KHAT (*Catha edulis*) EXTRACT USE AND ITS EFFECTS FOLLOWING WITHDRAWAL ON ENDOCRINE AND GONADAL FUNCTION IN MALE RABBITS

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October 2019
DECLARATION

This thesis is my original work and has not been presented for award of a degree or any other award in any other University

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DEDICATION

This thesis is dedicated to my parents whose sacrifice and financial support has seen me through my Master’s program. I also dedicate to my siblings for their support and encouragement during my entire study period.
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<td>Adrenocorticotropic Hormone</td>
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<td>ANOVA</td>
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<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary Deoxyribonucleic acid</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>Loop of Henle</td>
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<td>Luteinizing Hormone</td>
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PCT  Proximal Convoluted Tubule
PPE  Personal Protection Equipment
UN   United Nations
UNNL United Nations Narcotics Laboratory
UK   United Kingdom
USA  United States of America
RNA  Ribonucleic Acid
SER  Smooth Endoplasmic Reticulum
TMB  Tetra Methyl Benzene
TNF  Tumour Necrosis Factor
WHO  World Health Organisation
ABSTRACT

Gonadal and endocrine effects following withdrawal from sub-chronic to chronic khat (*Catha edulis*) use remains scanty. Most literature available, though contradictory, highlights on effects of khat on functional systems of the body including the reproductive system in humans and experimental animals during khat exposure. This information, to a large extent, does not provide insights into the possible recovery of ‘khat addicts’ from the vice. The aim of this study was to investigate effects of khat extract during sub-chronic exposure and accompanying effects on reproductive function in male rabbits following withdrawal from use. Fresh leaves and shoots of khat were weighed, crushed with mortar and pestle, and dissolved in distilled water in a conical flask for extraction. The working volume of the stock solution for each dose was obtained by factoring in the body weight of the rabbits. The final volumes were standardised by adding normal saline. Sixteen male rabbits were grouped into four groups of 4 rabbits each. The first group (G1) were controls and administered normal saline while the other three groups (G2, G3 and G4) were administered 1.0 g/kg, 10 g/kg and 20 g/kg body weight of khat extract respectively. The test animals were administered the doses of khat extract via oral gavage on alternate days of the week (Monday, Wednesday and Friday) for 12 weeks. Thereafter two animals from each group were sacrificed for histological evaluation of testis, epididymis and kidney. The remaining 2 animals from each group were taken through the withdrawal period of 4 weeks before sacrifice. Blood samples were collected, processed and plasma stored at -20°C until assayed for FSH and testosterone levels for treatment groups as well as controls. Hormonal data and data on clinical observation for difference in means among groups and over the experimental period were analysed by two-way ANOVA at 95% confidence interval followed by Tukey’s multiple comparison for post hoc test. Intergroup analysis was done using paired t test. The relationship between weight and levels of testosterone was done using spearman rank correlation analysis at 5% significance level. Khat extract at low dose increased plasma levels of testosterone and FSH while at high dose and over experimental time significantly suppressed testosterone production but the FSH levels were only slightly reduced. This was reversed during withdrawal period where plasma testosterone and FSH in both controls and treatment groups showed no significant difference in their measurements. Histological data on testis during sub-chronic exposure showed vacuolation in germinal epithelium of moderate and high dose treatment groups over experimental period. The same was observed in the epithelial lining of proximal convoluted tubules of kidney nephrons of high dose treatment groups at sub-chronic exposure. However, histological sections of the epididymis appeared unaffected at all doses of khat extract. Following withdrawal from sub-chronic exposure, testicular, epididymal and kidney histology of all treatment groups appeared to regenerate from cellular damage when compared to controls. These results in rabbits have demonstrated for the first time that structural alterations on reproductive system reported variously in literature on khat addicts are reversible following withdrawal from heavy and long-term use in man.
CHAPTER ONE: INTRODUCTION

1.1 Background information to the study

Khat is grown in different parts of the world including Middle East, Somalia, Eastern Africa (Nyongesa and Onyango, 2010). Under favourable conditions, it grows to a height 7 m but occasionally reaches as high as 15 to 25 m wide. Its leaves are simple, oblong and glossy green above but lighter and leathery below and stiff tapering to both ends (Nyongesa et al., 2014a).

Plate 1. Khat plant (Maua species)
In Kenya, there are different varieties of khat namely: Giza, Kangeta and Kata. Giza has short stems and is said to be more potent while Kangeta has long stems. The cost of khat varies with the quality and season with the most expensive being around KShs.1000 a bunch and the cheapest around KShs. 200 (Nyongesa et al., 2008). Khat is locally known as miraa while in other countries where grown it is referred to variously as African salad, Abbyssynian tea, Kuses-salatin tohai, “kat”, “qat”, “qad”, “qaad” and “jaad” (Vijal Basker, 2013). The young fresh leaves of khat are used as a recreational drug because they contain the naturally occurring alkaloid, cathinone which provides euphoric and psychostimulant effects to the user (Nyongesa et al., 2014b).

In areas where khat is grown, much of the activity seems to centre on the crop bringing together farmers, traders, middle men, consumers and transporters. Large sums of money change hands daily in market centres where khat business is transacted making it the most lucrative business in these regions (Nyongesa and Onyango, 2010). Khat is chewed commonly but a small number use it inform of dried leaves while others like to smoke it (Cox and Rampes, 2003). Chewing of khat is best when fresh as a pleasantly stimulating beverage (Mwenda et al., 2003). Chewing efficiently helps to extract the major components of khat which are absorbed in the oral mucosa. (Toennes et al., 2003).

Cathinone, the psychostimulant component of khat is released within 15 to 45 min during chewing (Graziani et al., 2008) and to a lesser extent cathine and norephedrine (Ambelu et al., 2015). Research findings on effects of habitual use of khat on reproductive function, urinary system as well as splenic function are scanty. Information on effects on these functional systems following withdrawal from long-term exposure to khat is obscure. Available reports on khat and body weight loss have been ascribed to anorexia in the user (Murray et al., 2008; Nyongesa et al., 2014a). Whether or not effect on body weight gain
complicates hormonal production hence alterations on reproductive function remains speculative. Further, information on potential resumption of reproductive and kidney functions following withdrawal from long-term use of khat and length of time it takes to normalization is lacking.

1.2 Statement of the problem

Khat use in areas where grown is increasingly being abused and leaves ‘addicts’ with hopelessness in life. This state of affairs has brought about engaged of youths in criminal activities such as prostitution, drug abuse, breakage of marriages among many social evils with no hope of recovery when compelled to withdraw from the vice. This has necessitated extensive research on the khat plant and its effects on different functional systems of the body. Findings from previous reports have offered more or less contradictory information as explained by differences in experimental designs, animal models, duration of study and sample size among other factors.

