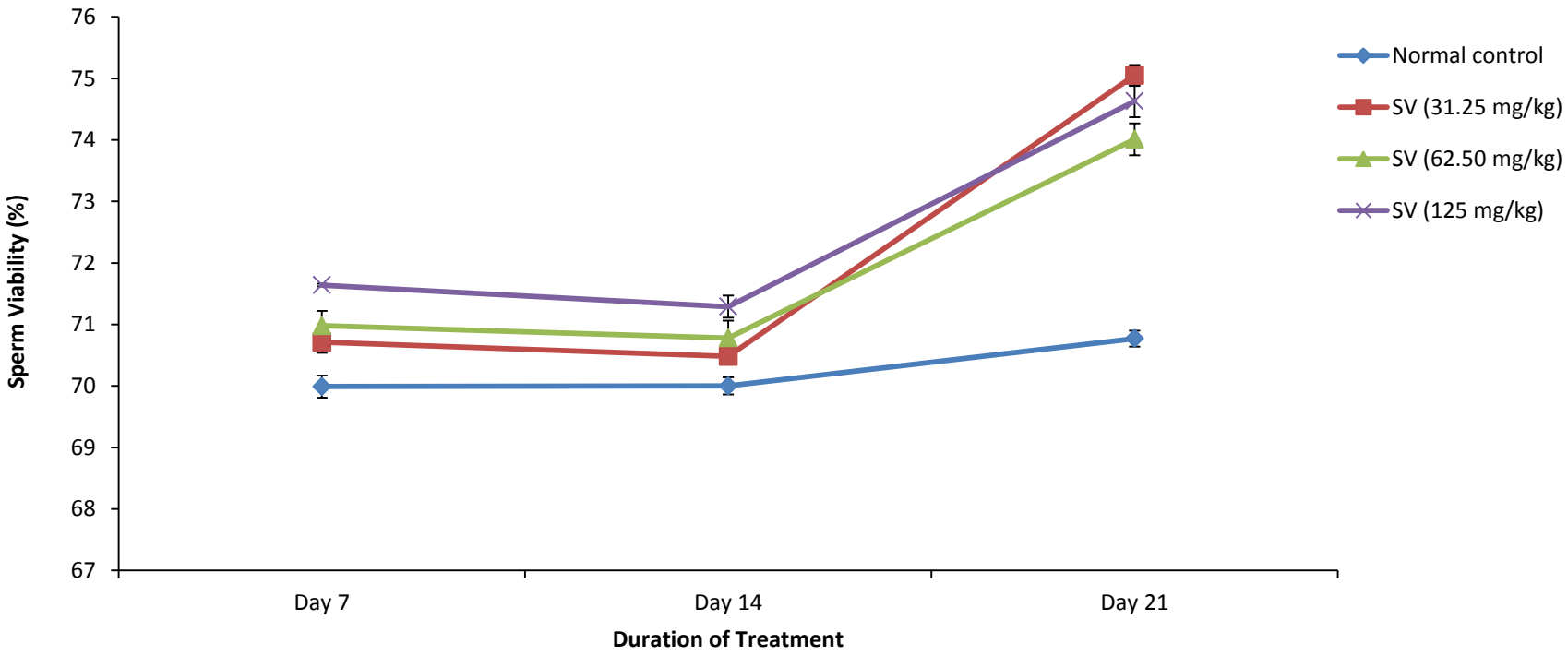


Figure 4.12: Profile of sperm Viability in adult male wistar rats treated with *Securinega Virosa* for twenty one (21 days). Normal control group (normal saline 1 ml/kg), SV= *Securinega Virosa*

4.13 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Weight of Vital organs of Adult Male Wistar Rats Treated for Twenty-one (21) days.

Table 4.13 shows changes in the organ weight in *S. virosa* treated and control rats. The result shows the weight distribution of the vital organs at the end of treatment. Following three weeks of treatment, there was a significant decrease ($P< 0.05$) in body weight of all the SV treated groups when compared to the control (237.60 ± 1.25); SV 31.25 mg/kg (219.80 ± 1.59 vs 237.60 ± 1.25), SV 62.50 mg/kg (218.40 ± 0.87 vs 237.60 ± 1.25); and SV 125 mg/kg (212.40 ± 1.97 vs 237.60 ± 1.25). There was however no statistically significant change in the weight of the liver, kidney, adrenal gland and spleen when compared to the controls.

