EFFECTIVENESS OF POMEGRANATE *Punica granatum L.* FRUIT EXTRACT ON THE SEXUAL FUNCTION IN RATS

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

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Recommendation

This thesis has been submitted for examination with our approval as official University supervisors.

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DEDICATION

To my husband, Mr. Collins Kirui and parents, Dr. Joseph Katore Katana and Pr. Naomi Kache Katana who established the foundation of my education.

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ABSTRACT

The worldwide prevalence of erectile dysfunction (ED) in 1995 was estimated to be 152 million males, and the figure is expected to rise to 322 million by the year 2025. The inability to achieve or sustain an erection and/or an inability to ejaculate is common among men over 40 years, where about 5% will experience ED and rates increase with age. Pomegranate (*Punica granatum* L.) has traditionally been believed to be of importance in male sexual disorder management. This investigation was undertaken so as to assess the effect of pomegranates fruit extract alongside its likely adversative effects and acute toxicity on healthy male rats as the animal model. Fifty (50) adult albino rats of Wistar strain weighing 250-350 g and 200-250 g, respectively, of both sex (male and female) were used in this study. The pomegranate fruit extract was orally ingested (500, 1000 and 1500 mg/kg) to different groups of male rats on a once-daily regime throughout the experiment period. The mating behaviour, libido, sexual potency and testosterone concentration were recorded and comparisons made with sildenafil citrate. The administration of pomegranate fruit extract orally at the dose of 1500 mg/kg led to a significant increase of sexual activity in male rats. The fruit extract significantly increased the Mounting frequency ($F = 383.9375$ $P = 1.34$), Intromission frequency ($F = 484.2$ $P= 1.34$), Intromission latency ($F = 65.81186$ $P = 3.17$) and created a significant decrease in the Mounting latency ($F= 687.1389$ $P = 4.23$), and Post Ejaculatory Interval ($F = 17047.4$ $P = 5.17$). It also produced a significant increase in the Mounting Frequency ($F = 109.486$ $P = 2.67$) with penile anaesthetization as well as Erections ($F = 383.9375$ $P = 1.34$), Quick Flips ($F = 502.4615$ $P= 9.4$), Long Flips ($F = 283.8$ $P= 2.62$), Total penile reflexes ($F = 2182.636$ $P = 4.27$) with penile stimulation. In addition, it significantly increased testosterone concentrations ($F = 13.84038$ $P = 0.000229$). The pomegranate fruit extract was without any adversative effects and acute toxicity. The resulting significant and continued increase in the sexual activity and testosterone concentration of normal healthy male rats with no visible antagonistic effects point out that the pomegranate fruit extract has aphrodisiac activity which might be due to its nervous stimulating components. This study make available a scientific perceptive for the traditional use of pomegranate in the management of male sexual disorders.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BCA	Bicalutamide
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
ED	Erectile Dysfunction
EDRF	Endothelium-derived Relaxing Factor
EL	Ejaculation latency
IF	Intromission frequency
IL	Intromission latency
IIEF	International Index of Erectile Function
LF	Long Flips
MF	Mount frequency
ML	Mount latency
NIH	National Institutes of Health
NO	Nitric oxide
NOS	Nitric oxide synthase
OR	Odd ratio
PDE	Phosphodiesterase
PEI	Post ejaculatory interval
ROS	Reactive oxygen species
SOD	superoxide-dismutase
TGF	Transforming growth factor
TOMHS	Treatment of Mild Hypertension Study
TPR	Total Penile reflexes
TT	*Tribulus terristrus*
QF	Quick Flips

CHAPTER ONE
INTRODUCTION

1.1 Background information

Sexual behaviour mirrors the normal functioning of the hypothalamo-pituitary-gonadal axis which involves sexual motivation and performance (Ojo *et al*., 2016). In males, sexual performance depends on the integration and coordination of different anatomical and physiological factors which eventually leads to an increase in the corporal system and offers the necessary penile tumescence as well as rigidity for successful sexual activity (Türk *et al.,* 2010). Sexuality is practiced in humans for purposes of reproduction, providing pleasure, boosting self-esteem, promoting intimacy, and lowering anxiety or tension. Human beings experience normal sexual activity all the way through adult life which declines with aging (Won *et al.,* 2012). Apart from old people, healthy people who usually suffer from psychogenic, organic, or mixed etiologies are not able to carry out sexual act hence experiencing erectile dysfunction (ED) (Ramya *et al.,* 2011). Erectile dysfunction, better known as impotence, is the inability to attain and maintain a penile erection that is sufficient to sustain satisfactory sexual activity for both partners (Njila, *et al*., 2018).

The global prevalence of erectile dysfunction (ED) in 1995 was evaluated to be over 152 million men. The estimates for 2025 show a prevalence of about 322 million with ED which makes an augment of approximately 170 million men (Rakuambo *et al.,* 2012). The largest projected increases were in the developing world (Africa, Asia and South America). Inability to achieve or sustain an erection and/or an inability to ejaculate is common among men over 40 years, where about 5% will experience ED and rates escalate with age (Kachroo and Agrawal, 2011). There are no present statistics existing on what age group is more susceptible to erectile dysfunction for Kenya, however, in USA nearly 10% of men are thought to be affected. By 45 years of age, most men have experienced erectile dysfunction at least once. The numbers rises with age where by 5% of men at the age of 40 and between 15% and 25% of men at the age of 65, and 70% as men reach 80 years of age suffer from erectile dysfunction (Kumar and Sahasudipta, 2013). At old age, men are reported to lose sexual desires as well as erectile dysfunction, which are unavoidable features of ageing (Jarvis *et al.,* 2010). Men with prolonged sicknesses, reduced testosterone level, those ingesting certain medications and alcohol consumers, are more vulnerable to experience. In addition, emotional flux, fatigue, physical injury (Hou *et al*., 2012) are some among other numerous causes of ED. Young adult male experiencing erectile dysfunction can time and again relate the condition to fundamental

health circumstances, like diabetes, heart disease, and side effects of some medicines and drugs. Psychological distress can lead to ED in men under 40 (Ahmad and Habib, 2014).

Throughout history, pomegranate symbolizes fertility and wellbeing, and has been postulated to enhance sexual drive (Türk *et al*., 2008). *Punica granatum* L. (pomegranate) is a fruit-bearing deciduous shrub which develops upto 5 and 8 metres (16 and 26 ft) tall (Al-Olayan *et al*., 2014). The fruit is usually in season as from September to February (Davis, 2014) as well as March to May. Pomegranate has been stated to have originated in the region of latter-day Iran. Studies show that it has been cultivated since olden times throughout the Mediterranean region as well as northern India (Ahmed *et al*., 2014). Pomegranates were identified in Ancient Israel as the fruits that spies carried to Moses to make evident as well as symbolizing the fertility of Canaan, the "promised land" (Elnagar, 2010). Pomegranates were made known into Spanish America and California by Spanish settlers in the late 16th century. In the present day, it is extensively cultivated in Indian subcontinent, north and tropical Africa, Central Asia, all through Middle East and Caucasus region and the drier parts of Southeast Asia.

Pomegranate fruit can be ingested as a whole fruit or as blended juice, and it's a healthy addition to any diet (Azra and Rafeeq, 2014). The seeds and its surrounding pulp are palatable and nutritious (Avdatek *et al*., 2018). Research has shown that the fruit raises oxygen levels in the body especially in the heart and fights arthritis (Amata *et al.*, 2014). Pomegranate fruit is packed with beneficial vitamins, minerals, phytonutrients and antioxidants. Clinical evidence reveals that it has abilities to protect against heart disease and cancer (Venkatesh *et al*., 2013). They are also associated with health, fertility and rebirth (Mansour *et al*., 2013). *Punica granatum* (pomegranate) has also been postulated to enhance sexual drive and believed to help fight erectile dysfunction among men but there was no scientific validation of these claims. This prompted an investigation on the effects of pomegranate fruit extract on sexual function in male rats hence the present study.

1.2 Statement of the problem

Erectile dysfunction is a global problem and is one of the most prevalent kind of sexual debilitation, which is set to increase over time. The inability to achieve or sustain an erection and/or an inability to ejaculate, is a condition common among men. Over 50% of all men experience erectile dysfunction (ED) and at age 40 and above, about 40% experience light to moderate ED and by age 70, men will experience 70% chance of erectile dysfunction. Impotence can trigger feelings of low-esteem and depression, which further leads to anticipatory anxiety. It also affects one's masculinity and confidence, and emotional fallout as well for their partners. Men going through sexual dysfunction can be succeed reversing these disorders by observing and considering on lifestyle factors rather than relying on medication. Pomegranate fruit is thought to alleviate the detrimental effects of ED, but little information is available on the effects of pomegranates on sexual function and this study therefore investigated the aphrodisiac activity of pomegranate in male rats.

1.3 Objectives

1.3.1 General objective

To investigate the effects of pomegranate *Punica granatum* L**.** fruit extract on the sexual function in healthy male rats.

1.3.2 Specific objectives

1. To evaluate the effects of pomegranate fruit extract on mating behaviour and libido in male rats.

2. To determine the effects of pomegranate fruit extract on sexual potency in male rats.

3. To determine the effects of pomegranate fruit extract on the concentration of testosterone in male rats.

1.4 Hypotheses

1. There is no significant difference in the effects of pomegranate fruit extract on mating behaviour and libido in male rats.

2. There is no significant difference in the effects of pomegranate fruit extract on sexual potency in male rats.

3. There is no significant difference in the effects of pomegranate fruit extract on concentration of testosterone in male rats.

1.5 Justification

The pomegranate fruit has been shown to have significant biological effects, but there is paucity of information regarding its sexual enhancement effects, and yet it is readily available. Pomegranate is believed to elevate testosterone levels in men which is known to be the key hormones behind sex drive (Emad and Nacer, 2014) and sperm quality (Türk *et al.*, 2008) but most men turn to conventional aphrodisiac drugs. Many drugs like Sildenafil (Viagra) have side effects ranging from sudden vision loss, chest pain, nausea, general ill health to irregular heartbeat. There is need, therefore, to find natural alternatives like pomegranate which have minimal side effects, if any.

CHAPTER TWO
LITERATURE REVIEW

2.1 Sexual function and dysfunction

The aspect of sexual function defined as being relevant to the assessment includes sexual craving, erection, orgasm and ejaculation (Diaz and Close, 2010). Sexual function characterizes how the body responds in various stages of the sexual response cycle or as a result of sexual dysfunction. Sexual disorder is struggle experienced by a person or a couple in the course of any phase of a usual sexual activity, inclusive of physical delight, desire, inclination, arousal or orgasm. As per Nolen-Hoeksema (2014). Sexual dysfunction call for an individual to feel great agony and relational strain for not less than 6 months, excluding medicine induced sexual dysfunction. Sexual dysfunction can have a deep influence on a person's apparent quality of sexual life (Eden and Wylie, 2009).

The brain is the main sense organ. Sensory and mental stimulation is the initial phase for an erection. Impulses travelling down the spinal column from the brain and impulses from the nerves in the penis relax smooth muscles in two spongy cylinders that run the length of the penis (Ferrini, 2009), parallel to the urethra. At the point when the impulses cause the muscles to relax, blood streams into the spongy tissue spaces, and this creates pressure making the penis to enlarge. Blood is trapped in the penis due to the membrane surrounding the cylinders hence maintaining an erection. The penis goes back to its drooping state if the muscles contract thus bring to an end the inflow of blood and opening outflow channels. Erection disorders can arise if any of the events in this order is interrupted; the problem may encompass psychological processes, physical processes such as responses in fibrous tissue, muscles, veins and arteries in the penis or nerve impulses.

2.2 Erectile dysfunction

Erectile dysfunction (ED), better known as impotence, is a sexual disorder in which male person are unable to maintain, or achieve an erection. The incapability to sustain or achieve an erection that is adequate for satisfactory sexual act impacts a substantial proportion of men occasionally (Njila, *et al*., 2018).

The relentlessness of erectile dysfunction is frequently termed as mild, moderate or severe as per the 5 item questionnaire on International Index of Erectile Function (IIEF-5) comprise scores of 1 - 7 signifying severe, 8 – 11 representing moderate, 12 - 16 symbolizing mild - moderate, 17 - 21 serving as mild, finally 22 – 25 for no erectile dysfunction (Forest, 2007).

Men with 18 years of age and above can go through some form of erectile dysfunction, because of exhaustion, tension, personal issues, anxiety and bad habits such as too much intake of alcohol and smoking. Growing rates of impotence with advancing age is a robust aspect of male senescence. The revealed age linked rise in erectile dysfunction was observed by the Massachusetts male aging study, and the upsurges have consecutively remained stated in various epidemiological studies (O'Leary, 2003).