For instance, cathine and norephedrine alkaloids have been shown to stimulate the sperm maturation and inhibition of acrosomal loss. Other findings showed that sub-chronic exposure to cathinone affects expression of gonadotropes hence luteinizing hormone (LH) synthesis and release that was interpreted to influence steroidogenesis and spermatogenesis in vervet monkeys. This research sets out to determine the possible resumption of reproductive function in male rabbits following withdrawal from sub-chronic khat exposure. Specifically, the study considered measurement of plasma FSH and testosterone as well as testicular, epididymal and kidney nephron histology during sub-chronic exposure of khat extract and subsequently through withdrawal phase.
1.3 Justification of the study

The present study was designed to investigate the effect prolonged intake of khat extract on the plasma FSH and testosterone that affect testicular function and consequently assessment of possible recover of these reproductive parameters following withdrawal from long-term exposure. There is dire need to quickly address the problem of ever-growing numbers of khat addicts with no formal information on negative impact of habitual khat use on their health. Since drug addiction entails both psychic and physical involvement with some level of tolerance seen in amphetamine abuse (Mwenda 2006), addicts lack the ability to control intake and so become driven by the drugs. Such individuals have no hope of recovery even when advised to withdraw from the habit. Other than reproductive impairment, habitual khat use poses risk of anorexia (Murray et al., 2008) hence poor nutrition, which might play role entirely or in connection with other parameters such as hormones on reproductive impairment. Follicular Stimulating Hormone (FSH) plays a critical role in sperm maturation (Hiller-Sturmhöfel and Bartke, 1998). In this regard, its role on reproductive function during long-term khat extract exposure and during withdrawal phase should not be ignored. Literature on effects of khat on FSH and its relationship with testosterone on reproductive function in the user as well as in individuals during recovery phase is lacking. In this research study the New Zealand white rabbit was chosen due to its recognized utility in the laboratory in terms of blood sample collection, adaptability to laboratory set-up, less expensive and has fewer health risks with a short reproductive cycle hence it is possible to have several experimental groups.
1.4 Research questions

i What are effects of long-term exposure of khat extracts at different dose rates on plasma levels of testosterone and FSH in male rabbits?

ii What is the effect of long-term exposure of khat extract at different dose rates affect testicular, epididymal and kidney histology of male rabbit?

iii Any effect of long-term exposure of khat on body weight changes of male rabbits?

iv Any reversal effect on testicular, epididymal and urinary histology following withdrawal from long-term khat extract exposure in male rabbits?

1.5 Hypotheses

i Khat exposure at different dose levels do not alter the plasma testosterone and FSH in male rabbits.

ii Different doses of khat at different dose rates do not interfere with testicular, epididymal and kidney histology in male rabbit

iii There is no change in body weight after administration of different doses of khat at long-term in male rabbit.

iv Withdrawal from long-term khat extract exposure does not revert reproductive function in the male rabbit.

1.6 Research objectives

1.6.1 General objective
To determine the effects of khat on gonadal and endocrine function as well as on body weight changes during treatment and following withdrawal from sub-chronic exposure in male rabbits.

1.6.2 Specific objectives

i To evaluate the effect of different doses of aqueous khat extract on plasma levels of testosterone and FSH in male rabbits following sub-chronic exposure.

ii To determine the effect of different doses of khat on testicular, epididymal and kidney histology as well as body weight changes in khat-treated male rabbits.

iii To evaluate the effects of khat extract on plasma FSH and testosterone levels, testicular, epididymal and kidney histology in male rabbits following withdrawal from sub-chronic exposure.

1.6 Significance of the study

This research demonstrates that long-term effects of khat use on some functional systems such as testes and kidneys are reversible following withdrawal from use. These findings offer a reprieve to counsellors, mentors, medical practitioners and other public health officers dealing with victims of khat addiction brought under rehabilitation. The findings also offer insights into what level of exposure and for what duration will be stimulatory or suppressive to different measures that were considered in this study. This is a pointer on possible use of khat as a contraceptive although more work needs to be done to this end.
CHAPTER TWO: LITERATURE REVIEW

2.1 Background information of Khat

Khat plant is best grown in areas of high altitudes in Eastern and Southern Africa (Nyongesa and Onyango, 2010). It’s best grown in black cotton and red soils at a pH of 6.0 to 8.2. In Eastern Africa, it is the basis of lifestyle and also contributes significantly to the government revenue. In Kenya for example, sales from khat fetch at least $150 million annually making it the source of livelihood to many peasant farmers (Lemessa, 2001). Chewing of khat is more prevalent in males often resulting into family strains (Al Motarreb et al., 2002).

2.2 Prevalence of khat use

An earlier study by Stefan and Mathew (2005) reported that over 20 million people worldwide are regular khat users and the number was projected to increase day by day. In a separate study by Odenwald et al. (2005), 31% of Somali men and 50% Ethiopia men were found to be current khat users. The prevalence of khat chewing was found to be 31.7% in a study conducted in the rural of Ethiopian community (Belew et al., 2000). The majority of khat chewers were between 16 and 25 years. There was a clear association of khat chewing and the use of alcohol and tobacco. In Kenya, of the youth appearing in juvenile courts, 6% used khat (Maru et al., 2003). Common khat use was reported in cross-sectional surveys among patients attending primary health centres in urban and rural areas of Kenya (Otieno et al., 2000). While khat consumption has been important for centuries around the horn of
Africa and south western Arabia, air transport has removed the major obstacle to the distribution of khat (Manghi et al., 2009).

2.3 Composition of khat

The phenylalkylamines and the cathedulins are the major components (Cox and Rampes, 2003). The phenylalkylamines comprise cathinone (S-(-)-cathinone), cathine (norpseudoephedrine) and norephedrine (1R, 2S-(-) - norephedrine. Cathinone is the main psychoactive component of khat; it is unstable and undergoes decomposition a few days following harvesting of the leaves (Al-Motarreb et al., 2005). Cathinone is stored in the young leaves and shoots and upon maturation, is broken down to cathine (Wabe, 2011). These compounds are structurally related to amphetamine and noradrenaline (Houghton et al., 2003) (Fig 1). The stimulating effects of khat are associated mainly with its alkaloid content cathinone and to a lesser extend cathine and norephedrine (Ambelu et al., 2015).

\[
\text{(-)-CATHINONE} \quad \text{(+)-NORPSEUDOEPHEDRINE} \quad \text{(+)- AMPHETAMINE}
\]

Fig 2.1: Chemical structures of cathinone, cathine and amphetamine (adapted from Kalix, 1996).
Amphetamines usually stimulate the central nervous system hence classified as psychostimulants (Sikiru et al., 2012). Cathine has been identified as an additional psychoactive ingredient in khat (Graziani et al., 2008). To maintain freshness after harvesting, leaves and shoots are wrapped in banana leaves for moisture preservation as shown in plate 2.

Plate 2: Bundles of khat wrapped in banana leaves

### 2.4 Pharmacology and Pharmacokinetics of khat

During chewing process 80% of cathinone and cathine, and over 90% of norephedrine is released (Toennes and Kauert, 2002). Khat is absorbed at two stages, the first stage occurs at the buccal mucosa which plays a major role in the absorption of alkaloids while the second stage occurs following swallowing of the juice, at the stomach or small intestine. Maximal plasma concentration of cathinone, cathine and norephedrine are reported to be reached at 2.3, 2.6 and 2.8 h, respectively (Toennes et al., 2003). Although cathinone appears to be only half as potent as amphetamine, cathine is roughly 7–10 times less potent than amphetamine.
The reviews by Kalix (1980) provide a comprehensive description of the progress in the understanding of khat pharmacology (Fig 2).