Table 4.13: Vital organ weight of adult male wistar rats treated with methanolic root bark extract of *Securinega virosa* for twenty one (21) days

Treatment Groups	Body weight (g)	Liver weight (g)	Kidney weight (g)	Adrenal weight (g)	Spleen weight (g)
Control	237.60± 1.25^{bcd}	7.03± 0.21	1.29± 0.08	0.04± 0.00	0.52± 0.02
SV 31.20 mg/kg	219.50±1.59^{ad}	7.12±0.13	1.31±0.08	0.03±0.00	0.51±0.00
SV 62.50 mg/kg	218.40±0.87^{ad}	7.41±0.51	1.37±0.08	0.03±0.00	0.61±0.03
SV 125 mg/kg	212.40±1.97^{abc}	7.43±0.42	1.36±0.11	0.04±0.00	0.68±0.08

Superscripts a,b,c,d indicate significant difference (P< 0.05) when compared to groups; Control, SV31.25, 62.50 and 125 mg/kg

4.14 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Weight of Reproductive Organs of Adult Male Wistar Rats Treated for Twenty-one (21) days

Table 4.4 shows changes in reproductive organ weights of *S. virosa* treated and control rats. There was statistical significant ($P< 0.05$) increase in testicular weight in all the SV treated groups when compared to control; SV 31.25 mg/kg (1.64 ± 0.01 vs 1.45 ± 0.00), SV 62.50 mg/kg (1.69 ± 0.01 vs 1.45 ± 0.00), and SV 125 mg/k (1.89 ± 0.02 vs 1.45 ± 0.00). However, there was only a significant ($P< 0.05$) increase in the epididymal weight of the SV 125 mg/kg treated group, following three weeks of treatment when compared to control (0.66 ± 0.03 vs 0.55±0.03. Although there was a decrease observed in the SV 31.25 mg/kg treated group, this difference was however not statistically significant ($P> 0.05$) compared to the control. The highest activity of SV on vas deferens weight was observed in the SV 125 mg/kg treated group following three weeks of treatment relative to all the groups across the weeks. Following three weeks of treatment, there was no statistically significant difference ($P> 0.05$) observed in testicular weight in all the treated groups when compared to the control.

Table 4.14: Effect of methanolic root bark extract of *Securinega virosa* (SV) on reproductive organs weights of adult male wistar rats treated for twenty one (21) days

Treatment Groups	Testicular weight (g)	Epididymal weight (g)	Vas deferens weight (g)	Penile weight (g)
Control	1.46±0.00^{bcd}	0.55±0.03^{d}	0.48±0.00^{bcd}	0.99±0.23
SV 31.20 mg/kg	1.64±0.01^{ad}	0.51±0.03^{d}	0.55±0.00^{acd}	1.10±0.25
SV 62.50 mg/kg	1.69±0.01^{ad}	0.62±0.03	0.60±0.00^{abd}	1.69±0.01
SV 125 mg/kg	1.89±0.02^{abc}	0.66±0.03^{ab}	0.67±0.01^{abc}	1.21±0.25

Superscripts a,b,c,d indicate significant difference ($P< 0.05$) when compared to groups; Control, SV31.25, 62.50 and 125 mg/kg

4.15 Effect of Methanolic Root Bark Extract of *Securinega virosa* (SV) on Testicular Histology in Adult Male Wistar Rats Treated for Twenty-one (21) days

The Photomicrographs showing testicular histology following administration of *Securinega virosa* at varying doses of 31.25 mg/kg, 62.50 mg/kg and 125 mg/kg is shown in plates I-IV. The control shows normal seminiferous tubules containing spermatogenic cells with orderly maturation of mature spermatozoa. The testicular tissues from animals treated with 31.25 and 62.50 mg/kg also showed no sign of testicular injury; instead, they show normal seminiferous tubules containing spermatogenic cells with slight indication of cellular proliferation compared to the control. In the 2^{nd} weak of treatment there is evidence of normal seminiferous tubules with a markedly increased concentration of mature spermatozoa relative to the control.

