EFFECTS OF KHAT EXTRACT AND CATHINONE ON REPRODUCTIVE PARAMETERS OF MALE RATS

By

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<td>Bmf</td>
<td>Bcl-2-modifying factor</td>
</tr>
<tr>
<td>CAT5</td>
<td>5 mg/kg dose of cathinone</td>
</tr>
<tr>
<td>EL</td>
<td>Ejaculation latency</td>
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<tr>
<td>ICI</td>
<td>Intercopulatory interval</td>
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<td>IF</td>
<td>Intromission frequency</td>
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<td>IR</td>
<td>Intromission ratio</td>
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<td>IL</td>
<td>Intromission latency</td>
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<td>K100</td>
<td>100 mg/kg dose of khat extract</td>
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<td>K200</td>
<td>200 mg/kg dose of khat extract</td>
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<tr>
<td>K300</td>
<td>300 mg/kg dose of khat extract</td>
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<td>MF</td>
<td>Mount frequency</td>
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<td>ML</td>
<td>Mount latency</td>
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<td>MPOA</td>
<td>Medial preoptic area</td>
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<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
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<td>PEL</td>
<td>Post ejaculatory latency</td>
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<tr>
<td>Rf</td>
<td>Retardation factor</td>
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<td>VMH</td>
<td>Ventral medial hypothalamic nucleus</td>
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**Abstract**

Khat (*Catha edulis*) is extensively used in East Africa and the Arabian Peninsula. It has also become a growing concern in the developed world. Khat is reported to alter reproductive behavior; however, conflicting results have been published in the literature. Khat is shown to increase sexual desire and suggested as an aphrodisiac. Moreover, constituents of khat have been demonstrated to stimulate the final stage of sperm maturation in mouse and human. On the other hand, khat is reported to impair the male reproductive system and also to induce a deleterious effect on morphology, count and motility of sperm. Controversy remains regarding the potential effects and mechanisms by which khat may affect reproductive functions. The aim of this work was therefore to add to the body of evidence by evaluating the effect of crude khat extract and cathinone on reproductive ability of male rats. Male Sprague-Dawely rats were treated with khat extract (100, K100; 200, K200; and 300, K300 mg/kg doses) and cathinone (5 mg/kg, CAT5) intragastrically and subjected to a battery of behavioral tests. This was followed by hormonal analyses, sperm count and morphologic-pathology studies.

The results illustrated that khat extract produced a biphasic response on sexual behavior of male rats. Both K200 and K300 decreased parameters showing sexual desire and performance. In contrast, K100 appeared to enhance sexual desire, with parameters showing sexual performance were unchanged. On the other hand, CAT5 seemed to reduce sexual desire and performance. Like sexual behavior, khat extract produced a biphasic effect on serum level of testosterone. Whilst serum level of testosterone was doubled in rats treated K100 (P<0.01), a significant drop was recorded in rats treated with K200 (by 18%, P<0.01) and K300 (by 50%, P<0.01). CAT5, however, was noted not to affect serum testosterone level. A different pattern emerged with cortisol level. K200 and K300 were associated with a rise in serum cortisol level by 83% (P<0.05) and 164.5% (P<0.01), respectively. By contrast, K100 and CAT5 failed to alter cortisol level. Khat extract demonstrated a dose dependant decline in epididymal sperm count of male rats: 50% with K100 (P<0.05), 78% with K200 (P<0.01) and 89% with K300 (P<0.01). Surprisingly, CAT5 did not produce any significant change in sperm count. All doses of khat extract and cathinone did not produce any discernible damage on the reproductive tissues studied.
The findings of the present study indicate that mild dose of khat improves sexual desire, without affecting performance. With increasing dose, however, khat reduces both desire and performance. Although testosterone level correlated well with desire, there was dissociation with sperm count, indicating that optimum level is required for normal spermatogenesis. Moreover, effect of khat on reproductive behavior seems to be a function of cathinone content of the extract.

*Key words:* Khat; Cathinone; Sexual desire; Sexual performance; Sperm count; Testosterone; Cortisol.
1. Introduction

1.1. Reproductive Behavior in Rats

Rats acquire copulatory ability between 45 and 75 days of age (Hull and Dominguez, 2007). Females of breeding age come into heat all year-round, every 4 to 5 days, unless they are pregnant, and even then, they may come in heat once or twice early in the pregnancy. Each female usually has a regular schedule that can be marked on the calendar, but it can vary. Each heat usually begins in the evening and lasts most of the night (Foster & Smith Educational Staff).