![Diagram of khat alkaloids](image)

**Fig 2.2** Summary of different classes of alkaloids in khat leaves (Source: EMCDDA, 2009C)

### 2.5 Khat and male reproduction

The hypothalamus and the anterior pituitary gland have sole regulatory functions, which are mediated by the hormones they synthesize and secrete (Nyongesa, 2013). The testes produce androgens which controls spermatogenesis and secondary sexual characteristics. Among other hormones, the hypothalamus produces gonadotropin releasing hormone (GnRH) which is secreted in pulses into a system of blood vessels within the pituitary stalk connecting the hypothalamus to the pituitary gland (Nyongesa, 2013).

#### 2.5.1 Hypothalamo - hypophyseo-gonadal axis in the male

The anterior pituitary gland produces gonadotropins, LH and FSH in response to GnRH. In the male, LH stimulates the interstitial cells of testes to produce androgens whereas FSH plays an important role in sperm maturation (Yan *et al.*, 2009). The testes consist of the
Seminiferous tubule and interstitium. Seminiferous tubules are composed of Sertoli cells and germ cells. Spermatogenesis takes place within the seminiferous tubules while steroidogenesis takes place within Leydig cells of the interstitium (Spierings et al., 2003). Sertoli cells play a critical role in sperm development by supporting and nourishing the germ cells during their development (Hiller-Sturmhöfel and Bartke, 1998).

2.5.2 Effect of khat on male reproductive system

In a study in rats exposed to cathinone enantiomers at different dosages, microscopic examination of testicles from these animals showed degenerative changes in the Leydig and Sertoli cells (Nyongesa, 2013). Olive male baboons when administered with crude khat extract caused penile erection, increase in plasma testosterone and a decrease in cortisol and prolactin levels (Mwenda et al., 2006). A study of male patients with history of infertility following regular use of khat and 31 human volunteers (control group) were examined. In this study khat addicts had a reduced semen volume, low sperm count and reduced sperm motility (Adeoya-Osiguwa and Fraser, 2005). The same researchers reported that the effects were more pronounced when khat was taken concurrently with coffee, alcohol and cigarettes. (Adeoya-Osiguwa and Fraser, 2005). These could be a possible source of infertility associated with khat chewing (Hakim, 2002).

2.5.3 Effects of khat extract on the action of Luteinising Hormone on the Leydig cells

Effects of khat on steroidogenesis have been reported in previous studies. A study of male Olive baboons showed enhanced plasma testosterone, decreased prolactin and cortisol levels following khat treatment (Mwenda et al. 2006). These findings were later confirmed on viable mouse interstitial cells in vitro where short-term exposure at low doses of khat extract enhanced while high doses suppressed testosterone production (Nyongesa et al., 2007).
Individuals who have been chronically exposed to drugs show both structural and functional testicular changes and male hormone production changes (Yucel and Lubman, 2007; Yucel et al., 2007). Findings from this study coupled with previous findings helps to understand the effects of prolonged use of khat and effects following withdrawal from the same.

2.5.4 The Process of Spermatogenesis

Spermatogenesis starts with mitosis of germ cells through meiosis and spermiogenesis in the seminiferous tubules of testis (Plate 3). This process must be under precise regulation to attain a homeostatic state that ensures the quality and quantity of mature spermatozoa (Spierings et al., 2003). The human sperm comprises three parts: head, mid piece and flagellum. Compared with most other mammals, the components of human sperm are highly variable within and between men and do not conform to discrete categories of size and shape (Menkveld et al., 2011). The phenotype of an ejaculated sperm is primarily the end result of spermiogenesis when the round meiotic spermatid develops into a spermatozoon.
Fig 2.3 - Spermatogenesis process (Adapted from Spierings et al., 2003).

2.5.5 Regulation of Spermatogenesis

The seminiferous epithelium has germ cells and the Sertoli cells. The Sertoli cell in the seminiferous epithelium is the target for both FSH and testosterone, which are the main hormonal regulators of spermatogenesis (Yan et al., 2009). Leydig cells present in the interstitial compartment are the source of testosterone in the testes thus there is a considerably higher concentration of testosterone within the testes than in blood serum (Sofikitis et al., 2008). The production of appropriate numbers of spermatozoa depends upon stimulation of the testes by FSH and LH. In response to LH, testosterone is synthesized by the Leydig cells (Zirkin, 1998). In addition, testosterone withdrawal results in changes in the adhesion of spermatids to the Sertoli cells with which they are associated, probably via effects on the
Sertoli cell cytoskeletal components, actin and myosin filaments, and thus on the junction between the spermatids and Sertoli cells. Loss of spermatid adhesion, in turn, precludes further maturation of these cells (Zirkin, 1998). It is also known that testosterone is crucial in the maintenance of elongate spermatid adhesion in the testis (Yan et al., 2009).

2.6 Medical and Socio-Economic impact of khat use

Although khat has an extreme social nature (individual feelings of sociability in social gatherings), it influences socio-economic consequences for individuals and the community (Yeshigeta and Abraham, 2004). In Kenya, thousands of farmers who cultivate khat depend on the income generated from local and internal market for their livelihood. Despite the 2014 international ban of khat trade to the UK and Netherlands following laws on smuggling of drugs, farmers in endemic areas of khat crop cultivation are still going strong due to support from the Kenyan government in terms of legalization of the crop as well as provision of funds to small-holder farmers. Local sales from the crop contribute significantly to government revenue in form of taxes hence providing economic value the country.

2.7 Effects of khat on body organ system

Khat use has effects on the cardiovascular, respiratory, gastrointestinal, uro-genital, metabolic, endocrine, and nervous system dysfunction and are more severe with prolonged khat use (Dalu, 2000; Hassan et al., 2000; Al-Habori, 2005; Hassan et al., 2005).

2.7.1 Effect on the central nervous system

Chewing of khat affects the nervous system in different ways where one is in a state of euphoria which is manifested with elation of feelings, the person becomes very alert, has a lot
of inertia by being ‘energetic’ and all the time aroused. Such people talk a lot and experiences vivid delusion, loquacity and mood surge (Al-Motarreb et al., 2002). The mood of the user keeps on changing with increased khat intake, but such mood surge end with each khat chewing session (Hassan et al., 2002a). Most people who chew khat believe that their sexual desire and excitement is attributed to the drug (Al-Motarreb et al., 2002). Studies done earlier have proven beyond reasonable doubt that chewing of khat has the opposite effect compared to their mind set. Such effects include reduced sexual potency accompanied by sperm loss, induced anorexia where the user always does not have the urge to eat food and insomnia resulting in late wake-up next morning and low work performance (Hassan et al., 2002). Drugs of abuse have a serious effect on the user both structural and functional and that the brain is also affected. (Yucel and Lubman, 2007; Yucel et al., 2007). Mostly, cerebral cells are affected by chewing of khat which exposes one to a significant risk factor for acute cerebral infarction (Mujlli et al., 2005). Neurobiological structural changes accumulate with continued use of the drug and become severe, This is aggravated by many factors which include the type of drugs and the pattern of use, as well as the interaction with pre-existing neural developmental factors (Dalley et al., 2007).