Because of erectile dysfunction humiliating form and the disgrace felt by victims, the matter has been a taboo for quite some time, and is the subject matter of several urban legends. Traditional remedies have for quite some time been upheld, with some being publicized generally since the 1930s. The introduction of maybe the primary pharmacologically successful solution for ineptitude, Sildenafil (trade name Viagra), during the 1990s led to an influx of open consideration, pushed to some extent by the news value of tales about it and overwhelming promulgation (Njila, *et al*., 2018). It is evaluated that about 30 million male persons in the United States and 152 million men globally suffer from erectile dysfunction. Conversely, low health literacy, social stigma and taboos prompted a less commentary, making an exact pervasiveness rate difficult to decide (Rakuambo *et al.*, 2012).

2.2.1 Causes of erectile dysfunction

There are various underlying origins of erectile dysfunction and they may be psychological or physical. The following are some of the causes of ED:-

Psychological causes

These are emotional factors that can be brought about by interpersonal or mental problems. Mental problems can be the consequence of melancholy, sexual feelings of trepidation or blame, past sexual injury, and sexual issue (Michetti *et al*., 2005). Sexual dysfunction is particularly common among individuals with anxiety disorders. Normal anxiousness can evidently lead to erectile dysfunction in men with no psychiatric complications, however clinically diagnosable conditions for example panic disorder commonly leads to evasion of intercourse and premature ejaculation (Coretti and Baldi, 2007).

Psychological problems hinder erection process by disrupting the male person from possessions that generally would bring about arousal. These issues cause a range between 10 to 40 percent of erectile dysfunction. Even in situations where the primary problem is physical, these factors can exhibit a vital secondary role, for example, when a man who has had some erectile difficulty begins to expect sexual failure. As a result, psychological factors play some

causal role in at least 80% of cases of erectile dysfunction (Michetti *et al*., 2005). Non-organic erectile dysfunction, better known as adrenaline mediated erectile dysfunction, has not been well studied but is an essential factor to consider when assessing and handling men with this condition (Coretti and Baldi, 2007).

Physical causes of erectile dysfunction

Physical factors prompting sexual dysfunction include the utilization of conventional medicine and drugs, such as sildenafil, alcohol, nicotine, narcotics, stimulants, antihypertensives, antihistamines and some psychotherapeutic drugs. Sexual activity can be affected by an injured back, likewise, problems with an enlarged prostate gland, as well as blood supply or nerve damage have an impact on sexual activity. Damaged smooth muscles, fibrous tissues and blood vessels of the penis are the commonest physical causes of erectile dysfunction. Blood vessel problems account for 48% of erection problems while those of the nerves contribute for 14% and those of the structure of the penis and surrounding tissues contribute for 3% (Shabbir, 2004).

Erectile disorders in male person over 50 years old are bound to have physical causes. Diseases affecting someone's body account for 70% of physical causes. These may include diabetes, kidney disease, and multiple sclerosis. Arteriosclerosis or "hardening of the arteries" can prevent adequate blood from entering the penis. Diseases such as cardiovascular disease, multiple sclerosis, kidney failure, vascular disease (Nolen-Hoeksema, 2013), diabetics neuropathy, tumors, and rarely, tertiary syphilis may also pose impacts to person's sexual activity, as could the failure of various organ systems (such as the heart and lungs), endocrine disorders (thyroid, pituitary, or adrenal gland problems), hormonal deficiencies (low testosterone, other androgens, or estrogen), and some birth defects (Shabbir, 2004). In addition type II diabetes (Banihani *et al*., 2014), hypertension and heart disease (Shabbir, 2004) and spinal cord injury are some of the basis of erectile dysfunction. Other health complications (Foresta, 2003) are all related with raised danger of ED even after age control.

Nonendocrine causes

Neurogenic

It is predictable that 10 to 19% of erectile dysfunction is neurogenicorigin (Abicht, 1991). Shortfall in nerve signals to the corpora cavernosa brings about neurogenic erectile dysfunction. Spinal cord injury, Parkinson's disease (Sachs, 1988), multiple sclerosis, lumbar disc disease, traumatic brain injury, radical prostatectomy, radical cystectomy,

abdominoperineal resection and diabetes (Sachs, 1988) are secondary discrepancies leading to neurogenic erectile dysfunction. The central nervous system (CNS) mediating control of erection can be hindered by the upper motor neuron lesions, which is above spinal nerve T10 and cause no consequence in local variations in the penis. In contrast, sacral lesions which are basically accountable for reflexogenic erections lead to functional and structural changes due to the lessened innervation (Brackett *et al*., 2010). The functional modifications, which was as a result of such injuries, is the reduction in nitric oxide capacity that is existing to the smooth muscle. The physical alterations on apoptosis of both the smooth muscle and endothelial cells of the blood vessels not forgetting the upregulation of fibrogenetic cytokines (Ferrini, 2009) leads to collagenization of the smooth muscle.

Vasculogenic

Erectile dysfunction (ED) is a demonstration of an underlying vascular ailment. Erectile dysfunction can be as an outcome of the endothelial dysfunction and vascular illness. These dysfunctions and disorders cause a decrease in blood inflow and blood arterial insufficiency. The most suggested common and primary cause of vasculogenic impotence is the failure of sufficient venous occlusion (Rajfer *et al*., 1988). The danger of developing vasculogenic erectile dysfunction is augmented in male person with hypertension, (odds ratio (OR) of 3.04 for those on anti-hypertensive medication, and 1.35 for those not on medication), diabetes (OR 2.57) and dyslipidaemia (OR 1.83) (Kupelian *et al*., 2010). Vasculogenic impotence do not advance from high blood pressure however is tributary to the arterial wall modifications in response to the rise in BP (blood pressure). Cigarette smoking and atherosclerosis related to diabetes, can develop arterial stenosis and multiple vascular damage.

Iatrogenic

The radical pelvic surgery is another cause of erectile dysfunction. Pelvic fissures cause erectile dysfunction due to nerve disruption damage and arterial distress. The harm occurring during these protocols is predominantly neurogenic in nature (Tal, 2009). Various treatments used to cure hypertension such as thiazide diuretics and β-blockers are mostly associated with erectile dysfunction. Antiandrogens and antiulcer drugs, psychotherapeutics, digoxin and opiates, are speculated to have a link with the growth of impotence (Francis *et al*., 2007). Nevertheless, whether impotence results straight from the underlying disease like hypertension, or rather from other form of medication, is challenging to define. Chlorthalidone, a diuretic drug used for hypertension treatment, has a greatest impact on sexual function at two years of treatment,

but the palliative accomplished is almost equal to those at four years. As a result, chlorthalidone might potentiate impotence earlier in those who are probable to develop the disorder in future.

Endocrine causes

During penile growth and physiology, androgens are well thought-out as the most important hormonal regulator (Boas, 2006). Nevertheless, the objective of testosterone replacement therapy in ED or impotence is contentious due to inconsistencies from clinical test results, and the fact that both hypogonadism and impotence are frequent in ageing. The rising association of impotence and the liberal deterioration of androgen concentrations with ageing do not automatically means a fundamental connection. Previous studies focusing to comprehend the task of lowered testosterone on erectile function concentrated on androgen ablation, a model that cannot be simply translated to erectile dysfunction in humans (Filippi, 2009). From these studies and metabolic models, three sites of action for androgens have been described: the nuclei in the CNS, the spinal neurons and pelvic ganglia, and the genital tissues (Isidori, 2014). Mediated sexual desire as well as male libido is part of the erectile response to testosterone (libido correlates with testosterone levels), nonetheless, studies have acknowledged a undeviating aim of testosterone on cavernous smooth muscle cells, linking nitric oxide, associated protein kinase, PDE5 and the adrenergic response (Isidori, 2014).

2.2.2 Peripheral nerve and limbic system control in male erection

The erect penis has continuously been a representation of a male's sexual competency and virility. Even though it is not a fatal condition, the subject on impotency and its therapies has endlessly remained all through the ages (Glina *et al*., 2008).

The reflex erection and psychogenic erection, are the main features of the male erection that can take part in the male person's impotence which are issues to therapeutic intervention. The reflex erection is accomplished through direct caressing the penile shaft. It is under the control of the peripheral nerves and the lower parts of the spinal cord. However the psychogenic erection is attained by erotic (emotional stimuli) in addition it uses the limbic system of the brain. Earlier, psychogenic ED was thought to be the most common type with 90% of ED male person assumed to suffer from this situation. Sexual behaviour as well as male erection is controlled by the: hypothalamus, the limbic system and cerebral cortex. For that reason, stimulatory or inhibitory messages can be sent to the spinal erection centres to enable or prevent erection (Steers, 1990). An animal study showed that the stimuli of sympathetic nerve lead to detumescence of the erect penis (Diederichs *et al*., 1991).

2.2.3 Nitric oxide (NO) and erection

Nitric oxide, better known as the endothelium derived relaxing factor (EDRF) is biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes. Nitric oxide is one of the rare gaseous signaling molecules known and it is the core vertebrate biological messenger, playing a role in various biological processes. It is a known bio-product in almost all types of organisms, ranging from bacteria to plants, fungi, and animal cells (Roszer, 2012).

Pomegranate lifts nitric oxide creation, on the other hand it's packed with antioxidants. Antioxidants aids in clearing systems of free radicals (Seeram *et al.*, 2005), which obstructs the metabolic processes of nitric oxide yields. It also rises nitric oxide production, and on the other hand, it makes nitric oxide more metabolically efficient. Observing nitric oxide status by saliva testing detects the bioconversion of plant derived nitrate into nitric oxide (Dessy and Ferron, 2004). A rise in salivary concentrations is symbolic of diets full of leafy vegetables which are frequently plenteous in hostile to hypertensive eating regimens.

The nitric oxide yields are raised in populaces habiting high altitudes, which helps human beings to avoid hypoxia by aiding in pulmonary vasculature vasodilation. Impacts incorporate vasodilatation, neurotransmission, regulation of the hair cycle, generation of responsive nitrogen intermediates and penile erections. Nitroglycerin and amyl nitrite are transformed to nitric oxide in the body so as to function as vasodilators. The vasodilating antihypertensive drug (minoxidil) contains a nitric oxide moiety, thus, acting like an agonist of nitric oxide (Dessy and Ferron, 2004). In like manner, sildenafil citrate, also known as Viagra, stimulates erections primarily by enriching signaling through the nitric oxide pathway in the penis. Sildenafil among other impotence medications, work effectively due to their activity on the NO pathway (Njila, *et al.*, 2018). Among other causes of ED, is unhealthy and matured arteries that nourish blood to the sexual organs. Sildenafil citrate function by manipulating enzymes in the nitric oxide (NO) pathway, provoking a series of enzymatic reactions that enhance nitric oxide, leading to more blood flow and better erection.

In warm blooded animals, nitric oxide is a vital cellular signaling molecule involved in numerous functional and pathological procedures. NO is an incredible vasodilator with a short half-life of a few seconds in the blood. Nitric oxide has been shown to be of importance in the following activities of the cell. It helps memory and behaviour through data transmission among nerve cells in the cerebrum. It assists the immune system at fighting off bacteria and

defending against tumors. It regulates blood pressure by dilating arteries. It reduces inflammation and improves sleep quality. It increases sense (smell) recognition, endurance and strength. Likewise it aids in gastric motility. Nitric oxide (NO) contributes to vessel homeostasis hindering vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. People with atherosclerosis, diabetes, or hypertension time and again show diminished nitric oxide pathways (Dessy and Ferron, 2004). Salt intake in high levels was shown to weaken nitric oxide manufacture to human beings hence possessing critical hypertension (Osanai *et al*., 2002).

Pomegranate symbolizes fertility (Mansour *et al*., 2013) and wellbeing, and has been postulated to enhance sexual drive. Some animal studies have shown that juice intake from pomegranate fruit upsurges testosterone concentration and sperm quality (Leiva, 2011; Türk *et al.*, 2008). Investigators discovered that consumption of pomegranate juice for three months lessened stress-induced ischemia, denoting that it facilitated better supply of blood and studies recommend that pomegranate extracts significantly prevented the growth of prostate tumors and rises salivary testosterone production (Emad and Nacer, 2014).

2.3 Pomegranate

Pomegranates are native to Pakistan, Iran, Afghanistan and northern India, and these days are farmed all through California, the Mediterranean, together with South Europe. Since roughly 3000 B.C. *Punica granatum* L. (pomegranate) were used as medicine and for culinary. In addition, they have been associated with health, fertility and rebirth (Mansour *et al.*, 2013), and they had a vital task in the cuisine and way of life of the Middle East. Pomegranates are named numerously in the Old Testament as the fruit speculated to have tempted Eve (Elnagar, 2010). Pomegranates are from the shrub well known as *Punica* granatum L. The pomegranate tree is the mostly thorny plant with small waxy leaves that grows well in various areas, could be in arid or semi-arid. In Kenya, it generally grows in both tropical and cold areas. Regions with high temperatures have tendencies to improve the flavor of the fruit making it more palatable. In clay, sandy, silt and gravel soils, the pomegranate tree thrives well (Farmers Trend, 2015).

Figure 1: Pomegranate fruit *Punica granatum* L.