The reproductive cycle of female rats is called estrous cycle and is characterized as proestrus, estrus, metestrus (or diestrus I) and diestrus (or diestrus II) (Fitzroy, 1971; Marcondes et al, 2002). Proestrus, which lasts 12 h, corresponds to the onset of mating behavior, known as behavioral estrus, or heat. The female ovulates at the end of proestrus and thus enters estrus, which lasts 36 h. After estrus it enters diestrus, a 48 h period that is subdivided into diestrus I (first 24 h) and diestrus II (second 24 h). After diestrus, it enters proestrus again (Nelson, 1995).

During behavioral estrus, the female solicits the male to prompt mounting by darting toward the male and then running away. This approach-retreat sequence may be repeated several times. The female may also pause near the male, and may intercept the male in pursuit of another female. The male finds these solicitation behaviors very attractive, and follows the female and mounts. The pressure the male exerts on the female’s flanks, lower back, and anogenital area triggers lordosis (Nelson, 1995).

Male sex drive is expressed after puberty, when the testes become active and start to secrete a hormone called testosterone. Male rats usually begin a sexual encounter by investigating the female’s face and anogenital region. Both partners may emit mutually arousing 50 kHz ultrasonic vocalizations (Hull & Dominguez, 2007). Then the male mounts and intromission becomes possible if the female is in heat and could exhibit lordosis posture (Nelson, 1995). Upon detection of the female’s vagina, the male gives a deeper thrust and then springs backward rapidly and grooms his genitals (Hull &
Dominguez, 2007). Intromission is accompanied by thrusting motions of the hindquarters and ejaculation. After mating, the male may emit ultrasonic vocalizations, and becomes sexually inactive and lethargic. He may groom himself, then lie down and sleep (Nelson, 1995). After 7–8 ejaculations males reach satiety and usually will not copulate again for 1 to 3 days (Hull & Dominguez, 2007).

The mount latency (ML) and intromission latency (IL) are frequently used as measure of sexual motivation. Intromission requires penile erection and coordinated activity of the striated penile muscles and is therefore not entirely determined by sexual motivation. The inter-ejaculation interval or post-ejaculatory latency (PEL) and mounting frequency (MF) may reflect a mixture of sexual motivation and potency (performance). The intromission frequency (IF) and the intromission ratio (IR) represent the potency, in other words the efficiency of erection and penile orientation (Agmo, 1997).

The medial preoptic area (MPOA) of the brain, which is just above the hypothalamus, appears to be the central brain structure in controlling sexual behavior for male rats. These areas contain high concentrations of androgen receptors, and their levels are regulated by androgen (Robbins, 1996). Stimulation of MPOA increases sexual behavior, and lesioning this area eliminates it in male rats, but does not affect the sexual behavior of female rats. Further, this area contains testosterone receptors. So, it is likely that this is the area of the brain where testosterone has its initiating effect. This may well be the case with human males too, in that autopsies indicate that males have larger medial preoptic, with more cells, than females (Hall, 1998).

With female rats, the ventral medial hypothalamic nucleus (VMH) appears to play a similar role in their sexual behavior. As with the MPOA in males, lesioning the VMH eliminates sexual behavior, stimulating it increases sexual behavior, and this area is rich in estrogen receptors (Hall, 1998).

1.2. Control of Sexual Desire in Male Rats

Many psychosexual and behavioral terms, including sexual appetite, desire and drive, sexual impulse and interest are used as synonyms of libido, a Latin word that means
‘desire’. The sex steroids, especially androgens and estrogens, have essential developmental (organizational), maintenance and activational functions in both males and females (Levin and Riley, 2007). Estrogen and testosterone stimulate the synthesis of oxytocin binding sites and recent evidence links oxytocin with the facilitation of sexual desire in both men and women (Graziottin, 2000).