Section of photomicrograph from testicular tissue of rats treated with normal saline (plate I), 31.25 mg/kg (Plate II), 62.50 mg/kg (plate III) and 125 mg/kg (plate IV) methanol root bark extract of *Securinrga virosa* (H and E stain, x250)

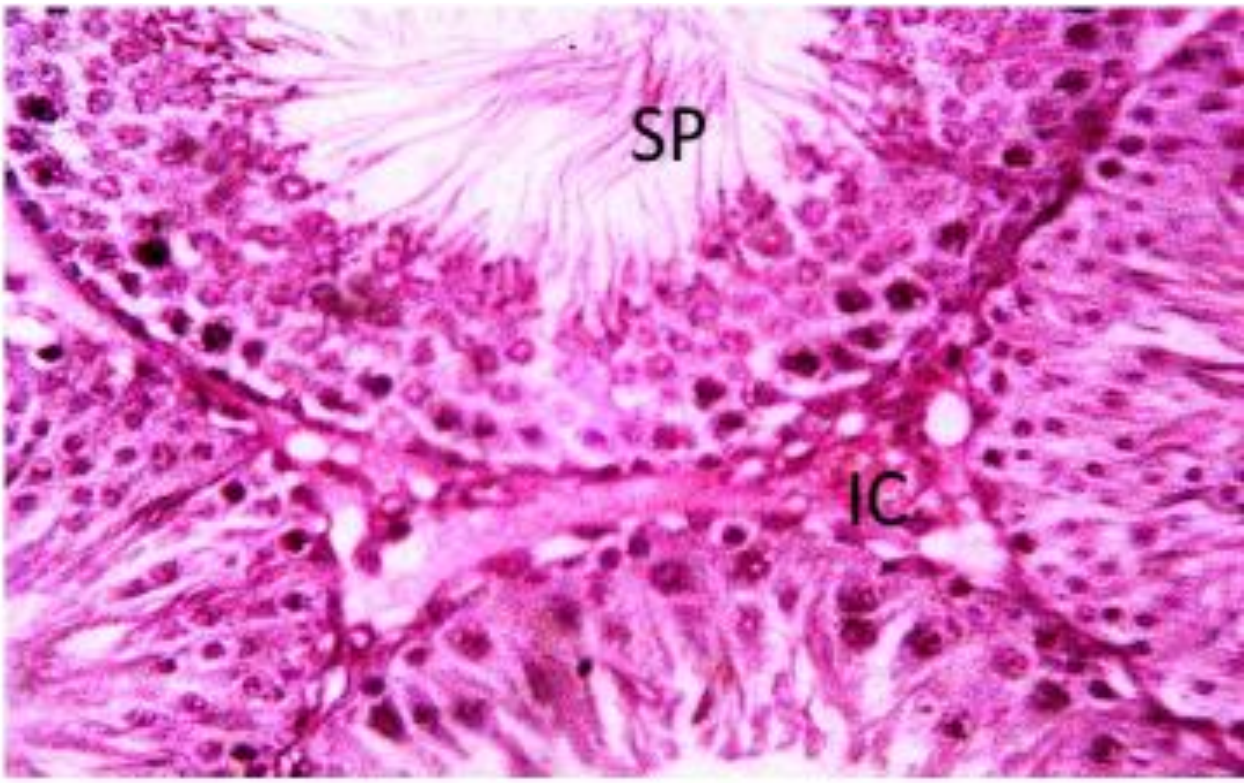


Plate I: Photomicrograph of seminiferous tubule in control rats showing normal spermatids (SP) and interstitial cells (IC) (x 250 H&E)