(Source: Gomez-Caravaca, 2013)

Pomegranate fruit (Figure 1) has a red surface over a hard yellow rind comprising sacs of seeds It originated from Persia in the early days (Farmers Trend, 2015). Pomegranate fruit is naturally round and 2.5 to 5 inches wide. The pomegranate fruit reflects a refreshing and palatable fruit that encompases sweet and tart taste. The fruit is filled with useful vitamins, minerals, phytonutrients and antioxidants (Venkatesh et al., 2013).

Pomegranate comprises roughly 600 jelly seeds, better known as arils that are enclosed in white pith. The pomegranate fruit contains raised levels of fiber, vitamins, phytochemicals and low levels of calories, which help to prevent cancer and promote heart condition. Nutritionists advocate for people to eat pomegranate seeds when still fresh and juicy, a state considered very valuable. One can also sprinkle the seeds on a fruit salad to utilize the values it offers to the body. Research shows that pomegranate fruit increases the level of oxygen in the body and helps fight erectile dysfunction among men in Kenya (Farmers Trend, 2015).

Punica granatum seeds are particularly rich in Vitamins C and K. A 100 g percentage of uncooked ripe seeds, delivers 10.2 mg of vitamin C equivalent to 17% of the required day to day value. The seeds provide to some extent, more than 16 mg of vitamin K an equivalence of 20 percent of the daily value (El-Nemr et al., 1990). Vitamin C is an essential element in wound healing, one's immune system function and promotion of healthy gums. It is the manufacturer of collagen and elastin. It facilitates the absorption of Iron. Vitamin K is vital in blood clotting and in the maintenance of strong, healthy bones. In addition, *Punica granatum* fruit contains 11 percent of a day's Vitamin B6 and 8% Vitamin E. the fruit also has 27 percent of the day to

day required intake of folate, 13 percent of a daily niacin, thiamin, riboflavin, choline and pantothenic acid. There are various most vital mineral nutrients contained in pomegranates. Copper is the highest contained mineral with one raw fruit having 22 % of the day's required quantity. The fruit also comprises 19% of a day's potassium, 17% of its manganese and 10% of a day's phosphorus. Pomegranates also offers lower levels of magnesium, iron, calcium, zinc and selenium.

Pomegranate, a predominant member of the Punicaceae family, has as well proven to exhibit imperative physiological characteristics, that include anticancer (Afaq *et al*., 2005; Lansky, 2005) anti-proliferative, apoptotic (Seeram *et al.*, 2005), HIV-I entry inhibition (Neurath *et al*., 2004), cardioprotective, antihyperlipidemic, anti-inflammatory, anti-mutagenic, anti-bacterial activities and as a powerful antioxidant and antifungal substance (Tehranifar *et al*., 2011). The seeds holds plenty of categorical polyphenols, such as tannins, quercetin and anthocyanins all of which may offer both heart health and anti-cancer importance. As powerful antioxidants, polyphenols may improve healthy cell survival, induce cancer cell death and prevent tumor growth (Seeram *et al*., 2005). Anthocyanins have anti-inflammatory, antiviral and antimicrobial properties (Seeram e*t al.*, 2005; Lansky, 2006). Pomegranates contain other vital nutrients like carbohydrates, mono and polyunsaturated fat. It also contains 9 percent of a day's protein and 223 mg of Omega-6 fatty acids.

Pomegranate juice inhibits oxidized LDL uptake and cholesterol biosynthesis in macrophages (Fuhrman *et al.*, 2005). Animal studies have shown that pomegranate juice intake raises testosterone concentration in men (Emad and Nacer, 2014), also increases the number of motile sperm (Fedder *et al*., 2014) and semen quality (Fayed *et al*., 2012; Türk *et al*., 2008). Therapeutically beneficial constituents of pomegranate include ellagic acid, ellagitannins, punicallagins, punic acid, flavonoids, anthocycanidins, antocyanins and estragenic flavones (Seeram *et al*., 2005; Bachoual *et al.*, 2011). Additionally, many investigators have reported that pomegranate and its derivatives have free radical scavenging and potent antioxidant activity (Sikka, 1996).

Reactive oxygen species (ROS) are exceedingly reactive oxidizing agents from the class of free radicals. The generation of reactive oxygen species in different organs like testis is a usual physiological event however, the variations in their synthesis stimulate the oxidation and DNA damage of cells (Sikka, 1996). The plasma membrane of sperm has a raised level of unsaturated fatty acids. Thus, it is especially vulnerable to peroxidative damage. The lipid peroxidation

wrecks the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the deformities of film trustworthiness. Antioxidants, in general, are compounds which dispose, scavenge, and suppress the formation of ROS and lipid peroxidation. Among the outstanding biological antioxidants, glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide-dismutase (SOD) have a significant role as a suppressor or scavenger of free radicals. Thus, the application of ROS scavengers is probably going to improve sperm (Qu, 2010).

2.4 Plant related natural aphrodisiacs

There are varying standard treatments of erectile dysfunction (ED) nevertheless, the scientific community has erudite that natural choices are effective at improving impotence. The following are plant related natural remedies for treating ED:

Red ginseng is the herbal Viagra and a type of ginseng with most reported aphrodisiac effects. Studies indicate that a dose of red ginseng at 600 - 1,000 mg consumed 3 times a day, was very good in improving male libido (Coon and Ernst, 2002). Jang *et al*., (2008) carried out an in-depth systematic assessment on the effect of red ginseng on the management of erectile dysfunction and testified that out of 28 published articles seven studies assessed 363 males aged between 24 - 70 years old. The meta-analysis reached a deduction that ginseng improved sexual performance more than placebo (Jang *et al*., 2008). The studies absorbed entirely on male impotence neglecting the capability to precisely evaluate the aphrodisiac effects of red ginseng in females. The mechanism of action of red ginseng is largely unidentified, with former animal studies reporting that the main active component of red ginseng better known as ginsenosides, results to an augmented production of nitric oxide from the smooth muscles of the cavernous tissue and side effect stated is normally mild gastrointestinal upset (Hong *et al*., 2002).

Potency wood (*Muira puama*) is a herb growing predominantly in Brazil with many conventional explanations that it can endorse sexual activities, enhance libido, sexual desire, fantasies and the ability to reach orgasm in 65 percent of women with previous sexual disorders especially in old male persons (Xin *et al*., 2003). In its original state, the *Muira puama* is a blossoming bush in the Brazilian Amazon forests. *Muira puama* roots and bark have been used by the Amazonian native individuals to treat different sexual dysfunctions because it upsurges ones testosterone concentration. Current tests have noticed ED improvement when men were given *Muira puama* supplements. In a study conducted, 60 percent of male persons with

originally low libido and 50 % of men primarily with poor erectile function, testified upturn in their sexual desire following ingestion of *Muira puama* herb (Tian *et al*., 2004).

Horny goat weed (*Epimedii herba*) is a plant used to manage several sexual problems in males, including impotence, fatigue, low libido and pain. *Epimedii herba* is used as an energy and erectile enhancing drug in customary Chinese medicine for centuries. Horny goat weed herb contains inhibitors that are in the same way used to treat men for erectile dysfunction.

Icariin is the major active component of E*pimedii herba* (Adaikan *et al*., 2000). Previous animal studies done have conveyed a significant rise in intracavernosal pressure after giving icariin (Gauthaman and Ganesan, 2008). This was merged to significant growth in the expression levels of inducible and neuronal nitric oxide synthases in the corpus cavernosum (Adaikan *et al*., 2000).

Tribulus terristrus (TT) is a continuing sidling plant of a global distribution. This plant has for a long period of time been used as an old-style sexual stimulant. Present studies and researches are merely restricted to animal studies that have shown significant proliferations in erectile function after oral administration of the plant extract (Gauthaman *et al*., 2003). Various findings have established that, certainly, *Tribulus terristrus* is capable to rise endogenous testosterone levels, which might be the fundamental capability.

Maca (*Lepidium meyenii*) is a root that have its place to the brassica (mustard) family. The root is cultivated in Adrean. Maca root is enrich with amino acids plus minerals (for example iodine, iron and magnesium). Conventionally, these roots have been utilized due to their aphrodisiac and fertility improving possessions (Rowland and Tai, 2003). Most carried out studies done on the aphrodisiac activities of Maca are minor and did not show dependable actions of sexual function (Zenico *et al.,* 2009). As of present studies, in a double-blinded, placebo-controlled study, there was no significant inconsistency in International Index of Erectile Function (IIEF) scores between men who consumed Maca and those who used placebo (Zenico *et al*., 2009). However, the overall sense of well-being was significantly greater in the Maca group. The reported findings are in agreement to previously affirmed results which suggested that the aphrodisiac effects of Maca are predominantly due to its nutritional characteristics for they are rich in vital amino acids and minerals (Balick *et al*., 2002).

Chocolate is initially prepared from Coco beans after fermentation and manifold processing. Every so often, chocolate has well been thought as the food with the greatest influence on

attitude, passion as well as feeling. According to Sies *et al.,* (2005), it has potrayed a possible impact on the overall health of human beings. Chocolate contains a wide range of chemical compounds that are pharmacologically active such as methylxanthines and Narachidonoylethanolamine, a brain lipid that can imitate the psychoactive impacts of cannabinoids. Fascinatingly, a number of researches acknowledged that some chocolate constituents, especially serotonin and flavinoids, may be involved in curbing ladies' genital sexual functioning (Matuszewich *et al*., 2000). Serotonin, which may act both as a vasoconstrictor and vasodilator, has been located in numerous regions of the female genital tract in both animals and humans. A recent study on examination of the effects of chocolate on women's sexual health (Salonia *et al*., 2006) using various female sexual function questionnaires, put forward a positive trend of greater scores and thus healthier sexual function in women ingesting daily chocolate and women who did not. Nevertheless, when the data was adjusted to age, this difference was lost. Discovering a correlation between chocolate and sexual health is very alluring, yet, further in-depth findings should be done prior to a much firm conclusion reached (Salonia *et al*., 2006).

Tongkat ali is a Malaysian herb used successfully in reproductive trials and studies. It aids in the production of LH (luteinizing hormone) cells. It brings about a chain reaction which raises the levels of testosterone (Ang *et al.,* 2003). *Tongkat ali* obstructs the harmful chemicals that blocking congested arteries and blood flow to the penile region.

The rhizomes of *Curculigo orchioides* have been generally utilized as sexual enhancer. In the examination ethanolic concentrate of rhizomes was assessed for its impact on sexual conduct in rodents. The treatment especially influenced sexual behaviour of animals as mirrored in decrease of mount latency (ML), an escalation in mount frequency (MF) and enhanced attraction of a stronger affinity towards female (Chauhan *et al.,* 2007).

In another investigation, the aphrodisiac constituents of *Microdesmis keayana* root extract and major secluded alkaloids were assessed by observing the sexual behaviour of male rats (Zamblé *et al.,* 2008). Aqueous extract of *Microdesmis keayana* (150 mg/kg body weight) and pure alkaloids (3 mg/kg body weight) of *Microdesmis keayana* were administered orally by gavage to male rats. The results have revealed that aqueous extract and alkaloids of *M. keayana* stimulate sexual parameters in rats' sexual behaviour.

2.5 Rat as an animal model for male erectile function

Sexual dysfunction in rats can be induced using drugs. The recent knowledge of physiology of erection is gotten from animal *in vivo* plus *in vitro* inquiries. In the second half of the 19th century, it was showed that the pelvic nerve is involved in the erection in dogs. Eventually, more animal models were elucidated and used. Some of different animals used were monkeys, dogs and rabbits

Erectile dysfunction in rats can also be caused by injury of the cavernous nerve, metabolic syndrome, hyperhomocysteinaemia, and hyperlipidemia which impairs erectile dysfunction in rats by causing cavernosal fibrosis (Snehlata *et al*., 2015). The use of intracavernous pressure (ICP) measurement in mice and rats similarly was available commercially, which further aided in studying the pathophysiology of ED (Snehlata *et al*., 2015). This versatile model should aid in the future development of research instrument, characterize additional signaling target molecules and pathways, and assist in one's comprehension of male sexual dysfunction.

2.6 The Laboratory rats

A laboratory rat is reared and reserved for scientific research. They served as vital animal model for investigation in psychology and biomedical science (Vandenbergh, 2000). Research using animal models has been central to many of the accomplishments of contemporary medicine (Lieschke and Currie, 2007). It has contributed most of the basic knowledge in fields such as human physiology and biochemistry. The use of organism as models (in vivo models) have widely been used to explore human disease when experimentation on human beings may possibly be unethical. Even though biological activity in an in vivo model does not ensure an effect in humans, they developed guidance for production of drugs, treatments and cures for diseases in human beings are (Chakraborty, 2009). The Wistar rat as shown in the figure below (Figure 2) is an outbred albino rat established at Wistar Institute in 1906 for use in biological and therapeutic research, as well as exceptionally the number one rat established to serve as an *in vivo* model used for lab inspection. The rat is at present one of the most widespread rats utilized in laboratory research. The features most found are wide head, long ears, and a tail length that is always less than its body length. Therefore, the use of Wistar rat to extrapolate people in this study, takes into consideration better comprehension of physiological procedures without the additional danger of harming a real human being.