2.7.2 Effect on the digestive system

Khat users have a higher incidence of oesophageal cancer (Balint et al., 2009). As a consequence of its mode of consumption khat seems to affect the oral cavity and the digestive tract. The studies to these complications show, however, conflicting results. A high frequency of periodontal disease has been suggested as well as gastritis and chronic recurrent subluxation and dislocation of the tempero-mandibular joint (Kummoona, 2001). Oral keratotic lesions at the site of chewing (Ali et al., 2004) and plasma cell gingivitis (Marker
and Krogdahl, 2002) have been reported. The tannins present in khat leaves are ascribed to observed gastritis in regular users (Halbach, 1972). Other studies rather suggested beneficial effects on the periodontium (Al-Hebshi and Skaug, 2005). Habitual users try to attenuate this undesirable effect by food adaptation, notably by eating a meal with high fat content like ground nuts prior to the khat session in order to facilitate intestinal transit (Hughes, 1973). Evidence has shown that chewing khat leaves significantly slows both the oro-caecal transit time and the whole gut transit time (Gunaid et al., 1999). These two mechanisms may contribute to the constipating effect of khat. Moreover, khat chewing was found to interfere with the absorption of some orally administered antibiotics, particularly ampicillin and tetracycline resulting in low bioavailability (Attef et al., 2000).

2.7.3 Psychostimulant drug addiction

There is a direct link between an individual’s genotype with predisposing/risk factors which can either be environmental (availability of drugs, poverty, social change, cultural norms, peer culture, occupation) or individual (genetic disposition, personality disorders, social deprivation, depression) that can direct a certain response to drug dependence (Nestler et al., 1996). In view of this, it is prudent to incorporate behavioural assessment when investigating genetic vulnerability of a certain individual in the understanding of development of drug addiction. The handicap, however, is the quantification of behavioural endpoints since they are more susceptible to environmental influence thus giving variance in behavioural manifestations. But this can be fine-tuned through allowing major focus on establishment of behavioural endpoints with same degree of sophistication and inter-rator reliability as earlier reported (Würbel, 2000). Environmental stimuli influences brain circuitry the same way as genetic involvement hence the need to combine specific phenotypes with genetic approaches in identification of development of addiction in humans and animals.
Other studies that have been done involve place-avoidance assay (Maldonado et al., 1996) and measurement of rate and degree of tolerance with opiate dependence (Bohn et al., 1999; Zhu et al., 1999). The stability of behavioural abnormalities that characterize addiction is a clear indicator that gene expression may be involved in drug-induced behavioural changes (Nestler and Aghajanian, 1997). Most studies have approached drug addiction cases from behavioural profiling in humans and experimental animals (Leshner, 1997; O’Brien, 1997; Heyne and Wolffgramm, 1998).

Previous studies reported genetic heritability of alcohol (Kendler et al., 1994), opiates and cocaine (Tsuang et al., 1998; Kendler et al., 1998). Opioid receptor genes for opioid dependence have reasonably been associated with opioid dependence (WHO, 2004). In other substances of abuse, a complex of genes may be involved. For instance, alcohol heritability has been associated with genes involved in drug metabolism, alcohol receptor genes, and genes responsible for synthesis of GABA, serotonin and dopamine (WHO, 2004; Thomasson et al., 1991; Chen et al., 1999).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Animal housing and Experimental Animals

The study was carried out at the Department of Veterinary Anatomy and Physiology, University of Nairobi. Sixteen male sexually mature New Zealand white rabbits sourced from Limuru Farm aged between 9 and 12 months and weighing 1.5 to 2.5 kg were used. The rabbits were fed on standard rabbit pellets from Unga feeds, Nairobi. Carrots, vegetables and fresh tap water were also provided *ad libitum*. The animals were kept under 10h light: 14 h dark cycle (light on: 07:00 to 17:00h and light off 17:00 to 07:00h). All the experimental procedures were conducted during the light cycle. Each animal was caged singly (16 inches x 16 inches x 13.3 inches) in the animal house where beddings were changed after every three days.

3.2 Preparation of aqueous crude khat extract

Fresh bundles of khat were bought from Kangemi Market, Nairobi while wrapped in banana leaves to preserve moisture hence their psychoactive potency. Three different doses (1.0, 10.0 and 20.0 g/kg body weight) of khat extract were prepared from the fresh leaves of khat blended in normal saline according to procedure described by Nyongesa (2007). Briefly, for a
dose of 1.0 g/kg body weight, 10 g of fresh khat were blended in 10 ml normal saline to give
a concentration of 1.0 g/ml. For a rabbit weighing 2 kg, 2 g of khat extract was used. Thus 2
ml of the stock solution containing 1.0 g/kg was mixed with 8 ml of normal saline to make a
total volume of 10 ml, which was administered via intra-gastric tube. To prepare a dose of 10
g/kg body weight 100 g of fresh khat were blended in 10 ml normal saline to give a
concentration of 10g/ml. For a rabbit weighing 2 kg, 20 g of khat extract was used. Thus 5 ml
of the stock solution containing 10 g/ml was mixed with 5 ml of normal saline to make a total
volume of 10 ml which was administered. For 20 g/kg, 120 g of fresh khat was blended in
6ml normal saline to give a concentration of 20 g/ml. For a rabbit weighing 2 kg, 10 ml of the
khat extract was administered via an intra-gastric tube.

3.3 Experimental design, sample size determination and drug administration

3.3.1 Experimental design

A total of sixteen male New Zealand rabbits were used in the study. The khat extract was
administered using a Curved oral Gavage needle, 14 Gauge, 2.9 inches long with a 4.0mm
rounded smooth designed to prevent any tissue damage during insertion. During
administration, the rabbits were restrained in a restrainer box measuring 380mm x 175mm x
230mm and neck slot of 220mm with a minimum height from the bottom and maximum
height of 470mm from the bottom. The animals were observed for any sign of discomfort
during the procedure. The animal house was covered with wire mesh to prevent entry of pests
and predators.

A first aid box with adequate bandages, surgical spirit, a small pair of scissors and forceps
was kept around all the time in case of accidental animal bite or injury during the procedure.
At the time of sacrifice and sample collection, the rabbits were restrained in the same manner
and euthanized through intramuscular injection of Sagatal at the dose of 26.4mg/kg for
harvest kidney, epididymis and testicular histological studies. This procedure was done in the small animal theatre and all precaution taken to ensure no injury to individuals participating in the procedure and no contact with animal body fluid or tissue.

Personal protective gear (gloves and lab coat) was used when handling the animals, nose masks when cleaning the house and treating the animals for any possible infection. The carcasses were disposed of by incineration. Animal beddings and housing waste were treated and disposed of as any other normal animal house wastes. All the chemicals that posed environmental danger were incinerated.

3.3.2 The administration of aqueous crude khat extract in male rabbits

The animals were first habituated to handling for 2 weeks. They were then grouped into 4 groups of 4 rabbits each. The first group (G1) were controls and each animal in this group was given 10 ml normal saline (0.9 % Sodium chloride) via intra- gastric tube thrice a week for 12 weeks. Groups 2, 3 and 4 received 1.0 g/kg, 10 g/kg and 20 g/kg body weight of khat extract respectively, via oral intubation thrice a week (Monday, Wednesday and Friday) at 10.00 am for 12 weeks. At the end of treatment period, two test animals from each group were sacrificed as stated under 3.3.1 above for tissue histology studies. The remaining two animals from each group were studied for effects following withdrawal from khat exposure for 4 weeks.

3.4 General Clinical Assessment

General clinical assessment includes the state of alertness, demeanour, general body condition, posture, any discharge through natural orifices and general responsiveness to handling.
3.4.1 Body weight and food consumption

The body weight was measured using a beam balance before administration of khat, during the experimental period and following withdrawal from khat administration. The change in body weight was determined by subtracting the body weight of the rabbits at the start of the experiment from any addition or reduction for different khat doses over the experimental period. This gave body weight gain or loss at different khat doses and over the experimental period. Food intake per day was determined by subtracting the weight of food remaining from the weight of the food given to the animal the previous day.