In males, consistent evidence shows that androgens are a necessary though not sufficient factor to maintain a satisfying human libido (Graziottin, 2000). Frequently, hypogonadism is associated with decreased libido and restoring androgen to the castrated animal restores sexual function (Robins, 1996; Mikhail, 2006). A study performed in castrated hamsters showed that administration of testosterone restored masculine sexual behavior (Arteaga-Silva et al., 2008). Similarly, hypogonadism in men usually results in loss of libido and potency which can be restored by androgen administration (Gooren, 2006). On the other hand, a study performed on Camels revealed that a positive correlation exists between circulating testosterone and sexual desire, which suggests the role of testosterone in governing sexual libido (Deen, 2008).

Since leutenizing hormone (LH) release is believed to be regulated by the hypothalmic decapeptide, gonadrotrophin releasing hormone (GnRH), presumably sexual stimuli cause LH release through increased secretion of GnRH. Prolonged elevations in glucocorticoids may reduce the response of LH to GnRH while a brief exposure of the anterior pituitary to raised cortisol levels may enhance LH release in response to GnRH (Wang et al., 1986).

1.3. The Role of Neurotransmitters in Male Sexual Behavior

Steroid hormones prime neural circuits for sexual behavior, in part by regulating enzymes, receptors, or other proteins affecting neurotransmitter function (Hull et al., 1997). Testosterone is thought to upregulate nitric oxide synthase (NOS) activity in the MPOA and enhances release of dopamine. NO has been reported to enhance catecholamine release and to inhibit reuptake, possibly by reversing the transporter (Hull et al., 2004).

Dopamine facilitates male sexual behavior in numerous species and stimuli from an estrous female and/or the act of copulation elicits dopamine release in three integrative neural
systems (the nigrostriatal tract, the mesolimbic system and the MPOA) (Fig 1). Dopamine in the nigrostriatal tract is thought to contribute to the somatomotor patterns of pursuit and mounting of the female. Dopamine in the mesolimbic system is critical for appetitive behavior and reinforcement. It has been implicated in feeding, drinking, brain stimulation reward, drug addiction, sexual behavior, and active avoidance of noxious stimuli. Dopamine in the MPOA facilitates genital reflexes, enhances specifically sexual motivation, and promotes somatomotor copulatory patterns (Hull et al, 1997).

![Diagram](image)

**Fig 1: Effects of dopamine in three integrative neural systems.** Stimuli from an estrous female and/or the act of copulation elicits dopamine release in each system. The nigrostriatal tract promotes the initiation of somatomotor patterns of copulation. The mesolimbic system enhances general appetitive behavior. The medial preoptic area (MPOA) facilitates genital reflexes, enhances specifically sexual motivation, and promotes somatomotor copulatory patterns (Hull et al, 1997).

In addition to dopamine, serotonin (5-HT) has also a role in male sexual behavior. However, it is primarily inhibitory, although stimulation of 5-HT$_{2c}$ receptors increase erections and inhibit ejaculation, whereas stimulation of 5-HT$_{1A}$ receptors has the opposite effects: facilitation of ejaculation and, in some circumstances, inhibition of erection.
Antidepressants of the selective serotonin reuptake inhibitor class (SSRIs) impair ejaculatory/orgasmic function and frequently inhibit erectile function and sexual interest as well. Microinjection of large doses of 5-HT into the MPOA impaired male sexual behavior in rats. Conversely, decreases in serotonergic activity, due either to lesions of cell bodies in the raphe nuclei or to synthesis inhibition, facilitated male copulatory behavior. 5-HT is released in the anterior lateral hypothalamus at the time of ejaculation (Hull et al, 2004).

Other neurotransmitters are also involved in the control of sexual behavior in male rats. Noradrenergic activity appears to increase sexual arousal. On the other hand, cholinergic agonists facilitate ejaculation, or in some cases, delay or prevent initiation of copulation and GABA agonists inhibit sexual responses (Bitran and Hull, 1987).

1.4. Regulation of Spermatogenesis

Spermatogenesis is the biological process of gradual transformation of germ cells into spermatozoa over an extended period of time within the boundaries of the seminiferous tubules of the testis (Rex, 1999). It is a complex, cyclic process that involves germ cells undergoing mitotic divisions, meiosis and terminal differentiation (Zirkin, 1998). The seminiferous epithelium is composed of germ cells and the Sertoli cell. The Sertoli cell in the seminiferous epithelium is the target for both follicle stimulating hormone (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. It is not surprising, therefore, that there is a considerably higher concentration of testosterone within the testes than in blood serum (Sofikitis et al, 2008).