Figure 2: Wistar rat

(Source: C&M Lab Pro)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

The study was carried out at Egerton University, Njoro, Kenya. The university is located approximately 25 km southwest of Nakuru town and roughly 182 km, by road, northwest of Nairobi, the capital city of Kenya. The university lies at 0°22'11.0" S, 35°55'58.0" E (Latitude: - 0.369734; Longitude: 35.932779) (Amata *et al*., 2014).

3.2 Experimental setup

3.2.1 Experimental Animals

Fifty (50) adult male and female albino rats of Wistar strain weighing 250-350 g and 200-250 g, respectively, and approximately three months old were used in this study. The rats were obtained from the University of Eldoret and transported to Egerton University. The rats were housed individually in discrete standard cages. They were kept under standard laboratory surroundings of temperatures between 24-28°C, relative humidity of 60-70% and 12/12 h light dark cycle (Dewsbury, 1972). They were fed with mice pencils from Unga Farm Care (E.A.) Ltd, Nakuru, Kenya, and water was provided *ad libitum.* Rats were distributed into three major experimental groups. The first experimental group (group 1) represented the control group, which received distilled water. The second experimental group (test group) represented group 2-4 and was treated with pomegranate fruit extract. The third experimental group (standard group) represented group 5 and was provided with suspension of the sildenafil citrate (Mazokopakis and Starakis, 2012).

3.2.2 Ethical approval

Ethical approval was obtained from the Biosafety, Animal use and Ethics Committee of the Faculty of veterinary medicine, University of Nairobi with the reference number FVMBAUEC/2019/213.

3.2.3 Pomegranate fruit extract preparation

Fresh pomegranate (*Punica granatum* L.) fruits were obtained from a market in Njoro, Kenya. They were transported in cartons to the laboratory in the Department of Biological Sciences, Egerton University, Kenya. *Punica granatum* fruits were washed and rinsed with water. They were dried under sunlight until water droplets completely evaporated. Using a surgical blade the fruits were cut into two halves and the seeds scooped into a mixing bowl. Using a blender, the seeds were blended until they were crushed and pulpy. Two pieces of cheese cloth were placed on a flat surface, one on top of the other. The blended seeds were poured into the centre

of the cheese cloth, then the ends of the cheese cloth were brought together to make a bag. The cheese cloth bag was held over a beaker and squeezed until juice stopped coming out. The seed casings remained in the cheese cloth, and the *Punica granatum* extract was collected in the container. The pomegranate extract was covered with a container lid and stored in a refrigerator at 4^0C (Banihani *et al.,* 2014).

3.2.4 Chemicals

Sildenafil citrate was procured from Teva Pharmaceuticals (Nairobi, Kenya). Estradiol and Progesterone was obtained from Sun Pharmaceutical Industries Limited (Mumbai, India), 5% xylocaine ointment was bought from Zawadi Healthcare Ltd (Nairobi, Kenya) and Bicalutamide (BCA) was obtained from Lexicare Pharma PVT. Ltd. (Nakuru, Kenya).

3.2.5 Drug preparation and administration

The drug solutions were made ready prior to the commencement of the experiment. All other chemical and reagent used were of analytical reagent quality. Pomegranate extract was administered orally as a fine suspension in Tween-80 (1%). Likewise, sildenafil citrate and ethinyl oestradiol were separately suspended in distilled water with the aid of a Tween-80 (1%) for oral consumption. However, progesterone was dissolved in olive oil there after used for subcutaneous injection (Sawada *et al.,* 2014).

3.3 Data collection

3.3.1 Adverse effects

Adverse effects were tested by the method described by Tajuddin *et al.,* (2005). Every treated rat was observed on a once-daily regime for evident signs of toxicity which include salivation, rhinorrhea, lachrymation, ptosis, struggling, fits and trembles. They were also observed for any signs of stress, such as fur erection and exophthalmia, and behavioural changes like cleaning of face, spontaneous and unconstrained movement in the cage, and climbing. Above all the intake of food and water was observed.

3.3.2 Acute toxicity test

Determination of acute toxicity of the pomegranate fruit extract was accomplished using the technique designated by Lorke (1983). Adult Wistar rats were divided randomly into five groups each consisting of five rats. Group 1, the control group was given 10 ml/kg of distilled water by use of mouth. The rats in group 2, 3, 4 and 5 were administered single doses (1000, 2000, 4000 and 5000 mg/kg body weight respectively) of the pomegranate fruit extract orally with the aid of an orogastric tube. All the animals used for the acute toxicity test were observed unceasingly for behavioural changes and mortalities for 48 hours (initial 4 hours and then

intermittently for the next 6, 24, and 48 hours after dosing). The behaviour parameters observed were convulsion, hyperactivity, sedation, grooming, loss of right reflex and increased respiration. The rats were set aside under the same laboratory conditions throughout the experiment.

3.3.3 Evaluation of the mating behaviour and libido

3.3.3.1 Mating behaviour test

Test for mating behaviour was performed as per the techniques designated by Dewsbury and Davis (1970). The healthy male rats were distributed into experimental groups of 5 rats each. The first experimental group was group 1 (control) which was given 10 ml/kg of distilled water orally, every day at 6:00 p.m. for seven days. Group 2-4 was the test group which was treated with pomegranate fruit extract at a dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, respectively, orally in a once-daily regime for seven days at 6:00 p.m. Finally group 5, the standard group was treated with sildenafil citrate which was administered by use of mouth at the dose of 5 mg/kg one hour before the beginning of the test. The rats were conveyed to the laboratory for acclimatization there after they were exposed to dim light (Dewsbury, 1972) in 1 W fluorescent tube in a laboratory of 14'x14' at the stipulated time of testing every day for six days prior to testing. Each rat was weighed (by the use of an electronic weighing balance) and its weight recorded once a week (Venkatesh *et al*., 2013).

Female rats permit copulating only during the period of oestrus stage. For this reason, they were artificially brought to oestrus as per the technique designated by Szechtman *et al.* (1981). They were ingested with a suspension of ethinyl oestradiol at the dosage of 100 µg/animal 48 hour preceding to the coupling, in addition progesterone was subcutaneously injected at the measure of 1 mg/animal six hours before experimentation (Srilatha, 1998). The receptiveness was affirmed in advance whereby the female rats were presented to male rats other than the standard, test and control groups of animals. Females who exhibited most receptive characters were identified, marked and chosen for the test. The experimental test was performed on the seventh day after the start of the treatment of the male rats. The experiment was done at exactly 8:00 p.m. East-African time, under the same light intensity in the laboratory. Receptive feminine rats were put into the male rat cages at a ratio of 1:1.

The mating stages as well as frequency of events were recorded on a pencil camera. The mating phases and frequencies were determined from the camera transcriptions: Mount frequency (MF) and Intromission frequency (IF); Mount latency (ML) and Intromission latency (IL);

finally, Ejaculation latency (EL) and Post ejaculatory interval (PEI). The test was completed as soon as each male rat started to mount the female once more after a short-lived period of inactivity. (Zeweil *et al*., 2013).

3.3.3.2 Test for libido

Test for libido was done using the technique described by Shravan *et al*. (2011). The Wistar male rats were distributed into various experimental groups of five rats each and put individually in discrete propylene cages throughout the study. The control group representing group 1 was issued 10 ml/kg distilled water, once every day for 7 days at 6:00 p.m. by use of mouth. The pomegranate-treated groups (group 2-4) were given the fruit extract orally at dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively every day for seven days at 6:00 p.m. The standard group was given sildenafil citrate suspension at the dosage of 5 mg/kg by use of mouth, one hour prior to the start of the libido test. The feminine rats were made receptive by hormonal management and each animal was accustomed or familiarized to the testing condition as earlier described in section 3.3.3.1. The rats were observed for the mounting frequency on the seventh day at 8:00 p.m. East-African time. A penile exposure was done through retraction of the sheath, later 5% xylocaine ointment was smeared 30, 15 and 5 minutes before beginning authentic observations (Teixeira *et al*., 2013). Every male rat was placed in an individual cage and the receptive feminine rat was introduced in the same cage. The number of mounting was noted and the rats were also observed for frequencies of intromission and ejaculation.

3.3.4 Test for sexual potency

Test for sexual potency was done by the approaches designated by Hart and Haugen (1968) as well as Hart (1979). The male rats were allocated three experimental groups, every sub-group comprised five rats. The rats were put independently in separate cages throughout the experimental period. The control group representing group 1 was given 10 ml/kg of distilled water, every day for 7 days by use of mouth. The pomegranate-treated groups (group 2-4) were given the fruit extract orally at the dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, respectively, every day for seven days. Finally the standard group (group 5) received suspension of the sildenafil citrate at the dosage of 5 mg/kg, by use of mouth, one hour before the start of the study. The test for penile reflexes was done on the 8^{th} day. Using a glass cylinders, the rats were positioned and lied down on their back, for partial restraint. Their preputial sheaths were pushed backwards for a time frame of about 15 minutes by so doing a

cluster of genital reflexes were to be elicited. The following parameters: Erection (E), Quick Flips (QF) and Long Flips (LF) were observed and recorded (Tehranifa *et al*., 2011).

3.3.5 Determination of the concentration of testosterone

The test was carried out on male rats and concentration of testosterone determined at the culmination of the study. The male rats were distributed into various experimental groups, every group contained five rats. The rats were put independently in individual cages throughout the experimental period. The control group representing group 1 was given 10 ml/kg of distilled water, every day for 7 days by use of mouth. The pomegranate-treated groups (group 2-4) were given the fruit extract orally at dose of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, respectively, every day for seven days. Finally the standard group (group 5) received suspension of the sildenafil citrate at the dosage of 5 mg/kg, by use of mouth, one hour before the beginning of the test. After eight weeks of treatment, the rats were anesthetized using ether. Thereafter, enough volume of blood was collected from each rat using the cardiac puncture method. The samples were gathered in the morning in order to lessen the diurnal variation of hormone levels. Bicinchoninic acid assay chemical (BCA) tablets were powdered to increase the dissolution surface area. Briefly, 25 mg of powder was transferred to a 100 ml volumetric flask. A volume of 60 ml methanol was added and solution centrifuged for total dissolution for 15 minutes at 1600 g. Methanol was added to the volume of 100 cm^3 and solution filtered through filter paper. Further dissolutions were made using methanol to get concentrations of 10 mg/ml. Absorbance was determined and the standard curve generated at 272 nm. The absorbance values were obtained from male rats treated with pomegranate extracts, sildenafil positive control and water negative control administered orally to compare sexual parameters based on those treatments. Testosterone concentrations were calculated based on the absorbance values (Sonu and Ashish, 2017).

3.4 Data analysis

Data collected was computed to find the mean values and summarized in graphs and tables. The occurrence of parameters as seen in standard, test and control groups was statistically analyzed by using one-way analysis of variance (ANOVA) method. The significance of difference between the mean was determined with post-hoc ‘t’ test. All the results were expressed as mean ± standard of the mean error (SEM) and the level of significance for comparisons set at $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

CHAPTER FOUR

RESULTS

4.1 Adverse effects

The results indicated absence of overt signs of toxicity, stress and changes in behavior. The ingested food (mice pellets) and water intake of the test group remained the same as those of animals treated with distilled water (control).

4.2 Acute toxicity studies

In all the treated and control groups of rats, there were no mortalities observed as well as behavioural changes, up to the dosage of 5000 mg/kg.

4.3 Effect of pomegranate fruit extract on mating behaviour and libido

4.3.1 Effect of pomegranate fruit extract on mating behaviour

Mounting Frequency (MF)

Mounting frequency in control group varied from 10 ± 0.2 to 13 ± 0.1 times per second (Table 1). While in pomegranate extract the range at the dosage of 500 mg/kg was 14 ± 0.1 to 15 ± 0.2 times per minute, 1000 mg/kg (23 ± 0.1 - 25 ± 0.2) and 1500 mg/kg (43 ± 0.2 - 45 ± 0.1) times per second. However in sildenafil, mounting frequency varied from 47 ± 0.3 to 49 ± 0.2 times per second. The mounting frequency of the rats in control test and standard group varied significantly (F = 383.9375 P = 1.34 E - 18).

Table 1: Mounting Frequency in seconds.

Test animal	**Control**		**Pomegranate**		**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	11 ± 0.2	14 ± 0.3	23 ± 0.1	43 ± 0.2	49 ± 0.2
2	12 ± 0.3	14 ± 0.1	25 ± 0.1	44 ± 0.3	48 ± 0.2
3	13 ± 0.1	14 ± 0.3	24 ± 0.3	44 ± 0.1	48 ± 0.1
4	11 ± 0.3	15 ± 0.2	23 ± 0.2	43 ± 0.3	47 ± 0.3
5	10 ± 0.2	15 ± 0.2	25 ± 0.2	45 ± 0.1	49 ± 0.2
Mean ± SEM	**11.40 ± 0.2**	**14.4 ± 0.2****	**24.00 ± 0.2*****	**43.80 ± 0.3*****	**48.20 ± 0.3*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01 and ***p < 0.001.