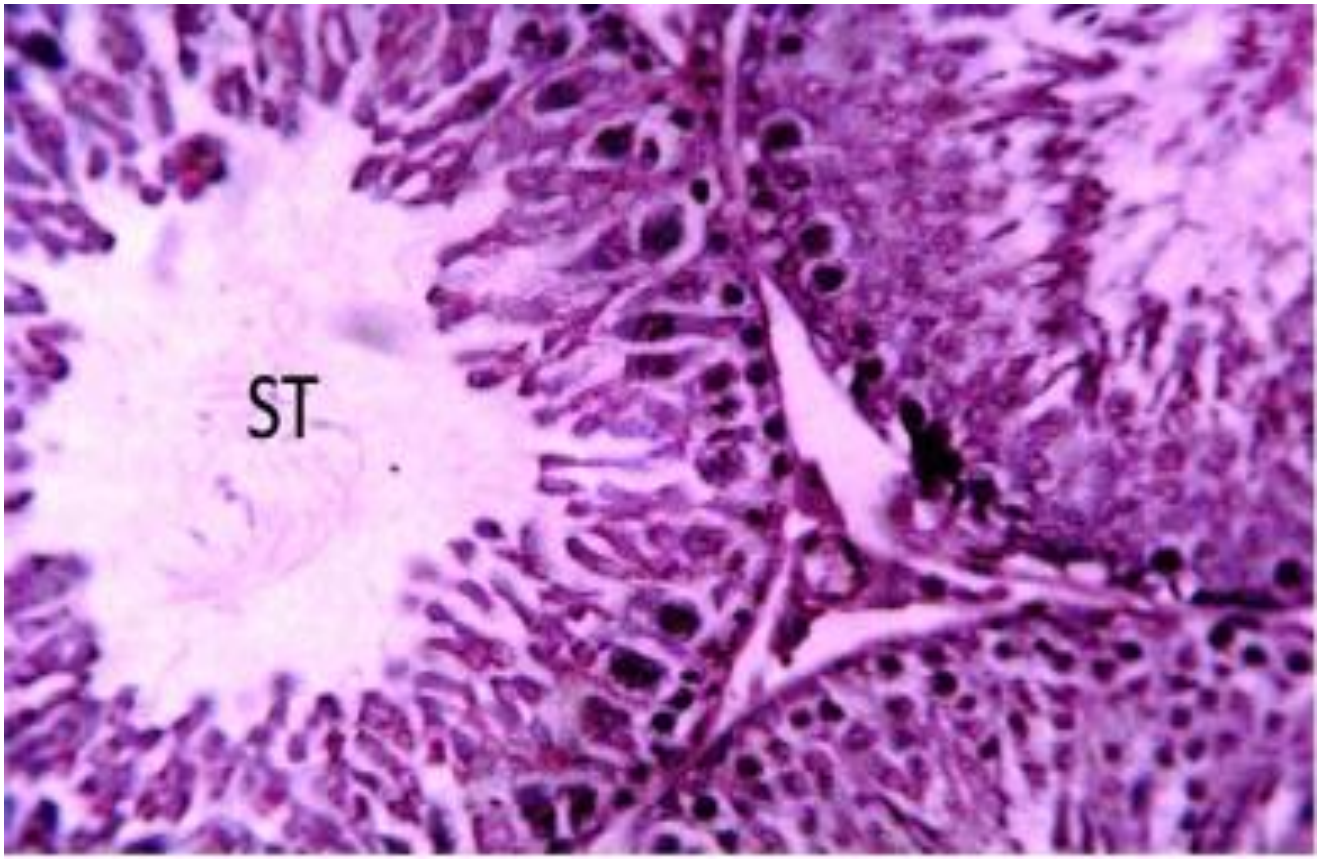


Plate II. Photomicrograph of seminiferous tubule in SV 31.25 mg/kg rats showing normal seminiferous tubule (x 250 H&E)