During the process, alterations occur in the male gamete nuclear proteins, cellular size, cellular shape, the position and size of pro-acrosomal granules and the localization of the centrioles. This fascinating process that converts a round immotile haploid gamete to an elongated cell with potential for movement is regulated by a complex of factors/mechanisms (Sofikitis et al, 2008). The hypothalamic–pituitary–testicular axis is a crucial regulatory axis for testicular function. Recent studies have shown that in the microenvironment of the seminiferous epithelium, locally produced autocrine and paracrine
factors are also involved in spermatogenesis, in particular at the level of cell junctions. These cell junctions at the Sertoli–Sertoli and Sertoli–germ cell interface are crucial for coordinating different events of spermatogenesis by sending signals back-and-forth between Sertoli and germ cells, in order to precisely regulate spermatogonial cell renewal by mitosis, cell cycle progression, meiosis, spermiogenesis, germ cell movement across the epithelium, spermiation and germ cell apoptosis (Yan et al., 2009).

The production of appropriate numbers of spermatozoa depends upon stimulation of the testes by the gonadotropic hormones, FSH and LH, both produced by the pituitary gland in response to GnRH from the hypothalamus. In response to LH, testosterone is produced by the Leydig cells (Zirkin, 1998).

FSH and LH are known to influence the germ cell fate. Their removal induces germ cell apoptosis. In human seminiferous tubuli, apoptosis is induced under serum-free conditions in vitro. The fact that this apoptosis is suppressed by testosterone indicates that testosterone in the human male is a critical germ cell survival factor. The mechanism by which androgen withdrawal induces germ cell death remains unclear. However, a recent study has suggested that the Bcl-2-modifying factor (Bmf) is likely to play an important role in germ cell death in response to reduced intratesticular testosterone profiles. Bcl-xl and Bcl-2 in the testis are altered following long-term anti-androgen treatment for prostate cancer (Sofikitis et al., 2008).

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 vinculin 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 elongating/elongated spermatid adhesion in the testis (Yan et al., 2009).

Besides, estrogens also play an important role in different aspects of germ cell development, such as in apoptosis. Recent studies have demonstrated that a cytochrome P450 enzyme called aromatase, which irreversibly converts androgens into estrogens, and estrogen
receptors (e.g., ERβ), which mediate the action of estrogens, are found in germ cells including spermatocytes, round spermatids, and elongated spermatids, as well as in Leydig and Sertoli cells. These findings thus illustrate that cells in the seminiferous epithelium are capable of producing estrogens from testosterone to regulate spermatogenesis (Yan et al, 2009).

1.5. Catha edulis

Khat, the edible part of Catha edulis Forsk, belongs to the score of vegetal materials that humans ingest not for their nutritive value but to experience their psychoactive effects (Graziani et al, 2008). The khat plant is a dense evergreen shrub belonging to the family Celastraceae (Kalix, 1996).

The shrub grows at altitudes between 1500 and 2500 m, requires high rainfall and grows best on acid, well-drained, clay soil. With irrigation and pruning, khat leaves can be harvested up to four times per year. Khat plants grow to a height of 6 m. The leaves are leathery, glossy, brownish green, with serrated edges, arranged in an alternate fashion on the straight branches (Fig. 2). The young shoots and leaves are the parts chewed for their psychoactive properties. Khat leaves are most commonly ‘preserved’ for transportation by being wrapped in banana leaves. A bundle of approximately 100-200 g of plant material makes up a measure of sale sometimes called, confusingly, a ‘kilo’ (Mela and McBride, 2000; Cox and Hagen, 2003; Nezar et al, 2005).
Khat is dominantly cultivated in East Africa and Arabian Peninsula. In these areas, a large part of the population has the habit of chewing khat leaves because of their stimulating effect, and therefore the plant is widely cultivated and commercialized (Kalix, 1996; Nezar et al, 2005; Abdulwehab and Muche, 2007). The khat habit was almost unknown in other regions of the world, and this is due to the fact that only fresh leaves have the desired stimulating effect. Owing to the possibility of air transport, however, khat has now made its appearance in Europe and North America (Kalix, 1996; Nezar et al, 2005; Abdulwehab and Muche, 2007).