Intromission Frequency (IF)

The intromission frequency in animals treated with distilled water varied from 3 ± 0.3 to 5 ± 0.2 per second (Table 2). In pomegranate extract, at the dose of 500 mg/kg, the range was 3 ± 0.3 to 5 ± 0.3, 1000 mg/kg (7 ± 0.3 - 9 ± 0.1) and 1500 mg/kg (11 ± 0.2 - 13 ± 0.3) times per second. In addition, the intromission frequency in animals treated with sildenafil varied from 23 ± 0.1 to 25 ± 0.3 times per second. The intromission frequencies varied significantly between control, test group and standard group (F = 484.2 P = 1.34 E - 18).

Table 2: Intromission frequency in seconds

Test animal	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	5 ± 0.1	5 ± 0.3	8 ± 0.1	12 ± 0.3	24 ± 0.3
2	5 ± 0.2	4 ± 0.1	8 ± 0.2	11 ± 0.2	23 ± 0.1
3	3 ± 0.3	5 ± 0.2	7 ± 0.3	13 ± 0.1	25 ± 0.1
4	4 ± 0.3	5 ± 0.3	9 ± 0.1	12 ± 0.1	24 ± 0.2
5	5 ± 0.2	3 ± 0.3	8 ± 0.3	13 ± 0.3	25 ± 0.3
Mean ± SEM	**4.4±0.2**	**4.4±0.3^{NS}**	**8±0.2****	**12.2±0.2*****	**24.2±0.1*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

Mounting Latency (ML)

The mounting latency in animals treated with distilled water (Control) varied from 34 ± 0.1 to 36 ± 0.2 seconds (Table 3). In pomegranate fruit extract and at 500 mg/kg dosage, the range was 35 ± 0.1 to 37 ± 0.2, 1000 mg/kg (28 ± 0.1 - 30 ± 0.3) and 1500 mg/kg (22 ± 0.1 - 24 ± 0.3) seconds. In addition, the mounting latency in animals treated with sildenafil varied from 11 ± 0.1 to 13 ± 0.2 seconds. The mounting latency varied significantly between control, test and standard group (F = 687.1389 P = 4.23 E - 21).

Table 3: Mounting latency in seconds

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	35 ± 0.3	35 ± 0.1	28 ± 0.3	22 ± 0.1	11 ± 0.1
2	34 ± 0.1	36 ± 0.2	28 ± 0.2	22 ± 0.1	12 ± 0.1
3	36 ± 0.2	36 ± 0.2	29 ± 0.2	23 ± 0.2	12 ± 0.2
4	36 ± 0.1	37 ± 0.2	30 ± 0.3	24 ± 0.3	11 ± 0.3
5	35 ± 0.1	35 ± 0.1	28 ± 0.1	23 ± 0.2	13 ± 0.2
Mean ± SEM	**35.2±0.2**	**35.8±0.2^{NS}**	**28.6±0.3****	**22.8±0.3*****	**11.8±0.1*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

Intromission Latency (IL)

The intromission latency in distilled water varied from 39 ± 0.3 to 41 ± 0.1 seconds (Table 4). In pomegranate, at a concentration of 500 mg/kg, the range was 37 ± 0.2 to 39 ± 0.1, 1000 mg/kg (34 ± 0.1 - 35 ± 0.3) and 1500 mg/kg (27 ± 0.2 - 28 ± 0.2) seconds. In addition, the intromission latency in sildenafil ranged from 15 ± 0.1 - 15 ± 0.3 seconds. The intromission latency varied significantly between control, test and standard group (F = 65.81186 P = 3.17 E - 11).

Table 4: Intromission latency in seconds

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	40 ± 0.3	37 ± 0.2	34 ± 0.3	27 ± 0.3	15 ± 0.2
2	40 ± 0.2	39 ± 0.1	34 ± 0.2	27 ± 0.2	15 ± 0.1
3	41 ± 0.1	37 ± 0.2	35 ± 0.3	28 ± 0.2	15 ± 0.2
4	40 ± 0.2	38 ± 0.3	34 ± 0.1	28 ± 0.2	15 ± 0.3
5	39 ± 0.3	38 ± 0.2	34 ± 0.1	28 ± 0.2	15 ± 0.2
Mean ± SEM	**40±0.1**	**37.8±0.2^{NS}**	**34.2±0.2***	**27.6±0.1****	**15±0.2*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

Ejaculatory Latency in the first series (EL1, in sec)

The ejaculation latency in the first series in animals treated with distilled water varied from 198 ± 0.2 to 200 ± 0.3 seconds (Table 5). In pomegranate, at a concentration of 500 mg/kg, the range was 198 ± 0.1 to 213 ± 0.1, 1000 mg/kg (217 ± 0.2 - 219 ± 0.1) and 1500 mg/kg (232 ± 0.2 - 236 ± 0.2) seconds. However, animals treated with sildenafil varied from 343 ± 0.1 to 350 ± 0.2 seconds. The ejaculation latency in the first series varied significantly between control, test and standard group (F = 1236.018 P = 2.866).

Table 5: Ejaculatory latency in the first series in seconds

Test animal	**Control**		**Pomegranate**		**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	198 ± 0.2	211 ± 0.2	218 ± 0.3	235 ± 0.3	344 ± 0.3
2	199 ± 0.1	213 ± 0.1	217 ± 0.2	236 ± 0.2	339 ± 0.3
3	198 ± 0.2	200 ± 0.2	219 ± 0.1	232 ± 0.2	350 ± 0.2
4	200 ± 0.3	212 ± 0.3	218 ± 0.2	236 ± 0.1	344 ± 0.3
5	198 ± 0.3	198 ± 0.1	218 ± 0.2	236 ± 0.1	343 ± 0.1
Mean ± SEM	**198.6±0.3**	**206.8±0.2^{NS}**	**218±0.3***	**235±0.2*****	**344±0.3*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

Ejaculatory Latency in the second series (EL2, in sec)

The ejaculation latency in the second series in control group varied from 295 ± 0.2 to 299 ± 0.2 seconds (Table 6). Pomegranate fruit extract at the dosage of 500 mg/kg, the range was 298 ± 0.2 to 301 ± 0.2, 1000 mg/kg (317 ± 0.1 - 319 ± 0.2) and 1500 mg/kg (332 ± 0.1 - 336 ± 0.3) seconds. However, animals treated with in sildenafil varied from 3390.3 to 350 ± 0.2 seconds. The ejaculation latency in the second series varied significantly between animals treated with distilled water, pomegranate extracts and sildenafil citrate (F = 457.449 P = 2.38 E - 19).

Table 6: Ejaculatory latency in second series in seconds

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	297 ± 0.1	300 ± 0.2	318 ± 0.1	335 ± 0.2	344 ± 0.2
2	295 ± 0.2	298 ± 0.3	317 ± 0.1	336 ± 0.3	339 ± 0.3
3	296 ± 0.3	301 ± 0.2	319 ± 0.2	332 ± 0.1	350 ± 0.2
4	298 ± 0.1	301 ± 0.1	318 ± 0.3	336 ± 0.1	344 ± 0.2
5	299 ± 0.2	298 ± 0.2	318 ± 0.1	336 ± 0.3	343 ± 0.1
Mean ± SEM	**297±0.2**	**299.6±0.3^{NS}**	**318±0.2^{NS}**	**335±0.2*** **	**398±0.3*** **

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

Post Ejaculation Interval (PEI)

Post ejaculation interval (PEI) in the control group varied from 360 ± 0.3 to 366 ± 0.1 seconds (Table 7). Although pomegranate fruit extract at a concentration of 500 mg/kg, the range was 333 ± 0.1 to 339 ± 0.2, 1000 mg/kg (299 ± 0.1 - 304 ± 0.2) and 1500 mg/kg (223 ± 0.2 - 226 ± 0.1) seconds. However, the ejaculation interval in sildenafil varied from 98 ± 0.1 to 99 ± 0.3. The PEI varied significantly between animals treated with distilled water, pomegranate extracts and sildenafil (F = 17047.4 P = 5.17 E - 35).

Table 7: Post ejaculation interval in the second series in seconds

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	364 ± 0.1	336 ± 0.3	301 ± 0.2	224 ± 0.3	99 ± 0.2
2	365 ± 0.2	338 ± 0.3	300 ± 0.3	223 ± 0.3	99 ± 0.3
3	360 ± 0.3	335 ± 0.2	299 ± 0.1	226 ± 0.1	98 ± 0.1
4	366 ± 0.1	339 ± 0.2	304 ± 0.2	224 ± 0.2	98 ± 0.1
5	365 ± 0.1	333 ± 0.1	302 ± 0.2	223 ± 0.2	99 ± 0.2
Mean ± SEM	**364±0.2**	**336.2±0.2***	**301.2±0.3*** **	**224±0.2*** **	**98.6±0.3*** **

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *p < 0.05, **p < 0.01; ***p < 0.001.

4.3.2 Effect of pomegranate fruit extract on libido

Test for libido showed that the mounting frequency varied significantly between animals treated with distilled water, pomegranate fruit extracts and sildenafil citrate (F = 109.486 P = 2.67 E - 13). It displayed that the pomegranate extract at the dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, significantly raised the mounting frequency ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) as related to group 1 (control). Similarly, sildenafil citrate significantly raised the mounting frequency ($p < 0.001$) with respect to animals in treated with distilled water. However, intromission and Ejaculation were not available in control, pomegranate and sildenafil groups (Table 8).

Table 8: Effect of pomegranate fruit extract on libido test in male rats

Parameters	**Mean Frequency ± SEM**				
	Control		**Pomegranate**		**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Mounting Frequency (MF)	6.40 ± 0.24	7.40 ± 0.51*	8.00 ± 0.71**	14.60 ± 0.51***	22.60 ± 1.03***
Intromission Frequency (IF)	0	0	0	0	0
Ejaculation (Ej)	0	0	0	0	0

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant, *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$.

4.4 Effect of pomegranate fruit extract on sexual potency

Erections (E)

Test for sexual potency on number of Erections (E) showed that the pomegranate at a measure of 1500 mg/kg, and 1000 mg/kg significantly augmented E ($p < 0.001$) and ($p < 0.05$), respectively, in contrast with the group 1 (control). While the pomegranate at the dose of 500 mg/kg, did not alter the E, however the sildenafil citrate drug significantly raised the E ($p < 0.001$) with respect to the animals in control group.

The number of erections in control group varied from 7 ± 0.1 to 8 ± 0.1 per second (Table 9). While the pomegranate fruit, at a dosage of 500 mg/kg, the range was 7 ± 0.1 to 8 ± 0.3 erection frequencies per second, 1000 mg/kg (8 ± 0.1 - 9 ± 0.3) and 1500 mg/kg (12 ± 0.1 - 13 ± 0.2) erections per second. However the number of erections in sildenafil varied from 18 ± 0.2 to 20 ± 0.3 per second. The results in sexual potency revealed that varied significantly between

animals treated with distilled water, pomegranate extracts and sildenafil (F = 383.9375 P = 1.34 E - 18).

Table 9: Frequency of erections (E) in male rats per second

Test animal	Co ntrol	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	7 ± 0.1	8 ± 0.2	8 ± 0.2	12 ± 0.1	19 ± 0.3
2	8 ± 0.1	7 ± 0.1	9 ± 0.3	13 ± 0.1	20 ± 0.3
3	7 ± 0.2	7 ± 0 .1	8 ± 0.1	13 ± 0.2	19 ± 0.1
4	8 ± 0.1	8 ± 0.3	8 ± 0.1	12 ± 0.3	18 ± 0.2
5	8 ± 0.1	7 ± 0.2	8 ± 0.2	12 ± 0.3	19 ± 0.2
Mean ± SEM	**7.6±0.1**	**7.4±0.2^{NS}**	**8.2±0.3***	**12.4±0.2*****	**19±0.2*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

Quick Flips (QF)

Test for sexual potency on quick flips (QF) exhibited that pomegranate fruit at a dosage of 1500 mg/kg significantly raised the number of QF ($p < 0.001$). Nonetheless it did not significantly affect the QF at the measure of 1000 mg/kg in comparison with the group treated with distilled water. However the sildenafil citrate significantly upsurge the QF ($p < 0.001$) with respect to the animals in group 1.

Quick flips varied from 5 ± 0.1 to 6 ± 0.2 times per second in control (Table 10). Whereas the extract at the dose of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, the variation was from (5 ± 0.1 - 6 ± 0.2), (5 ± 0.1 - 6 ± 0.3) and (8 ± 0.1 - 9 ± 0.1) respectively, per second. However sildenafil, the variation in QF was 17 ± 0.1 to 18 ± 0.2 per second. There was a significant variation in Quick flips between control test and standard group (F = 502.4615 P = 9.4 E - 20).

Table 10: Quick flips (QF).