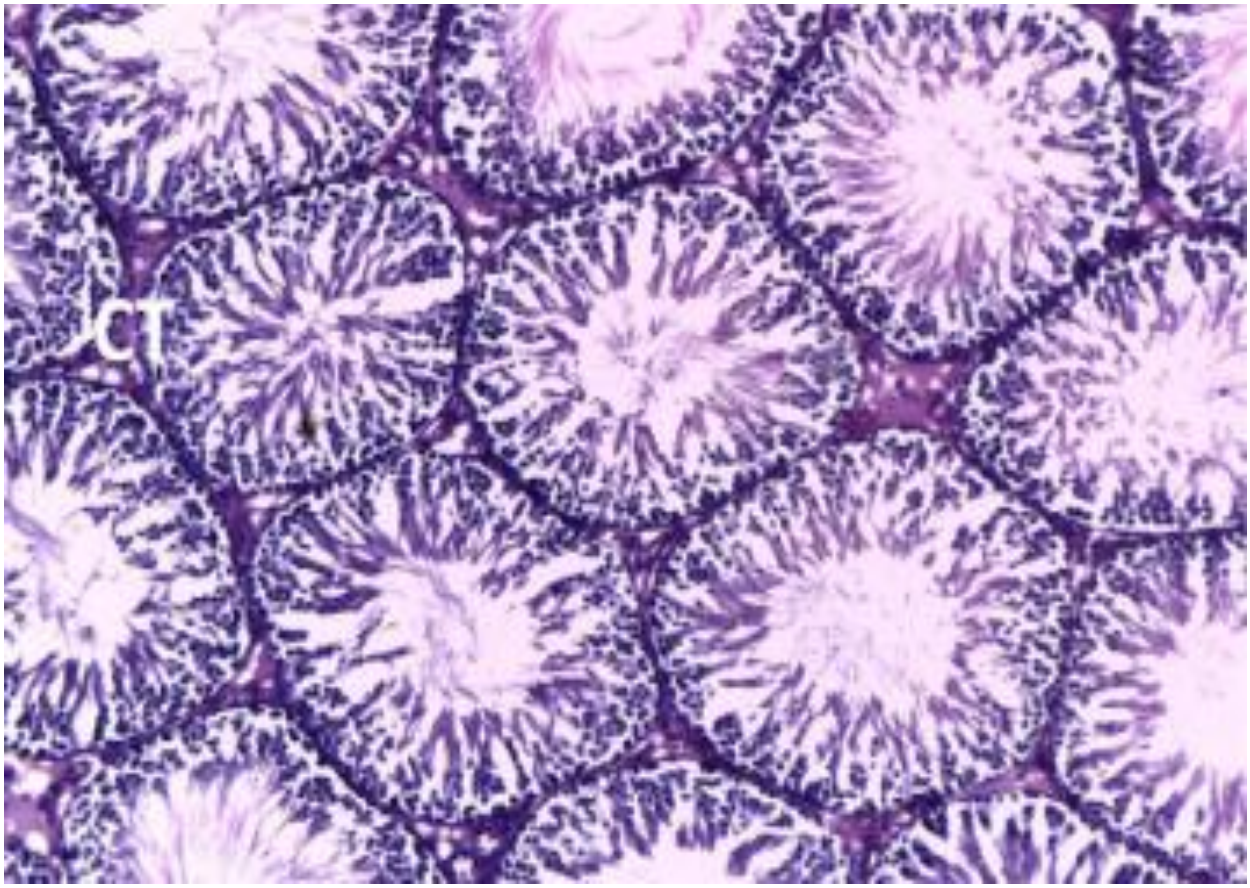


Plate III: Photomicrograph of seminiferous tubule in SV 62.50 mg/kg rats showing normal connective tissues (x 250 H&E)