3.5 Blood Sampling

The rabbits were habituated to blood collection procedure daily for a week while in the restraint box. The marginal ear vein was aseptically cannulated using a 20 G blood vessel cannula a day before commencement of blood collection to habituate them. The marginal ear vein was made visible by shaving the area of the ear using a surgical blade and swabbed with 70% alcohol. The cannula was inserted in the vein and small amount of heparinised saline introduced into the cannula to prevent blood clotting inside the lumen. One millilitre (1 ml) samples of blood were collected into heparinized LP3 tubes every week at the 10th min after khat administration. The samples were then centrifuged for 5 min at 1 x 1500 g to obtain the plasma which was stored at -20°C until assayed for testosterone and FSH.

3.6 Tissue sampling and processing

The histological examination was done as described by Yakubu and Afolayan (2009). Tissue samples of testes, pituitary gland, spleen, kidney and epididymis were harvested from 2 animals in each group while the remaining 2 animals were taken through the withdrawal period for 4 weeks. To obtain tissues for histological studies, animals were euthanized
through intravenous injection of Sagatal (Rhone Merieux Ltd.) at 26.4mg/kg body weight. For perfusion to be done the animals were fastened on the dissecting board and the procedure performed as follows: a mild incision was made from the neck to the groin to expose thoracic and visceral organs. The intercostal muscles were incised to allow introduction of a needle into the heart through the apex of the left ventricle to flush out all blood from the system using 0.1 M phosphate buffer while taking care not to introduce air bubbles.

An outlet was made at the caudal vena cava to flush out all the blood and phosphate buffer as the heart pumped until the fluid coming out was near clear as the saline being introduced. The tissues were then fixed in 10% (v/v) formaldehyde through the same route of the phosphate buffer, dehydrated through ascending grades of ethanol (70%, 80%, 90%, and absolute alcohol v/v) and cleared. The samples were then kept in the fixative for about 2 weeks for proper fixation, cleared in xylene, and embedded in paraffin wax (melting point 56 ºC). Semi-thin tissue sections were stained with hematoxylin and eosin, dried and viewed under light microscope.

3.7 Hormonal analysis

Hormonal assay for testosterone and FSH were done as described below;

3.7.1 Enzyme immunoassay for testosterone

Hormonal assay for testosterone was done by use of enzyme linked immunoassay technique according to the manufacturer’s manual provided with the kit. (Novatec Immune Diagnostica Gmbh). The technique uses the principle of competition of hormone in sample with enzyme conjugated hormone for limited binding sites on the specific antibody.

3.7.2 Enzyme immunoassay for Follicle Stimulating Hormone
Hormonal assay for Follicle Stimulating Hormone was done by use of enzyme immunoassay technique using the kit according to the manufacturer’s (Novatec Immune Diagnostica GmbH). The technique used the principle of competition of hormone in sample with enzyme conjugated hormone for limited binding sites on the specific antibody. The hormone assay procedure used was according to the manufacturer’s protocol provided with the kit.

3.8 Ethical Approval

Ethical approval for this study was obtained from University of Nairobi Bio-safety, Animal use and Ethics committee. Ref: FVMBAUEC/2017/132 and NACOSTI Ref: I56/27968/14

3.9 Data processing and analysis

The statistical analyses were performed using SPSS software version 22.0. Hormonal data and data on clinical parameters for difference in means among groups and over the experimental period were analysed by two-way ANOVA at 95% confidence interval followed by Tukey’s multiple comparison post hoc test. Inter group analysis was done using paired t test. The relationship between body weight change and plasma testosterone was done using spearman rank correlation analysis at 5% significance level.
CHAPTER FOUR: RESULTS

4.1 Clinical observations of body temperature and body weight gain

The body temperature of the rabbits remained within normal range during the study period (38±1°C); no animal showed adverse clinical signs during the study period that could be attributed to a disease condition/infection. Khat at 20.0g/kg (high dose) and 10.0g/kg (medium dose) levels suppressed body weight gain P>0.05 and the pattern was time and dose-dependent. High dose caused a significant decline in body weight gain. At 1.0g/kg (low dose) group there was no significant difference in mean body weight gain when compared to controls. (Fig3)
Fig 4.1. Effect of khat extract on body weight change in saline and aqueous khat- treated rabbits over an experimental period of 12 weeks. Dose 20.0g/kg led to a significant suppression in mean body weight gain (P<0.05) when compared to other treatment groups and controls (n=16).

4.2. Hormonal analysis

4.2.1 Effect of khat extract on testosterone hormone

Plasma levels of testosterone were higher in the low and medium dose groups but significantly decreased at high dose (P <0.05) (Table 4.1). After treatment using high dose of khat extract, the plasma levels of testosterone recorded over time showed a significant decrease (p< 0.05). At all doses of khat extract, plasma testosterone at first increased and subsequently decreased over blood sampling period of time (Fig 4.2).
Fig 4.2 Pattern of mean plasma testosterone levels following sub-chronic khat exposure to rabbits at different dose levels. At the beginning of sampling period, high and medium doses showed an increase and later a significant decrease in testosterone production (P<0.05) compared to controls. The suppressive effects were highest at the 9th to 11th sampling period. n=16

Table 4.1: Mean values of testosterone doses of khat as well as controls following a 12 week experimental period.

<table>
<thead>
<tr>
<th>Khat dose (g/kg)</th>
<th>Mean±SEM (nmol/l)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>20g/kg (High)</td>
<td>1.96±0.55</td>
<td>0.29</td>
<td>6.12</td>
</tr>
<tr>
<td>10g/kg (Medium)</td>
<td>2.25±0.92</td>
<td>0.06</td>
<td>10.71</td>
</tr>
<tr>
<td>1g/kg (Low)</td>
<td>3.02±0.88</td>
<td>0.20</td>
<td>9.31</td>
</tr>
<tr>
<td>0g/kg (Control)</td>
<td>4.65±0.42</td>
<td>1.18</td>
<td>11.67</td>
</tr>
</tbody>
</table>
4.2.2 Effect of khat on Follicle Stimulating Hormone

Khat extract at high and medium doses suppressed serum FSH with significant decrease at high (1.72 ± 0.19) and medium (1.75 ± 0.05) doses compared to low dose group (2.65 ± 0.70) and controls (6.61±0.19) (Table 2). At low dose plasma levels of FSH progressively increased over sampling time (Fig 4.3).
Fig 4.3 Mean plasma levels of FSH in saline treated controls and khat treated rabbits as a function of dose. FSH levels were significantly reduced at high dose n=16

Table 4.2 Plasma levels of FSH at low, medium and high doses of khat as well as controls following a 12 week experimental period.