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	5 ± 0.2	5 ± 0.3	5 ± 0.3	8 ± 0.1	17 ± 0.1
2	5 ± 0.3	6 ± 0.2	6 ± 0.3	8 ± 0.3	17 ± 0.2
3	5 ± 0.2	5 ± 0.3	5 ± 0.1	8 ± 0.2	17 ± 0.3
4	6 ± 0.2	5 ± 0.1	6 ± 0.1	9 ± 0.2	18 ± 0.2
5	5 ± 0.1	6 ± 0.2	6 ± 0.2	9 ± 0.1	17 ± 0.1
Mean ± SEM	**5.2±0.1**	**5.4±0.1^{NS}**	**5.6±0.2^{NS}**	**8.4±0.1*** **	**17.2±0.2*** **

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

Long Flips (LF)

Test for sexual potency on long flips (LF) showed that the pomegranate at a dose of 1500 mg/kg and 1000 mg/kg significantly augmented the frequency of LF ($p < 0.001$) and ($p < 0.01$), respectively, in comparison with the control group. The pomegranate at a dosage of 500 mg/kg altered not the LF. However the sildenafil citrate significantly raised the LF ($p < 0.001$) with respect to the control animals.

Long flips in animals treated with distilled water varied from 2 ± 0.1 to 3 ± 0.2 per second (Table 11). In 500 mg/kg pomegranate, the variation was from 3 ± 0.1 to 4 ± 0.3, 1000 mg/kg (4 ± 0.2 - 5 ± 0.3) and 1500 mg/kg (8 ± 0.1 - 9 ± 0.3) per second. However, in sildenafil, the variation in long flips was 12 ± 0.1 to 13 ± 0.3 per second. There was a significant variation in long flips between distilled water, pomegranate extracts and sildenafil citrate (F = 283.8 P = 2.62 E - 17).

Table 11: Long flips

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	2 ± 0.1	3 ± 0.3	5 ± 0.1	8 ± 0.2	12 ± 0.2
2	3 ± 0.2	4 ± 0.3	4 ± 0.3	8 ± 0.2	13 ± 0.1
3	3 ± 0.2	4 ± 0.1	5 ± 0.3	9 ± 0.3	13 ± 0.3
4	2 ± 0.3	3 ± 0.2	5 ± 0.2	8 ± 0.1	12 ± 0.1
5	2 ± 0.1	3 ± 0.1	4 ± 0.2	9 ± 0.2	12 ± 0.1
Mean ± SEM	**2.4±0.2**	**3.4±0.2^{NS}**	**4.6±0.1****	**8.4±0.2*****	**12±0.3*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

Total Penile Reflexes (TPR)

The results for sexual potency showed that the pomegranate fruit at a dosage of 1500 mg/kg and 1000 mg/kg significantly augmented the collective of these penile reflexes at ($p < 0.001$) and ($p < 0.05$), respectively, in comparison to the group treated with distilled water. The fruit extract at 500 mg/kg did not alter the Total Penile Reflexes (TPR). However the sildenafil citrate significantly augmented the TPR ($p < 0.001$) with respect to the animals in group 1.

The Total Penile Reflexes (TPR) in animals treated with distilled water varied from 14 ± 0.1 to16 ± 0.3 per second (Table 12). However, in 500 mg/kg of the fruit extract, the variation was from 16 ± 0.1 to 17 ± 0.3, 1000 mg/kg (18 ± 0.1 - 19 ± 0.1) and 1500 mg/kg (28 ± 0.2 - 30 ± 0.3) per second. In addition, in sildenafil, the variation was 48 ± 0.1 to 49 ± 0.2 per second. There was a significant variation in the aggregate of these penile reflexes between control, test and standard group (F = 2182.636 P = 4.27 E - 26).

Table 12: Total penile reflexes

Test animal	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	14 ± 0.1	16 ± 0.2	18 ± 0.3	28 ± 0.2	48 ± 0.1
2	16 ± 0.2	17 ± 0.2	19 ± 0.2	29 ± 0.3	49 ± 0.2
3	15 ± 0.2	17 ± 0.3	18 ± 0.1	30 ± 0.1	48 ± 0.3
4	16 ± 0.3	16 ± 0.2	19 ± 0.1	29 ± 0.2	48 ± 0.2
5	15 ± 0.2	16 ± 0.1	18 ± 0.2	30 ± 0.3	48 ± 0.2
Mean ± SEM	**15.2±0.1**	**16.4±0.2^{NS}**	**18.4±0.2***	**29.2±0.3*****	**48.2±0.1*****

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

4.5 Effect of pomegranate fruit extract on the concentration of testosterone

The data collected with the assessment for testosterone concentration showed that pomegranate fruit extract at the dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg, significantly increased testosterone levels ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) as related to group 1 (control). Similarly sildenafil citrate significantly augmented the testosterone levels ($p < 0.001$) with respect to the rats treated with distilled water (Table 13). The data therefore, affirms evidence for the significant difference in mean testosterone concentration between the treatment groups.

Table 13: Testosterone concentrations in mg/ml x 10^{-3}

Parameters	Mean ± SEM				
	Control		Pomegranate		Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Testosterone (mg/ml*10^{-3})	2.41 ± 0.57	3.11 ± 0.20 *	3.99 ± 0.12**	4.58 ± 0.20***	4.77 ± 0.36***

Tabular values are mean ± SEM, n = 5; significant difference from control, NS: Not significant. *p < 0.05, **p < 0.0 and ***p < 0.001.

Absorbance of light by testosterone

The absorbance values increased from 0.01 at a concentration of 0 mg/ml x 10^{-3} to 1.02 at a concentration of 14 mg/ml x 10^{-3} (Figure 3). However, there was a strong relationship between concentration (r = 0.9995).

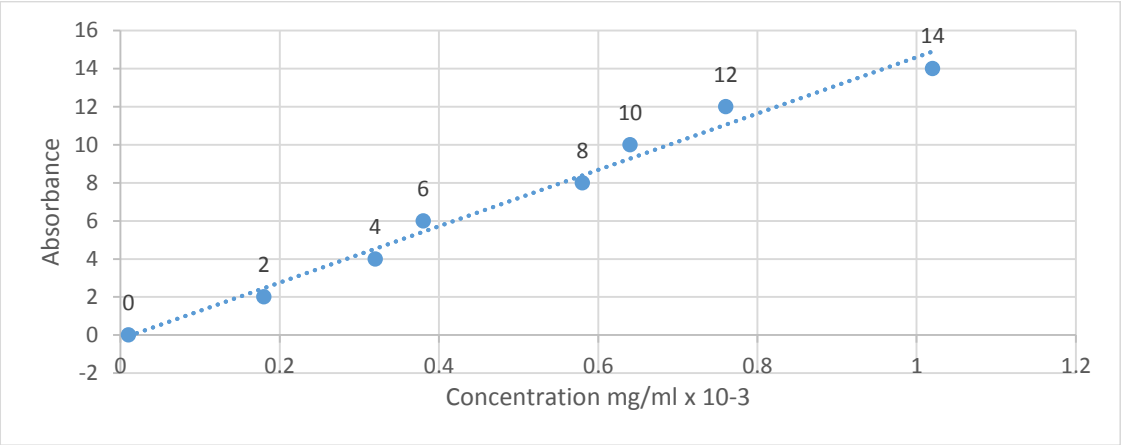

Figure 3: Relationship between BCA absorbance and concentration

CHAPTER FIVE

DISCUSSION

This study was aimed at investigating the effect of pomegranate fruit extract on the sexual function along with its acute toxicity using an animal model. The search reveals a distinct change in sexual behaviour of male rats.

5.1 Effect of pomegranate fruit extract on mating behaviour and libido

5.1.1 Effect of pomegranate fruit extract on mating behaviour

This investigation confirm that pomegranate fruit extract, significantly augmented the Mounting Frequency (MF) and Intromission Frequency (IF) as related to rats treated with distilled water. Nevertheless, sildenafil citrate exhibited a higher increase in these parameters. This may be attributed to the nature of pomegranate as compared to the pure form of sildenafil (Adukondalu *et al*., 2010). The results agreed with a previous study by Fedder *et al*., (2014). The possible results could be attributed to extraction of the same compounds in the two studies.

The significant escalation in the Ejaculatory Latency (EL) put forward that pomegranate fruit extract and sildenafil citrate elongated the period of coitus. The significant rise in the EL in both first and second series and the reduction in Post Ejaculatory Interval (PEI), which is the refractory period between first and second series of mating, indicates that the pomegranate fruit extract portrayed an intensity in sexual activity in an unremitting way. The results coincided with those of a study carried out in Japan by Misaka *et al*., (2011).

The pomegranate fruit extract showed a significant fallout in the Mounting Latency (ML) with comparison to group 1, while a highly significant reduction was noted in the ML, of rats administered with the reference drug, Sildenafil citrate. This agreed with a study carried out by Zeweil *et al*., (2013) in Egypt. This may be attributed to the plants from which the extracts were obtained accumulating the same active compounds. This also gives proof for aphrodisiac effect of the pomegranate fruit extract. These outcomes indicate that pomegranate fruit extract yields a remarkable improvement of all round sexual performance of ordinary animals.

The pomegranate fruit extract also lead to a significant decline in mean Intromission latency (IL) as likened to group 1 (control group). This can be associated with increased concentration of the active compounds within the pomegranate extracts. This disagreed with an earlier study by Qu *et al*., (2010). The variations in the results in the two studies may be associated by the differences in the ecological environment in which the pomegranates were growing.

5.1.2 Effect of pomegranate fruit extract on libido

Since Mounting Frequency (MF) after penile anaesthetization of rats is a dependable index of 'pure' libido or intrinsic sexual desire while reflexes of the penis in rats are good model of 'pure' sexual potency (Davidson, 1982), the pomegranate extract in this study was also evaluated for effect on these constituents of sexual behaviour.

The effectiveness of pomegranate fruit extract, on sex drive was investigated by evaluating the Mounting Frequency (MF) after genital anaesthetization where by it usually do away with the reinforcing effect of intrinsic sexual desire. In this study, or rather experiment, pomegranate fruit extract formed a significant rise in MF of sexually normal male rats despite the fact that, MF was much lowered in control, pomegranate and sildenafil as compared with the mounting frequency of corresponding groups in mating behaviour where the penis had not been anaesthetized. This could be attributed to low concentration of the active compounds in crude pomegranate (Leiva *et al*., 2011). Nevertheless, the experiment produced results which indicated that Intromission as well as Ejaculation parameters were absent in group 1, 2, 3, 4 and 5. The genital sensations were absent due to penile anaesthetization, thus, it may be inferred that the pomegranate fruit extract, had an outstanding augmentation in the intrinsic libido (sex drive).

The mounting frequency and intromission frequency are well-thought-out as the directories of both sex drive and sexual potency. Therefore, the upsurge in the Mounting Frequency and Intromission Frequency point out that pomegranate fruit extract, along with cumulative libido, perhaps it proliferates potency as well.

5.2 Effect of pomegranate fruit extract on sexual potency

The sexual potency test demonstrated that pomegranate fruit extract significantly augmented the occurrence of all the constituents of penile reflexes: Erections (E), Quick Flips (QF) and Long Flips (LF) in comparison to group 1, nevertheless, relatively lower than sildenafil citrate, group 5. The combination of these reflexes (Total Penile Reflexes) was also significantly raised in animals treated with pomegranate. However, sildenafil showed a great presence of the active metabolites associated with high concentration of the extracts. The results were comparable to those of a similar study in effects of pomegranate peel as antıoxıdant supplementatıon on dıgestıbılıty, blood bıochemıcal and rabbıt semen qualıty (Fayed *et al*., 2012). The imaginable reason for the difference may possibly be the soil physico-chemical features in which the plants were growing (Tang *et al*., 2017). Riad *et al*., (2016) asserted that the composition of the soil

that pomegranate grows, greatly influences the metabolites it will synthesis (ShahuAlakh, 2014).

This indicates that the pomegranate fruit extract increases sexual potency. This agreed with a study carried out on protective role of pomegranate peel extract on testis in adult male rabbits treated with pomegranate crude extracts by (Hussen and Arrack, 2014). Possible reason could be use of the same solvents with the same polarity in extracting active compound from pomegranate in the two studies (Chauhan and Dixit, 2010).

5.3 Effect of pomegranate fruit extract on concentration of testosterone

The findings of this test similarly confirmed that regular intake of pomegranate for eight weeks significantly increased testosterone levels at a dosage of 1500 mg/kg as equated to the group treated with distilled water but rather lower than sildenafil citrate. These results agree with the findings of Türk *et al.,* (2008) on male rats treated with pomegranate juice and those of Emad and Nacer (2014) who reported similar results on healthy male and female volunteers.