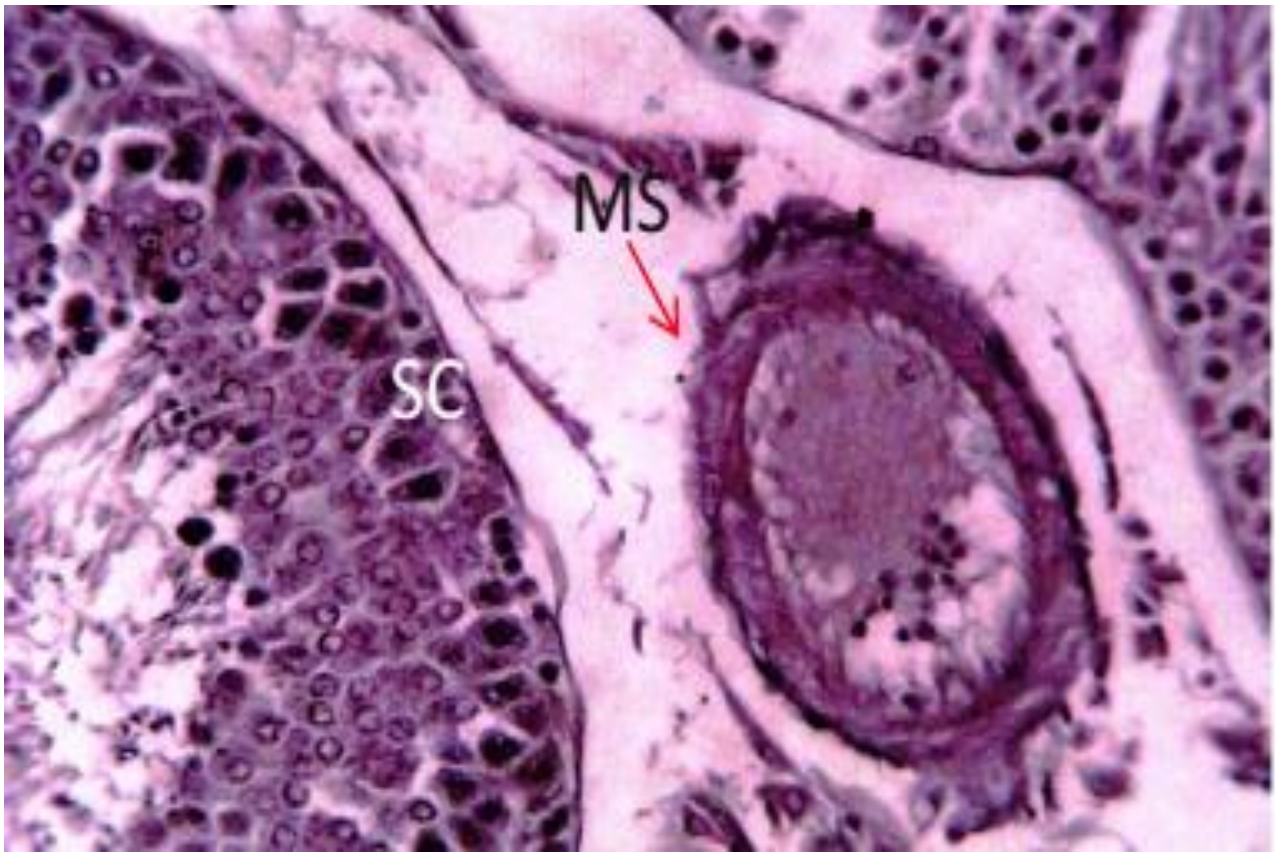


Plate IV: Photomicrograph of seminiferous tubule in SV 125 mg/kg rats showing matured spermatozoa (MS) and Sertoli cells (SC) (x 250 H&E)

CHAPTER FIVE

5.0 DISCUSSION

The root of *Securinega virosa* enjoys wide patronage as aphrodisiac among the Hausa people of North western Nigeria. Therefore the present study attempted to evaluate the aphrodisiac and reproductive activity of the methanol root bark extract of *Securinega virosa.* Phytochemical screening of the extracts revealed the presence of tannins, steroids/tarpenoids, saponins, flavonoids, alkaloids, cardiac glycosides, resins, carbohydrates and anthraquinones. Glycosides are known to increase feed intake and calcium level which plays a role as a neurotransmitter as well as hormone release. Flavonoids, Tannins and Saponins play vital roles in scavenging free radicals which aids in alleviating oxidative stress, resulting in optimal synthesis of sex hormones as well as performance (Adedapo *et al*., 2009). Alkaloids are known to have replenishing activities of depleted antioxidants in plant extracts (Cajuday and Pocsido (2009). Alkaloids are aphrodisiac (Harisaranraj *et al*., 2009) while phlobatanins can stimulate the synthesis of sex hormones (Okwu, 2001; Edeoga *et al*., 2005).

Sexual drive is usually marked by characteristic features like a shorter mounting latency (ML) and a higher mounting frequency (MF), under the regulatory influence of testosterone (Wallen, 2001). Steroids present in the extract could have also played roles in directly enhancing libido or indirectly, serving as building blocks for the synthesis of testosterone hormone via steroidogenesis. Testosterone supplementation has been shown to improve libido and intensifying orgasm and ejaculation (Fabbri, 2001). The ability of the extract to increase intromission and ejaculation frequency suggests that it enhances libido and potency (Bitran and

Hull, 1987; Meisel and Sachs, 1994); and improves sexual arousal and performance (Abdulwaheb *et al*., 2007). Substances that enhance sexual function may act via an increase in circulating testosterone levels, in enhancing sexual function via central and peripheral effects (Mills *et al*., 1996).