<table>
<thead>
<tr>
<th>Khat dose (g/kg)</th>
<th>Mean±SEM (nmol/l)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>20g/kg (High)</td>
<td>1.72±0.19</td>
<td>1.50</td>
<td>1.90</td>
</tr>
<tr>
<td>10g/kg (Medium)</td>
<td>1.75±0.05</td>
<td>1.60</td>
<td>1.95</td>
</tr>
<tr>
<td>1g/kg (Low)</td>
<td>2.65±0.70</td>
<td>1.78</td>
<td>6.12</td>
</tr>
<tr>
<td>0g/kg (Control)</td>
<td>6.61±0.19</td>
<td>5.88</td>
<td>7.24</td>
</tr>
</tbody>
</table>

4.3 Effects of aqueous crude khat extract on tissue histology
Microscopic examination of slides revealed significant histological changes to testis and kidney of rabbits treated with low, medium and high doses of khat extract. The study did not, however, consider a morphometric approach for comparison of relative weights of testes and kidneys which could be a pointer in quantifying tissue damage.

**4.3.1 Effect of sub-chronic exposure of khat extract on testicular histology**

Animals treated with 1.0g/kg body weight in acute phase did not show any observable lesions on testicular histology (Plate 4b) when compared with controls (Plate 4a). Seminiferous epithelium of testicular sections showed intact spermatocytes while the lumen was filled with spermatozoa. At sub- chronic, like for acute phase of 1.0 g/kg body weight, the seminiferous epithelium remained firmly attached on the basement membrane and onto each other with no morphological aberrations. The interstitial cells also appeared unaffected. At sub-chronic phase of 10 g/kg body weight, there was vacuolation in the spermatogonia and spermatocytes with evidence of piknotic nuclei as evidence of cellular degeneration (Plate 4c).

At a dose of 20 g/kg body weight of khat extract (Plate 4d) there was vacuolation in spermatogonia with peripheral nucleus indicative of degenerative changes. There was remarkable sloughing off the seminiferous epithelium and degeneration of the spermatocytes evident by piknosis of nuclei hence impairment of spermatogenesis.
Plate 4. Histology of the testicular tissues of control rabbits (4a), low dose (4b), Medium (4c) and high dose (4d) of khat extract groups. Note the intact seminiferous epithelium at different stages of development with spermatogonia lying intimately on the basement membrane (BM) in controls as well as low dose group. Sub–chronic exposure of khat at medium dose (4c) and high dose (4d) showed piknosis of nuclei in spermatocytes (arrow) with accompanying vacuolation and nuclei marginalization in spermatogonia (arrowhead). Interstitial cells (IS) appeared unaffected at all does of khat extract exposure compared to controls (Haematoxylin and Eosin staining X400)
4.3.2. Effect of sub-chronic exposure of khat extract on the epididymal histology

In this study, different doses of khat extract appeared to impact no significant effect on epididymal histology. In caput epididymis of controls (Plate 5a) and animals at high khat extract dose (Plate 5d), there were columnar cells with stereocilia having ovoid nuclei and numerous micropinocytotic vesicles in the cytoplasm. In both cases the histological sections appeared similar. The epithelial cells in the corpus epididymis of controls (Plate 5a) had normal cell height as those of high khat extract dose (Plate 5b) with short apical microvilli in comparison to caput epididymis (Plate 5).

Plate 5. Epididymis of rabbit showing caput (5c and 5d) and corpus (5a and 5b). Note that there were no observable lesions on the epididymal epithelial lining of high dose group of corpus epididymis (5b) compared controls (5a). The same picture is projected in caput epididymis where high dose group (5c) showed no observable lesions when compared to controls (5d). The slides show smooth muscle (SM), Spermatozoa (SZ) in the lumen (L) and columnar cells (CC) lying intimately on the basement membrane (BM) (Haematoxylin and Eosin staining X400)
4.3.3. Effect of sub-chronic khat extract exposure on kidney histology

The results showed pronounced effect of khat extract at high dose with evidence of vacuolation in epithelial cells lining proximal convoluted tubes indicating loss of secretory and re-absorptive function at this level of kidney nephrons (Plate 6d). However, at medium (Plate 6c) and low dose groups (Plate 6b) the kidney histology appeared normal compared to that of controls (Plate 6a) with no observable lesions. The results also showed evidence of congestion with prominent blood vessels in animals at high dose compared to other dose groups and controls. The epithelial lining proximal and distal convoluted tubules and the general architecture of the glomerulus and Bowman’s capsule of low and medium dose appeared normal.
Plate 6. Kidney nephron of rabbit controls (6a) and treatment groups (6b-6d). Note vacuolations in epithelial lining of proximal convoluted tubules (PCT) (arrowheads) at high khat extract dose (6d). Similar structures for controls, low and medium dose groups appeared unaffected by khat extract sub-chronic exposure. (H&E staining x400 Magnification)
4.4 Effect on body weight change following withdrawal from khat exposure.

The effect in terms of body weight gain differently with animals at medium and high dose exposure recovering the least compared to controls and low dose group (Fig 4.4). There was a significant effect (P<0.05) of time following withdrawal on body weight measurements with medium and high dose groups recording the least body weight gain (Fig 4.4).

![Bar chart showing change in body weight gain](chart.jpg)

**Fig 4.4** Mean body weight gain during withdrawal period in animals that had been exposed to different khat dose treatments. There a lag phase on recovery to body weight gain of treatment groups with significant effect (P<0.05) at high and medium doses compared to low dose group and controls. n=8
**Fig 4.5** Effect of time following withdrawal from sub-chronic khat extract exposure on mean body weight gain in animals that had been exposed to different khat dose treatments. N = 8

### 4.5 Effects on Testosterone secretion following withdrawal from sub-chronic khat exposure

The results showed recovered of testosterone production by animals that had been subjected to sub-chronic exposure to khat treatment. The increment was significant with animals that had been subjected to low dose (P<0.05) compared to medium and high dose treatment groups (Fig 4.2). The results also showed that recovery by Leydig cells to secrete testosterone enhanced with time from withdrawal period such that the longer it took from withdrawal the more the resumption of testosterone production (Fig 4.6). The mean plasma testosterone during withdrawal period in animals that had been treated with low dose (2.22±1.04) was
slightly higher than those at medium (1.98 ± 0.38) and high dose (1.34 ± 0.58). The controls showed a significant increase when compared to the treatments (3.85 ± 0.05) (Table 4.3).

**Fig 4.6** Pattern of plasma testosterone levels during withdrawal from sub-chronic khat extract treatment. Low dose group showed quicker recovery hence significant increase in testosterone secretion compared to medium and high dose groups. Note the increase in plasma testosterone in all treatment groups compared to controls.
Fig 4.7: Pattern of testosterone release over time during withdrawal period. Note the significant increase in testosterone levels at 13th and 20th April of animals that had been exposed to high and medium doses of khat extract. N=8

Table 4.3: Plasma testosterone levels following withdrawal from sub-chronic exposure to high, medium, low dose treatment as well as controls

<table>
<thead>
<tr>
<th>Khat dose (g/kg)</th>
<th>Mean ± SEM (n/mol)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>20g/kg (High)</td>
<td>1.34±0.58</td>
<td>0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>10g/kg (Medium)</td>
<td>1.98±0.38</td>
<td>1.12</td>
<td>1.25</td>
</tr>
<tr>
<td>1g/kg (Low)</td>
<td>2.22±1.04</td>
<td>0.48</td>
<td>2.12</td>
</tr>
<tr>
<td>Controls</td>
<td>3.85±0.05</td>
<td>0.70</td>
<td>3.97</td>
</tr>
</tbody>
</table>
4.6 Effects on plasma FSH following withdrawal from sub-chronic exposure to khat

During withdrawal period, plasma levels of FSH in rabbits that had been treated with low dose of khat (1.92 ± 0.11) was higher than those at medium dose (1.76± 0.07), and high dose (1.79±0.04) compared to controls (1.74 ± 0.03). There was, however, no significant effect on FSH levels following withdrawal from khat exposure among all treatment groups compared to controls (Fig 4.7).