In addition, the absorbance of light increased with concentration of testosterone (Figure 3). The results agreed with previous studies carried out on effect of pomegranate pretreatment on the oral bioavailability of buspirone in male albino rats (Shravan *et al*., 2011). Similarity in the metabolic activities of the rats may be a contributing factor (Sharma *et al*., 2012).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Pomegranate fruit extract gives the impression of possessing sexual improvement properties as mirrored in the sexual behaviour studies and testosterone concentration measurements.

a) The aphrodisiac effects of the pomegranate fruit extract may be due to its nervous stimulating properties and it shows an outstanding improvement of overall sexual act of normal rats.
b) The pomegranate fruit extract exhibited a remarkable rise in the intrinsic sex drive or 'pure' libido.
c) Along with increasing libido, pomegranate fruit extract also increased potency.
d) The pomegranate fruit extract increases levels of testosterone which promote sexual behaviour, further increasing libido and sexual activity.

The resulting significant and continued rise in the sexual activity and testosterone concentration of healthy rats with on visible adversarial effects and acute toxicity, put forward that the pomegranate fruit extract contains clinical aphrodisiac properties, and that it offers support to the assertions for its outdated use as sexual function improving medicine.

6.2 Recommendations

There is a positive relationship between pomegranate fruit extract consumption and sexual function parameters. However, pomegranate merits:

a) Long-term studies with a larger population and using pomegranate from different climatic conditions are required to corroborate these effects and to provide direction for future recommendations.
b) Further study is required to identify and isolate the active component (s) liable for sexual function enhancing activities.
c) Future studies should consider identifying the mechanism by which the pomegranate fruit extract augments sexual function.
d) Future studies should be explored further, if the drug improves nitrergic transmission in the cavernous tissue to promote sexual behaviour.

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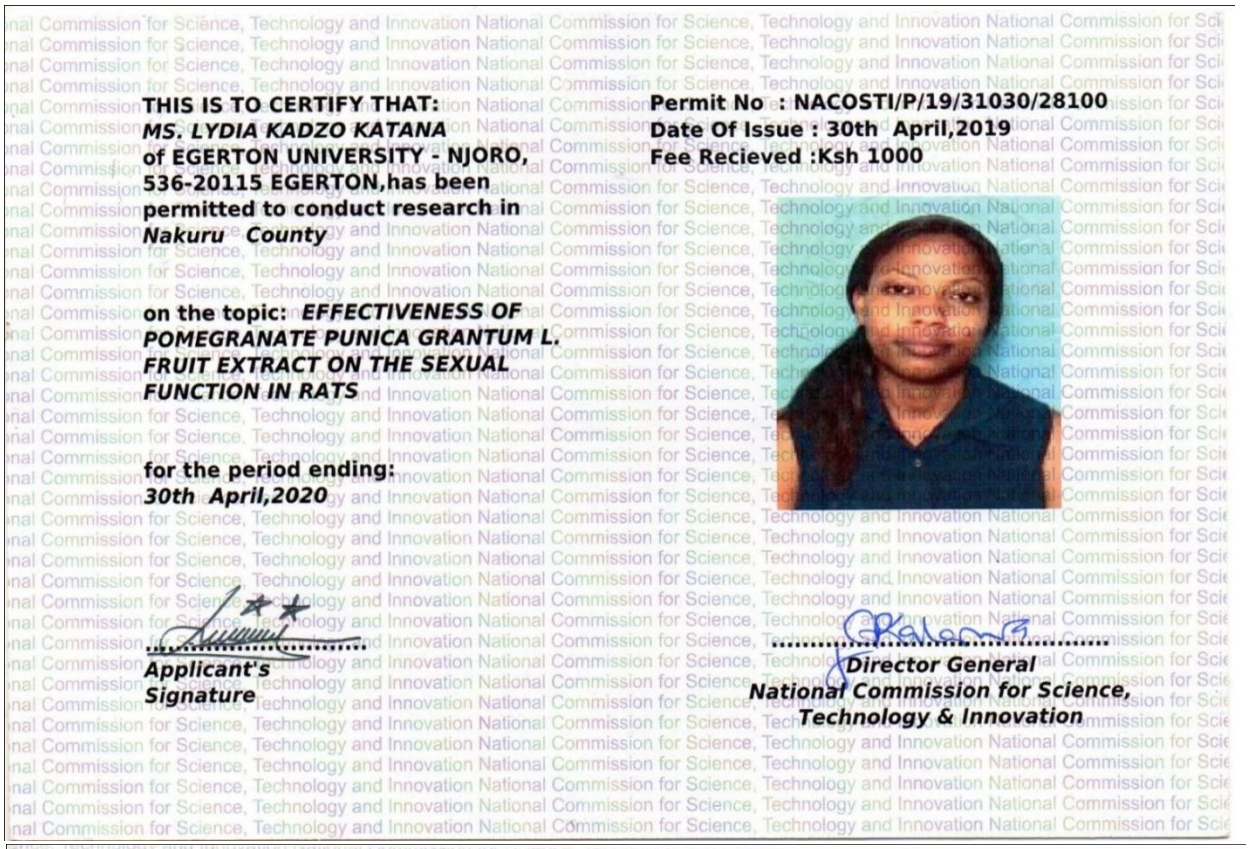
APPENDICES

Appendix 1: Research permit



THIS IS TO CERTIFY THAT:
MS. LYDIA KADZO KATANA
of EGERTON UNIVERSITY - NJORO, 536-20115 EGERTON,has been permitted to conduct research in *Nakuru County*

on the topic: *EFFECTIVENESS OF POMEGRANATE PUNICA GRANTUM L. FRUIT EXTRACT ON THE SEXUAL FUNCTION IN RATS*

for the period ending:
30th April,2020

Permit No : NACOSTI/P/19/31030/28100
Date Of Issue : 30th April,2019
Fee Recieved :Ksh 1000

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014.

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CONDITIONS: see back page

Appendix 2: Research Authorization

THE PRESIDENCY
MINISTRY OF INTERIOR AND
CO-ORDINATION OF NATIONAL GOVERNMENT

Telegram: "DISTRICTER" Nakuru
Telephone: Nakuru 051-2212515
When replying please quote

COUNTY COMMISSIONER
NAKURU COUNTY
P.O. BOX 81
NAKURU.

Ref No. CC. SR . EDU/12/1/2 VOL.IV/109

11th June, 2019

Deputy County Commissioner
NJORO SUB COUNTY

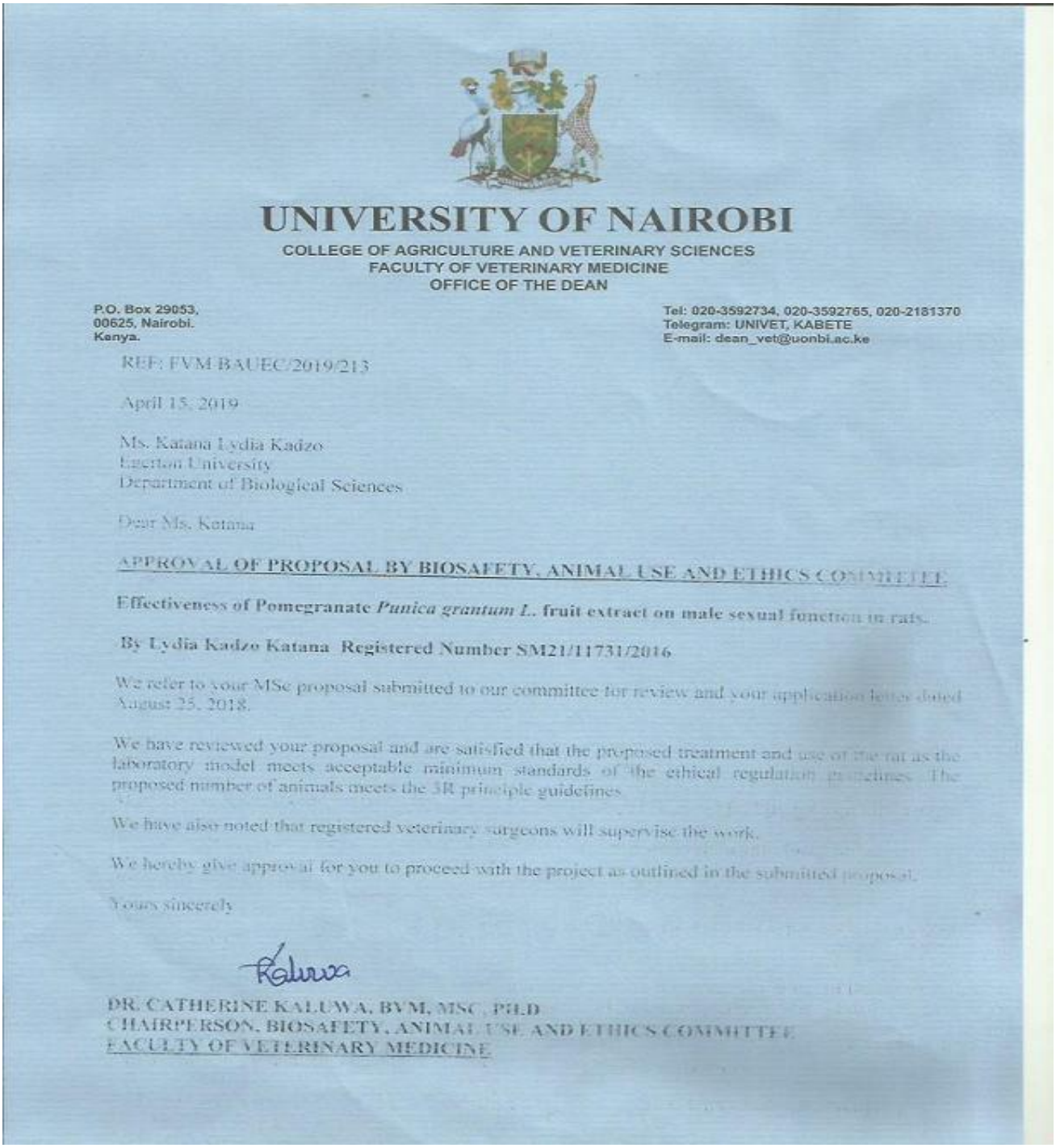
RE:- RESEARCH AUTHORIZATION – LYDIA KADZO KATANA

The above named student from Egerton University has been authorized to carry out research on **"effectiveness of pomegranate punica grantum l. fruit extract on the sexual function in rats""** in all sub counties in Nakuru County for a period ending 30th April, 2020

Please accord him all the necessary support to facilitate the success of his research.

MARY W. MWANGI
FOR COUNTY COMMISSIONER
NAKURU COUNTY

Appendix 3: Bioethics permit



UNIVERSITY OF NAIROBI

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES
FACULTY OF VETERINARY MEDICINE
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REF: FVM-BAUEC/2019/213

April 15, 2019

Ms. Katana Lydia Kadzo
Egerton University
Department of Biological Sciences

Dear Ms. Katana

APPROVAL OF PROPOSAL BY BIOSAFETY, ANIMAL USE AND ETHICS COMMITTEE

Effectiveness of Pomegranate *Punica grantum L.* fruit extract on male sexual function in rats.

By Lydia Kadzo Katana Registered Number SM21/11731/2016

We refer to your MSc proposal submitted to our committee for review and your application letter dated August 25, 2018.

We have reviewed your proposal and are satisfied that the proposed treatment and use of the rat as the laboratory model meets acceptable minimum standards of the ethical regulation guidelines. The proposed number of animals meets the 3R principle guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

DR. CATHERINE KALUWA, BVM, MSC., PH.D
CHAIRPERSON, BIOSAFETY, ANIMAL USE AND ETHICS COMMITTEE
FACULTY OF VETERINARY MEDICINE

Appendix 4: Effects of pomegranate fruit extract on mating behaviour

a) Mounting Frequency (MF)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	11	14	23	43	49
2	12	14	25	44	48
3	13	14	24	44	48
4	11	15	23	43	47
5	10	15	25	45	49
Sum >	**57**	**72**	**120**	**219**	**241**
Mean >	**11.4**	**14.4**	**24**	**43.8**	**48.2**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	38	7.6	0.3
Pomegranate (500 mg/kg)	5	37	7.4	0.3
Pomegranate (1000 mg/kg)	5	41	8.2	0.2
Pomegranate (1500 mg/kg)	5	62	12.4	0.3
Sildenafil (5 mg/kg)	5	95	19	0.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	491.44	4	122.86	383.9375	1.34E-18	2.866081
Error	6.4	20	0.32			
Total	497.84	24				

b) Intromission Frequency (IF)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	5	5	8	12	24
2	5	4	8	11	23
3	3	5	7	13	25
4	4	5	9	12	24
5	5	3	8	13	25
Sum >	**22**	**22**	**40**	**61**	**121**
Mean >	**4.4**	**4.4**	**8**	**12.2**	**24.2**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	22	4.4	0.8
Pomegranate (500 mg/kg)	5	22	4.4	0.8
Pomegranate (1000 mg/kg)	5	40	8	0.5
Pomegranate (1500 mg/kg)	5	61	12.2	0.7
Sildenafil (5 mg/kg)	5	121	24.2	0.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	1355.76	4	338.94	484.2	1.36E-19	2.866081
Error	14	20	0.7			
Total	1369.76	24				

c) Mounting Latency (ML, in sec)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	35	35	28	22	11
2	34	36	28	22	12
3	36	36	29	23	12
4	36	37	30	24	11
5	35	35	28	23	13
Sum >	**176**	**179**	**143**	**114**	**59**
Mean >	**35.2**	**35.8**	**28.6**	**22.8**	**11.8**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	176	35.2	0.7
Pomegranate (500 mg/kg)	5	179	35.8	0.7
Pomegranate (1000 mg/kg)	5	143	28.6	0.8
Pomegranate (1500 mg/kg)	5	114	22.8	0.7
Sildenafil (5 mg/kg)	5	59	11.8	0.7