Historically, the original source of khat seems to be obscure. However, there is general agreement that its use was prevalent in Ethiopia and from there, around the fifteenth century, the practice spread to the south-west of the Arabian Peninsula. Arab sources suggested that khat was in Yemen in the sixth century, when the Ethiopians conquered Yemen (Dhaifalah and Santavy, 2004; Nezar et al, 2005).
1.5.1. Epidemiology

Fresh leaves from khat trees are chewed daily by over 20 million people on the Arabian Peninsula and East Africa. The khat chewing habit is deeply rooted in the sociocultural traditions of these countries. Many of the users originate from countries between Sudan and Madagascar and in the southwestern part of the Arabian Peninsula. Khat use is particularly widespread in Ethiopia, Kenya, Djibouti as well as Yemen, where its use is socially sanctioned and even prestigious. Khat is consumed at parties in combination with smoking cigarettes and drinking tea and soft drinks. The biggest population of chewers is in Yemen, where the plant is used as a social stimulant. Recent reports suggest that 80–90% of the male adult and 10–60% of the female adult population in East Africa consume khat on a daily basis (Dimbal et al., 2004; Feyissa and John, 2008).

There is a high prevalence of khat chewing in Ethiopia. Belew et al. (2000) performed a house-to-house survey on a representative sample of 1200 adults from a rural Ethiopian community and the prevalence was found to be 31.7%. However, other data presented by Alem et al. (1999) shows the prevalence to be 50%.

1.5.2. Chemical Constituent of Khat

Many different chemical substances are found in the leaves of khat and these include alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins (7–14% by weight), amino acids, trace quantities of vitamins, and certain elements (Feyissa and John, 2008).

The phenylalkylamines and the cathedulins are the major alkaloids. Recently, 62 different cathedulins from fresh khat leaves were characterized. The khat phenylalkylamines comprise cathinone [S-(−)-cathinone], and the two diastereoisomers cathine [(+)-norpseudoephedrine] and norephedrine [1R, 2S-(−)-norephedrine] (Assessment of khat, 2006). The cathinone content of khat plants of different origins varies but on the average it is estimated that 100 g fresh khat would contain about 36 mg cathinone, 120 mg norpseudoephedrine, and 8 mg norephedrine (Kalix, 1996).

Most of the effect of chewing khat is thought to come from two phenylalkylamines—cathinone and cathine. Chemically, (−)-cathinone bears a close resemblance to
amphetamine, the only difference being that the two hydrogen’s on the first carbon of the amphetamine side-chain are substituted by oxygen (Fig. 3) (Abdulwehab and Muche, 2007).

![Chemical structures of cathinone, cathine and amphetamine](image)

Fig 3: Chemical structures of cathinone, cathine and amphetamine (Kalix, 1996).

### 1.5.3. Pharmacokinetics of Khat

Khat is usually chewed, occasionally brewed as a tea, and rarely smoked. The leaves are removed from their branches and thoroughly chewed; they are then kept for a while in the cheek as a ball of macerated material and later expectorated. The chewers fill their mouths to capacity with the tenderest leaves and shoots and then chew intermittently to release the active components or keep it in buccal vestibules (Feyissa and John, 2008). Cathinone and cathine are isolated from the leaves of the *Catha edulis* plant by the action of enzymes in saliva. Chewing khat has been shown to be an efficient way of extracting cathinone and cathine (Michael, 2005).

During the khat session the leaves and the bark of the plant are chewed slowly over several hours, usually for 2–10 h and an average 100–500 g of khat is chewed. The juice of the masticated leaves is swallowed, but not the residues (Feyissa and John, 2008). The absorption of the constituents of khat is said to have two phases, the first being at the buccal mucosa, plays a major role in the absorption of alkaloids. The second phase is following swallowing of the juice, at the stomach and/or small intestine (Soufi *et al*, 1991). The euphoric effect appears shortly after the chewing begins, suggesting absorption from the oral mucosa. The effect of cathinone is maximum after 15–30 min (Cox and Hagen, 2003).
Metabolism of cathinone is rapid, occurring mainly during first passage through the liver. Its metabolism to cathine involves reduction of the ketone group to an alcohol, a fairly common metabolic pathway in humans, catalyzed by liver microsomal enzymes. Only 7% or less of the absorbed (−)-cathinone is excreted unchanged in the urine, and is mainly excreted in the form of norephedrine and cathine (Feyissa and John, 2008).