**Fig 4.8** Plasma FSH during withdrawal phase from sub-chronic exposure of khat extract to rabbits. Note that treatment groups at low, medium and high dose recovered their FSH secretion following withdrawal from khat extract exposure. N=8
Table 4.4: Plasma levels of FSH following withdrawal from sub-chronic exposure to high, medium, low as well as controls

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean ± SEM (nmol/l)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1.59±0.04</td>
<td>1.69</td>
<td>1.90</td>
</tr>
<tr>
<td>Medium</td>
<td>1.76±0.07</td>
<td>1.62</td>
<td>1.95</td>
</tr>
<tr>
<td>Low</td>
<td>1.92±0.11</td>
<td>1.60</td>
<td>2.23</td>
</tr>
<tr>
<td>Control</td>
<td>2.74±0.03</td>
<td>1.64</td>
<td>1.81</td>
</tr>
</tbody>
</table>
4.7 Effects of khat on testicular histology following withdrawal from sub-chronic exposure.

Plate 7. Histology of the testicular tissues of control rabbits (7a), low dose (7b), Medium (7c) and high dose (7d) of khat extract groups. Note the intact seminiferous epithelium at different stages of development with spermatogonia lying intimately on the basement membrane (BM) in controls as well as low dose group during the withdrawal period compared to controls (Haematoxylin and Eosin staining X400)
4.8 Effects of khat on kidney morphology following withdrawal from sub-chronic exposure

Plate 8. Kidney nephron of rabbit controls (8a) and treatment groups (8b-8d). Note intact cells of PCT and DCT. No pikynosis in any of the cells. Glomerulus capillaries appeared intact when compared to the controls. (H&E staining, x400 Magnification)
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Effect of khat on body weight change

The general body assessment showed a significant effect of khat on the body weight changes during treatment period. The results showed that high and medium doses of khat extract at sub-chronic exposure resulted in a significant reduction in the body weight gain. During withdrawal phase of study, there was recovery in terms of body weight gain with lowest dose recording highest recovery while highest dose recording the least. Similar findings on khat and effect on body weight in humans and experimental animals were earlier reported (Abdul Ghani et al., 1987, Patel 2000, Nyongesa, 2007, Murray et al., 2008, Mahmood and Lindequist, 2008, Girma et al., 2015, Lemieux et al., 2015.). Research by Islam et al. (1990), showed that cathinone (active ingredient of khat) has suppressive effect on the body weight of rats in dose- dependent manner. These earlier findings, however, did not consider studies following withdrawal from long-term khat exposure on the same measure. Another study associated reduction of the weight in rats fed on khat with effect on metabolic rate and oxygen consumption due to cathinone, tannins and the inorganic ions present and their role in delayed absorption of glucose and gastric emptying. In a study by Al-Habori and Al-Mamary, (2004) using rabbits, khat leaves significantly reduced cholesterol and glucose concentrations. It is not clear whether the observed reduction in body weight gain and subsequent recovery during withdrawal period in the present study followed similar mechanism since the study did not consider measurement of serum leptin and its association with body weight gain during and after sub-chronic administration of khat extract.
5.1.2 Effect of sub-chronic khat extract exposure on plasma testosterone and FSH

The results of the present study showed that medium and high khat extract dose at sub-chronic exposure significantly suppressed (P<0.05) plasma testosterone while low dose enhanced plasma testosterone levels. These findings are consistent with those in mice (Nyongesa et al., 2007) and rats (Mohamed and Engidawork, 2011) that indicated a biphasic response in testosterone production following khat extract exposure. The decline in plasma testosterone may be as a result of direct effect of khat extract on Leydig cell function (Nyongesa et al., 2008, 2017) or indirectly due to decreased LH levels thus affecting steroidogenesis (Wagner et al., 1982; Nyongesa et al., 2015). It is possible that the observed decline in testosterone levels was as a result of sympathomimetic effects of cathinone in khat (Kalix, 1981). In a study by Khan and Rai (2004), it was reported that FSH stimulates the Sertoli cell receptors for the attachment of spermatozoa. From the findings of the present study, the receptors of Sertoli cells were affected by the high khat extract dose thereby resulting in vacuolation of germ cells and degeneration in spermatocytes. However, other studies have reported khat as a booster to reproductive function.

The suppressive effects of khat at long-term exposure on testosterone can be attributed to decreased spermatogenesis reported in a recent study in rabbits (Nyongesa et al., 2017). Taken together, these earlier observations with findings of the present study point at three possible mechanisms through which high doses of khat extract suppressed testosterone production: 1) it could have been direct effect on Leydig cells or their receptors, 2) impaired LH production by pituitary gland, 3) production of cytokines by the hypothalamus hence impaired pituitary function and, 4) a combination of some or all these factors. It is possible
from the findings that low dose stimulated Leydig cell function while high doses of khat extract interfered with Leydig cell histology or receptors hence impairment of steroidogenesis. Although the Leydig cell ultra-structure was not studied, further studies will be crucial in identifying effect of khat on cellular components involved in steroidogenesis. From the findings the levels of FSH decreased with the increase in khat doses with high dose showing a significant reduction. Previous studies in lizards showed role of FSH in spermatogenesis either by stimulating Sertoli cells or Leydig cell androgen production in lizards (Khan and Rai, 2003). However, earlier studies have indicated that cathinone has an amphetamine-like effects at central dopaminergic synapses (Kalix, 1983) and peripheral neurotransmitters (Nencini et al., 1984; Kalix and Braenden, 1985).

Another possibility through which khat extract influenced FSH levels is its direct effect on hypothalamic neurons in charge of GnRH synthesis. The decrease in FSH production could be due to dopamine-induced stress which affects the hypothalamo-pituitary gonadal axis by either modifying receptor function thereby inhibiting secretion of GnRH from the hypothalamus and subsequent reduction in FSH and LH release (Wagner et al., 1982). Khat may also have led to production of large amounts of hypothalamic concentration of Interleukin – 1 (IL-1) (Albers and Sonsall, 1995). Specifically, IL-1 has been shown to inhibit release of hypothalamic GnRH in the rat (Kaira et al., 1990).

Alkaloids inhibit cAMP production (Nyongesa et al., 2007) and the high concentrations of khat could have either prevented generation of cAMP or interfered with its function by combining with specific receptors like β-adrenergic receptors thus affecting steroidogenesis (Nyongesa et al., 2007). At low dose, the FSH level was significantly higher because at low dose, khat stimulated production of testosterone. The level of FSH was low in high dose
which agrees with previous findings that khat extract affected cell viability in dose dependent manner and possibly damaging the FSH receptors leading to less production of FSH by negative feedback. This agrees with earlier findings which showed that khat causes degenerative changes in interstitial tissue, atrophy of Leydig cells and Sertoli cells and subsequent decrease in sperm count in rats (Islam et al., 1990).