The extract increased sexual orientation activity both towards the female and self. The extract induced increase in sexual orientation could be consequence of the increased testosterone which is known to influence sexual behaviour in males. Androgens regulate the magnitude of penile erectile response, partly by regulating the venous outflow from cavernous spaces (Mills *et al*., 1994). Therefore, the increase in serum testosterone concentration by the extract might be responsible for the improved sexual behavior and libido in male rats (Bahmanpour *et al*., 2006). In fact, it has been shown that copulatory behaviour is maintained in castrated rats by treatment with testosterone (Meisel and sachs, 1994; Mills *et al*., 1994).

It was also observed in this study, a significant increase in the non-genital and genital grooming at all doses tested. Anogenital sniffling in rodents is an indication of interest intended to end in copulatory activities (Mills *et al*., 1994).

The increase in serum testosterone produced by the extract at higher could be due a possible direct stimulatory activity of the extract on the Leydig cells within the testes (Padashetty and Mishra, 2007). These cells are known to produce testosterone under the influence of luteinizing hormone (LH). Therefore, the extract may have stimulated the release of LH which acted on the Leydig cells thereby producing more testosterone. Serum testosterone level also increased with increasing dose of the extract which indicates a dose dependent relationship between the dose

and testosterone level. The increase could also have been due to the presence of alkaloids in the extract which stimulate testosterone production (Adedapo *et al*., 2009; Cajuday and Pocsido, 2009). The resultant increase in serum FSH obtained from the study could be attributed to a possible stimulatory action of the extract on hypothalamic cells resulting in the release of gonadotropin releasing hormone (GnRH), hence the release of FSH as observed. Steroids present in the extract shown from the phytochemical screening could also have served to boost the general synthesis of these sex hormones. From this current study, it was observed that serum level of FSH was highest in the SV 62.50 mg/kg treated group by third week of administration of the extract, which suggests that with increased duration, the effect of the extract is dose dependent.

Sperm indices such as concentration, motility, vitality and morphology are commonly used to determine the fertilization potential of sperm (van Der Horst *et al.,* 1999). Spermatozoa are produced in the seminiferous tubules of the testis from where they are transported to the epididymis. There is a gradient of concentration, maturation and increased motility as the spermatozoa move through the corpus epididymis and continues to improve through the cauda epididymis and vas deferens (van Der Horst *et al.,* 1999). The increase in sperm count from this study observed could have been due to a possible spermatogenic activity of the extract indirectly on the testes via the actions of increased LH and FSH as reported earlier. LH and FSH are known to cause proliferation of testicular cells and inhibit atrophy within the seminiferous tubules, all of which could result to increased spermatogenesis hence, increased sperm count. This increase could be due to the increased testosterone level in all the extract treated groups compared to the control. Testosterone is known to stimulate spermatogenesis in the testes. The production of

spermatozoa requires high intra-testicular concentration of testosterone, produced by the Leydig cells of the testis (Walker, 2011; Aprioku, 2013a). The highest increase was however observed following third week of treatment. This activity in sperm motility could also be attributed to the agonistic action of the extract on the primary reproductive hormones which play important roles in facilitating motility of spermatozoa. Sperm motility is a contributing factor during fertilization as sperms must move through the female genital tract from point of entry to site of fertilization (Aprioku, 2013a).

The reduction in body weight of the treated animals could be indicative of toxic action of a substance (Hiremath *et al*. 1997). There was a dose dependent decrease in the body weight observed in all the treated groups from this study. This activity could be due to the lipolytic action of testosterone hormone which involves the breaking down of lipids into glycerol and fatty acids. Testosterone binds to a receptor in the cell wall of the adipocytes which causes cyclic adenosine monophosphate (cAMP) to be generated inside the cell. The cAMP activates a protein kinase which phosphorylates and in turn activates a hormone sensitive lipase in the fat cell. This lipase cleaves free fatty acids from their attachment to glycerol in the fat stored in the fat droplet of the adipocytes. This process could cause a reduction in body weight over a period of time. There was however no statistically significant ($P > 0.05$) difference observed in the weight of the vital organs following administration of the extract, in the extract treated groups when compared to the control. And comparison between the body weight and the vital organ weight was without a statistically significant difference as well. The action of testosterone on body weight could also have been indirectly through the activity of testosterone on hunger hormones; ghrelin and leptin.