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Treatments	1978.96	4	494.74	687.1389	4.23E-21	2.866081
Error	14.4	20	0.72			
Total	1993.36	24				

d) Intromission Latency (IL, in sec)

Test animals	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	40	37	34	27	15
2	40	39	34	27	15
3	41	37	35	28	15
4	40	38	34	28	15
5	39	38	34	28	15
Sum >	**200**	**189**	**171**	**138**	**75**
Mean >	**40**	**37.8**	**34.2**	**27.6**	**15**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	200	40	0.5
Pomegranate (500 mg/kg)	5	192	38.4	1.3
Pomegranate (1000 mg/kg)	5	177	35.4	6.8
Pomegranate (1500 mg/kg)	5	151	30.2	30.2
Sildenafil (5 mg/kg)	5	75	15	0

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Treatments	2042.8	4	510.7	65.81186	3.17E-11	2.866081
Error	155.2	20	7.76			
Total	2198	24				

e) Ejaculatory Latency – In first series (EL1, in sec)

Test animals	Control		Pomegranate		Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	198	211	218	235	344
2	199	213	217	236	339
3	198	200	219	232	350
4	200	212	218	236	344
5	198	198	218	236	343
Sum >	**993**	**1034**	**1090**	**1175**	**1720**
Mean >	**198.6**	**206.8**	**218**	**235**	**344**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	993	198.6	0.8
Pomegranate (500 mg/kg)	5	1034	206.8	51.7
Pomegranate (1000 mg/kg)	5	1090	218	0.5
Pomegranate (1500 mg/kg)	5	1175	235	3
Sildenafil (5 mg/kg)	5	1720	344	15.5

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Treatments	70700.24	4	17675.06	1236.018	1.24E-23	2.866081
Error	286	20	14.3			
Total	70986.24	24				

f) Ejaculatory Latency – In second series (EL2, in sec)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	297	300	318	335	344
2	295	298	317	336	339
3	296	301	319	332	350
4	298	301	318	336	344
5	299	298	318	336	343
Sum >	**1485**	**1498**	**1590**	**1675**	**1720**
Mean >	**297**	**299.6**	**318**	**335**	**344**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	1485	297	2.5
Pomegranate (500 mg/kg)	5	1498	299.6	2.3
Pomegranate (1000 mg/kg)	5	1590	318	0.5
Pomegranate (1500 mg/kg)	5	1675	335	3
Sildenafil (5 mg/kg)	5	1720	344	15.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	8709.84	4	2177.46	457.4496	2.38E-19	2.866081
Error	95.2	20	4.76			
Total	8805.04	24				

g) Ejaculatory Latency – In second series (EL2, in sec)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	297	300	319	358	398
2	295	298	321	360	390
3	296	301	315	353	401
4	298	302	320	359	401
5	299	279	319	360	399
Sum >	**1485**	**1480**	**1594**	**1790**	**1989**
Mean >	**297**	**296**	**318.8**	**358**	**397.8**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	1485	297	2.5
Pomegranate (500 mg/kg)	5	1480	296	92.5
Pomegranate (1000 mg/kg)	5	1594	318.8	5.2
Pomegranate (1500 mg/kg)	5	1790	358	8.5
Sildenafil (5 mg/kg)	5	1989	397.8	20.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	38446.64	4	9611.66	371.3934	1.86E-18	2.866081
Error	517.6	20	25.88			
Total	38964.24	24				

h) Post Ejaculatory Interval in second series (PEI, in sec)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	364	336	301	224	99
2	365	338	300	223	99
3	360	335	299	226	98
4	366	339	304	224	98
5	365	333	302	223	99
Sum >	**1820**	**1681**	**1506**	**1120**	**493**
Mean >	**364**	**336.2**	**301.2**	**224**	**98.6**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	1820	364	5.5
Pomegranate (500 mg/kg)	5	1681	336.2	5.7
Pomegranate (1000 mg/kg)	5	1506	301.2	3.7
Pomegranate (1500 mg/kg)	5	1120	224	1.5
Sildenafil (5 mg/kg)	5	493	98.6	0.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	227753.2	4	56938.3	17047.4	5.17E-35	2.866081
Error	66.8	20	3.34			
Total	227820	24				

Appendix 5: Effect of pomegranate fruit extract on libido

a) **Mounting Frequency (MF)**

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	6	7	8	14	23
2	7	9	6	15	25
3	6	6	7	16	22
4	6	8	10	13	24
5	7	7	9	15	19
Sum >	**32**	**37**	**40**	**73**	**113**
Mean >	**6.4**	**7.4**	**8**	**14.6**	**22.6**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	32	6.4	0.3
Pomegranate (500 mg/kg)	5	37	7.4	1.3
Pomegranate (1000 mg/kg)	5	40	8	2.5
Pomegranate (1500 mg/kg)	5	73	14.6	1.3
Sildenafil (5 mg/kg)	5	113	22.6	5.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	937.2	4	234.3	109.486	2.67 E-13	2.866081
Error	42.8	20	2.14			
Total	980	24				

b) **Intromission Frequency (IF)**

Test animals	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	NIL	NIL	NIL	NIL	NIL
2	NIL	NIL	NIL	NIL	NIL
3	NIL	NIL	NIL	NIL	NIL
4	NIL	NIL	NIL	NIL	NIL
5	NIL	NIL	NIL	NIL	NIL
Sum >	**0**	**0**	**0**	**0**	**0**
Mean >	**0**	**0**	**0**	**0**	**0**

c) **Ejaculation (Ej)**

Test animals	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	Absent	Absent	Absent	Absent	Absent
2	Absent	Absent	Absent	Absent	Absent
3	Absent	Absent	Absent	Absent	Absent
4	Absent	Absent	Absent	Absent	Absent
5	Absent	Absent	Absent	Absent	Absent
Sum >	0	0	0	0	0
Mean >	0	0	0	0	0

Appendix 6: Effects of pomegranate fruit extract on sexual potency

a) Erections (E)

Test animals	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	7	8	8	12	19
2	8	7	9	13	20
3	7	7	8	13	19
4	8	8	8	12	18
5	8	7	8	12	19
Sum >	**38**	**37**	**41**	**62**	**95**
Mean >	**7.6**	**7.4**	**8.2**	**12.4**	**19**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	38	7.6	0.3
Pomegranate (500 mg/kg)	5	37	7.4	0.3
Pomegranate (1000 mg/kg)	5	41	8.2	0.2
Pomegranate (1500 mg/kg)	5	62	12.4	0.3
Sildenafil (5 mg/kg)	5	95	19	0.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	491.44	4	122.86	383.937 5	1.34E-18	2.866081
Error	6.4	20	0.32			
Total	497.84	24				

b) Quick Flips (QF)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	5	5	5	8	17
2	5	6	6	8	17
3	5	5	5	8	17
4	6	5	6	9	18
5	5	6	6	9	17
Sum >	**26**	**27**	**28**	**42**	**86**
Mean >	**5.2**	**5.4**	**5.6**	**8.4**	**17.2**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	26	5.2	0.2
Pomegranate (500 mg/kg)	5	27	5.4	0.3
Pomegranate (1000 mg/kg)	5	28	5.6	0.3
Pomegranate (1500 mg/kg)	5	42	8.4	0.3
Sildenafil (5 mg/kg)	5	86	17.2	0.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	522.56	4	130.64	502.4615	9.4 E-20	2.866081
Error	5.2	20	0.26			
Total	527.76	24				

c) Long Flips (LF)

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	2	3	5	8	12
2	3	4	4	8	13
3	3	4	5	9	13
4	2	3	5	8	12

5	2	3	4	9	12
Sum >	**12**	**17**	**23**	**42**	**62**
Mean >	**2.4**	**3.4**	**4.6**	**8.4**	**12**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	12	2.4	0.3
Pomegranate (500 mg/kg)	5	17	3.4	0.3
Pomegranate (1000 mg/kg)	5	23	4.6	0.3
Pomegranate (1500 mg/kg)	5	42	8.4	0.3
Sildenafil (5 mg/kg)	5	62	12.4	0.3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	340.56	4	85.14	283.8	2.62E-17	2.866081
Error	6	20	0.3			
Total	346.56	24				

d) Total Penile Reflexes (TPR)

Test animals	**Control**		**Pomegranate**		**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	14	16	18	28	48
2	16	17	19	29	49
3	15	17	18	30	48
4	16	16	19	29	48
5	15	16	18	30	48
Sum >	**76**	**82**	**92**	**146**	**241**
Mean >	**15.2**	**16.4**	**18.4**	**29.2**	**48.2**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	76	15.2	0.7
Pomegranate (500 mg/kg)	5	82	16.4	0.3
Pomegranate (1000 mg/kg)	5	92	18.4	0.3
Pomegranate (1500 mg/kg)	5	146	29.2	0.7
Sildenafil (5 mg/kg)	5	241	48.2	0.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	3841.44	4	960.36	2182.636	4.27E-26	2.866081
Error	8.8	20	0.44			
Total	3850.24	24				

Appendix 7: Testosterone absorbance values

Test animals	**Control**	**Pomegranate**			**Sildenafil**
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
1	0.086	0.16	0.225	0.284	0.308
2	0.236	0.194	0.219	0.219	0.319
3	0.16	0.137	0.202	0.275	0.274
4	0.132	0.18	0.233	0.264	0.236
5	0.049	0.199	0.241	0.248	0.218
Sum >	**0.663**	**0.87**	**1.12**	**1.29**	**1.355**
Mean >	**0.1326**	**0.174**	**0.224**	**0.258**	**0.271**

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	0.663	0.1326	0.005151
Pomegranate (500 mg/kg)	5	0.87	0.174	0.000656
Pomegranate (1000 mg/kg)	5	1.12	0.224	0.00022
Pomegranate (1500 mg/kg)	5	1.29	0.258	0.000656
Sildenafil (5 mg/kg)	5	1.355	0.271	0.001929

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	0.067447	4	0.016862	9.789858	0.000149	2.866081
Error	0.034447	20	0.001722			
Total	0.101894	24				

Appendix 8: Absorbance of Bicalutamide vs. Concentrated at 272 nm obtained from rat serum testosterone.

Concentrated verses Absorbance of Bicalutamide at 272 nm

Concentration mg/ml x 10^{-3}	0	2	4	6	8	10	12	14
Absorbance	0.01	0.18	0.32	0.38	0.58	0.64	0.76	1.02

Y= 57.36 x -0.0048

R^2 = 0.9995

Correlation coefficient

Appendix 9: Effect of pomegranate fruit extract on testosterone concentration

F = 9.112653; P = 0.000232; FCRI = 2.866081 n = 5

Since F calculated (9.112653) is greater than F Critical (2.866081) we reject H_0 and conclude that the data provides enough evidence for the significant difference in mean concentrations.

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (10 ml/kg)	5	12.04	2.408	1.62352
Pomegranate (500 mg/kg)	5	15.55	3.11	0.2049
Pomegranate (1000 mg/kg)	5	19.95	3.99	0.06705
Pomegranate (1500 mg/kg)	5	22.9	4.58	0.19845
Sildenafil (5 mg/kg)	5	23.86	4.772	0.64892

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Treatments	19.99564	4	4.99891	9.112653	0.000232	2.866081
Error	10.97136	20	0.548568			
Total	30.967	24				

Appendix 10: Research publication

ABSTRACT

Background: Pomegranate (*Punica granatum* L.) has been mentioned to be of value in the management of male sexual disorders. This study investigated the effects of pomegranate fruit extract on healthy male rats as the animal model.

Materials and methods: 50 adult male and female Albino rats of Wistar strain weighing 250-350 g and 200-250g respectively were used in this study. The pomegranate extract was administered (500, 1000 and 1500 mg/kg) to different groups of male rats on a once-daily regime throughout the experiment period. The general mating behaviour, libido, potency and testosterone concentration were studied and compared with sildenafil citrate.

Results: Administration of the pomegranate extract orally at the dose of 1500 mg/kg produced significant augment ofsexual activity in male rats. The mounting frequency, intromission frequency, mounting latency, intromission latency, ejaculation latency and post ejaculation interval did not vary significantly. However, the mounting frequency varied significantly between control, pomegranate crude extracts and sildenafil. In addition, the potency of the extracts did not vary significantly between control, pomegranate crude extracts and sildenafil. The testosterone levels varied significantly between pomegranate, sildenafil and control.

Conclusion: Pomegranate extracts have the potential of increasing sexual behaviour in rats. There is need for mass production of pomegranate extracts for use in enhancing sexual

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