5.1.3 Effect on plasma testosterone levels following withdrawal from sub-chronic khat extract exposure

Results of the present study showed that withdrawal from sub-chronic khat extract exposure caused reversal effects on plasma testosterone in all treatment groups compared to controls. The significant recovery was observed in low dose group indicating that the damage on testicular steroidogenic structures was not enormous. However, medium and high dose groups also showed recovery from suppressive effects of sub-chronic khat extract exposure though this was time-dependent from withdrawal time. However, following withdrawal from long-term drug exposure, undesirable binge-intoxication experience has been associated with withdrawal from drug abuse. The mechanism through which these reversal effects were achieved is not clear. It is possible that the faster rate of testosterone production at low dose group was because no steroidogenic structural alterations were encountered during long term exposure. At medium and high dose groups, steroidogenic cells which could have been structurally altered either recovered or new cells were being formed during withdrawal period and carried normal functions.

Studies in vervet monkeys showed that long-term exposure to cathinone causes disorganization in smooth endoplasmic reticulum (SER), lipid droplets, Golgi apparatus and loss of integrity of inner cristae of mitochondria of Leydig cells (Nyongesa, 2013). It is also
possible that the observed resumption in seroidogenesis was as a result of enhanced steroidogenic enzymes that had been suppressed at high khat extract doses of sub-chronic exposure. Previous studies showed involvement of 3β-hydroxysteroid dehydrogenase type I (3β-HSD 1) and 17β-hydroxysteroid dehydrogenase type I (17β-HSD 1) to play a crucial role in steroidogenesis (Payne and Hales, 2004). These enzymes are located on membranes of SER and mitochondrial cristae (Nussdorfer et al., 1980; Mazzocchi et al., 1982). It is therefore possible that expression of testicular steroidogenic enzymatic resumed following withdrawal with significant resumption at low dose group. This is the first study that is reporting on positive health outcomes from drug abuse in the user. Most literature reports on withdrawal symptoms as an aftermath of drug abuse, which has not given much hope to victims of psychostimulant use and misuse.

5.1.4 Effect on plasma levels following withdrawal from sub-chronic khat extract exposure.

The results showed that following withdrawal from sub-chronic khat extract exposure, plasma levels of FSH resumed in a dose- and time-dependent manner with animals that had been exposed to low dose having a significant resumption while those at medium and high dose the least recovery. The data presented here provides a mechanistic link supported by published data suggesting involvement of changes in cyclic AMP, cyclic GMP, arachidonic acid, the lipoxygenase pathway as well as diacylglycerols and lipid derivatives of phospholipase D as a result of intracellular signalling, leading to gonadotrophic hormone release (Dan-Cohen et al., 1992). These earlier findings provide insights into the possible mechanism through which treatment groups at different doses resumed FSH production following withdrawal from sub-chronic khat extract exposure.
Another possible mechanism through which FSH levels resumed following withdrawal is removal of stress stimulus to the animals. Cathinone and amphetamine have been shown to induce stress that in turn alters mesocorticolimbic dopamine neurotransmission (Marinelli and Piazza, 2002). The stress also has suppressive effects on GnRH secretion from hypothalamus thereby leading to reduction in LH and FSH production by the pituitary gland. To this end, withdrawal of sub-chronic exposure to khat meant removal of stress in treatment groups hence resumption of gonadotrophic function. Earlier studies reported alterations in hormonal profiles following cathinone (Wagner et al., 1982) and khat extract (Nyongesa et al., 2008,) exposure to humans and experimental animals. Some studies have, however, shown discrepancies to these findings. For instance, Nyongesa et al. (2015) showed that high dose (6.4mg/kg body weight) of cathinone at long-term exposure in vervet monkeys led to proliferation of gonadotrophs with decrease in lactotrophs and corticotrophs. However, researchers in this area have not offered further information on gonadotrophic function following withdrawal from drug abuse. This gap of information necessitated the grafting of objectives of the present study.

5.1.5 Effect on testicular, epididymal and kidney histology following withdrawal from sub-chronic khat extract exposure

From the findings of the present study, the resumption of optimum Leydig cell function in treatment groups at all doses during khat extract exposure was evidenced by lack of histological abberations at all dose groups when compared to controls. This same scenario was observed in plasma testosterone measurement where medium and high dose groups showed recovery of steroidogenesis in time-dependent manner during withdrawal period. The study did not consider Leydig cell ultrastructural assessment to ascertain degree of recovery. However, it is possible that sub-chronic khat exposure that caused structural alterations in SER, lipid droplet and mitochondria could have been reversed following withdrawal.
Studies by Nyongesa (2013) in vervet monkeys showed that long-term exposure to cathinone causes degeneration of mitochondria, reduction in number of SER, Golgi apparatus and lipid droplets in the Leydig cells. It is also possible that sub-chronic khat extract had caused Leydig cell atrophy and following withdrawal the Leydig cells underwent regeneration. Earlier studies by Islam et al. (1990) reported atrophy of Leydig cells, epididymis, seminal vesicles, testicular cellular infiltration and lymphatic engorgement following cathinone treatment. Previous studies also showed that khat extract and cathinone depress cell proliferation and inhibit RNA, DNA and protein synthesis in dividing cells responsible for reduced spermatogenesis (Al-Meshal et al., 1989).

From these earlier reports, it could be that withdrawal of khat extract eliminated the effect of inhibition on RNA, DNA and protein synthesis thus regaining normal histology as observed in the present study. This thesis also reports that there were no observable structural changes on epididymis both during sub-chronic khat extract exposure and following withdrawal period. This finding is supported by studies on Olive baboons which reported lack of effect on epididymal structure following khat treatment (Mwenda et al., 2006). Lastly, results showed normal histology of kidney nephron following withdrawal from sub-chronic khat extract exposure. It is possible that epithelial lining of proximal convoluted tubes at sub-chronic exposure of high dose khat extract underwent regeneration although mechanism through which this was achieved was not investigated.
5.2 Conclusions and Policy Recommendations

i. This thesis has reported for the first time, the reversal effects of hormone profiles, body weight and histology of testis, epididymis and kidney nephron of rabbits that had been exposed to sub-chronic khat extract.

ii. This information is significant since it offers hope to habitual khat abusers who lose hope of recovery either because of addiction or the accompanying undesirable withdrawal effects.

iii. Most literature has leaned towards giving more focus on withdrawal effects rather than recovery effects following withdrawal from drug abuse.

iv. This information is handy to public health service providers and personnel dealing with victims of drug abuse in rehabilitation centres that there is hope of recovery.

5.3 Suggestions for further studies

(i) This study did not consider ultrastructural studies to establish steroidogenic structural effects following khat extract exposure but can be a good starting point for future research in this area.

(ii) In the present study, effect of khat extract was examined on serum levels of testosterone and FSH in male rabbits. It is recommended also to examine effect of khat extract and cathinone on other hormones.

(iii) Although studies done on animals are indicative of the situation in humans, they cannot fully represent effect of a substance in humans. Thus, further works in humans are recommended regarding effect of khat chewing on reproductive behaviors such as plasma levels of hormones having association with sexual behaviors.
(iv) The central mechanisms by which khat may affect reproductive functions is not yet clearly known although neurotransmitters such as dopamine and serotonin are thought to play a role. Therefore, effect of khat extract and cathinone on brain neurotransmitter levels should be assessed in further works.

(v) Khat is commonly chewed together with other drugs such as coffee, tea and cigarette. In addition, alcohol is sometimes taken after the chewing session is over. The effect of concomitant use of such drugs on reproductive hormonal levels and tissue morphology should also be comprehensively studied.
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