Leptin is proportional to body fats which mean that a decrease in leptin levels would stimulate hunger (Elbetieha *et al.,* 2001).

In the testis, the production of sex steroid hormones and male gametes is regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH); which are secreted by the pituitary gland. Impairment in the normal functioning of this gland could, then, interfere with the development and functioning of the male reproductive system (Mahony and Hodgen 1995). In this study, a dose dependent increase ($P< 0.05$) was observed in testicular weight when compared to the control. Increase in spermatogenesis is usually accompanied by increased testicular weight since the bulk of testicular weight is made up of seminiferous tubules that house spermatids and spermatozoa (Oyewopo *et al*., 2011; Kenjale *et al*., 2008; Chauhan *et al*., 2007). This could also be as the result of the anabolic action of testosterone on the testicular growth rate as part of the necessary requirement for maturity and spermatogenesis. Although there was observed some statistically significant differences in both vas deferens and epididymal weight, there was however a non significant ($P> 0.05$) difference observed in the penile weight of the rats treated with the extract when compared to the control. However, despite the increased testosterone levels, it is worth noting that there was no significant change in accessory sex organs weights.

In this study, testicular histology following administration of extracts shows normal seminiferous tubules containing spermatogenic cells with orderly maturation of mature spermatozoa in the control. The testicular histoarchitecture of the rats showed no sign of testicular injury; instead, it showed normal seminiferous tubules containing spermatogenic cells with slight indication of

cellular proliferation compared to the control. The highest dose of the extract produced normal seminiferous tubules with a markedly increased concentration of mature spermatozoa. These changes observed could be attributed to the activities of pituitary FSH, LH and testosterone (Oyewopo *et al*., 2011)

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CHAPTER SIX

6.0CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The aim of the study was to provide pharmacological rationale for the ethnomedical use of the root of Securinega *virosa* as aphrodisiac as well as its general male reproductive function; sperm parameters and sex hormones. Oral administration of *Securinega virosa* has been found to improve mating behavior and sexual orientation both toward self and the female with increased sexual orientation behaviours. It has also increased the reproductive hormone levels, consequently, improving sperm parameters with no evidence of testicular tissue damage. There was no evidence of significant organ weight changes during the treatment period.

6.2 Recommendations

i. A study on the oxidative stress status of the plant should be considered in subsequent research by assaying its direct effect on reactive oxygen species (ROS) level, serum malondialdehyde concentrations (MDA), antioxidant enzymes (SOD, CAT and GPx) due to the presence of saponins, tannins and alkaloids observed from the phytochemical screening. This will help to elucidate a possible oxidative stress ameliorative pathway for its aphrodisiac and testicular function enhancing effects.
ii. Molecular studies like gene expressions and receptor assays could also be considered to further ascertain the precise mechanism and exact site of action of this plant extract which could open other portals of research for possible drug development.

6.3 Contributions to knowledge

1) *Securinega virosa* improves mating behaviour by increasing intromission frequency (IF) at 125 mg/kg (6.00 ± 1.15 vs 3.25 ± 0.25) and decreasing ejaculatory latency (EL) at 31.20 mg/kg (94.50 ± 24.49 vs 143.50±48.28) with an overall increase in sexual orientation activities.
2) *Securinega virosa* increases serum testosterone, LH and FSH (ng/ml) levels as well as sperm motility (71.50%), sperm count (90.34%), sperm morphology (99.03%) and sperm vitality (75.05%).
3) *Securinega virosa* administered orally decreases body weight (g) at 31.25, 62.50 and 125 mg/kg; 219.50±1.59 g, 218.40±0.87 g and 212.40±1.97 g respectively. At SV 125 mg/kg, *Securinega virosa* increases testicular, epididymal and vas deferens weight (g); 1.89 ± 0.02 g, 0.66 ± 0.03 g and 0.67 ± 0.01 g, respectively. Histology of SV 125 mg/kg treated rats showed normal seminiferous tubules with a markedly increased concentration of mature spermatozoa relative to the controls in a dose dependent manner.

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