Cathine has a slower onset of action, with a serum half-life in humans of about 3 h. It is excreted unchanged in the urine within about 24 h. When taking khat, large amounts of non-alcoholic drinks are consumed. There is pharmacological synergism with drinks containing methylxanthines (e.g. tea and cola), which therefore enhances the effects of khat (Cox and Hagen, 2003). Cathine has been found in breast milk in several lactating women who were chewing the leaves of khat (Feyissa and John, 2008).

1.5.4. Pharmacodynamics of Khat

The general analogy between the effects of cathinone and those of amphetamine (Table 1) as well as their chemical similarity (Fig. 3) suggested that the two substances might have the same mechanism of action. Amphetamine produces its effects by activating neurotransmission mediated by the catecholamines, noradrenaline and dopamine, in particular by releasing these neurotransmitters from their physiological storage sties. This is also the case for cathinone, since it has been shown that it is capable of releasing dopamine from synaptic terminals in the central nervous system. Similarly, cathinone releases noradrenaline from the terminals of peripheral sympathetic nerves. There is also evidence from animal studies that cathinone, like amphetamine, causes the release of neurotransmitters at serotonergic synapses (Kalix, 1996; Michael, 2005).

Further, it was found that in rats that had been trained to distinguish between amphetamine and placebo, cathinone substituted fully for amphetamine. Similarly, in monkeys conditioned to self-inject stimulants, cathinone elicited very high rates of responding, rates that were even higher than those attained with amphetamine. Such animals inject cathinone frequently day and night, they finally stop upon exhaustion but resume self-administration
of the drug after a rest period of one or several days; this cyclic pattern of drug-taking is
typical for amphetamine abuse by humans (Kalix, 1996).

Table 1: Experimental models in which cathinone acts as an amphetamine-like
compound (Graziani et al, 2008).

<table>
<thead>
<tr>
<th>Model</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingestive behavior</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Motor activity</td>
<td>Increased locomotor activity</td>
</tr>
<tr>
<td>Self-administration</td>
<td>Maintained</td>
</tr>
<tr>
<td>Drug discrimination</td>
<td>Amphetamine-like</td>
</tr>
<tr>
<td>Nociception</td>
<td>Analgesia</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Pressure and heart rate stimulation</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Lipolysis; increased oxygen consumption</td>
</tr>
<tr>
<td>Endocrine &amp; Neurotransmission</td>
<td>NE and DA release; ACTH and corticosterone release</td>
</tr>
</tbody>
</table>

1.5.5. Pharmacological Effects of Khat

The effects observed following khat consumption are generally of central stimulation and
include euphoria, excitation, anorexia, increased respiration, hyperthermia, logorrhea,
analgesia, and increased sensory stimulation (Brenneisen et al, 1990; Feyissa and John,
2008). Khat extract or cathinone has been also known to enhance sympathetic nervous
system (Kalix, 1996). Khat chewing has been linked as well to the cause of GI problems,
which may include lesions of the oral mucosa, stomatitis, esophagitis, gastritis and
constipation (WHO Advisory Group, 1980; Graziani et al, 2008). In addition to its effect in
the GIT, khat-chewing and its main constituent, cathinone, have been associated with
systemic hypertension and increased heart rate (Dhaifalah et al, 2004). Khat has been also
reported to affect male and female reproductive health (Jansson et al, 1988; Tariq et al.,
1990; Islam et al, 1990; Islam et al, 1994; El-shoura et al, 1995; Taha et al, 1995; Mwenda
et al, 2003; Adeoya-Osiguwa and Fraser, 2005; Jason et al, 2006; Abdulwehab et al, 2007;
Nyongesa et al, 2007; Bedada and Engidawork, 2009).
**Effect of Khat on Male Reproductive Health**

Khat and its alkaloid cathinone have been reported to affect male sexual potency. Contradictory reports are found in different animal species regarding the association between khat chewing and sexuality. In the ancient time, khat has been reported to be used as an aphrodisiac, and to treat premature ejaculation (Feyissa and John, 2008). Recently, Bentur *et al* (2008) reported that a capsule containing illicit cathinone have been marketed in Israel as a stimulant and an aphrodisiac drug. In addition, study done by Adeoya-Osiguwa and Fraser *et al* (2005) showed the effects of cathine and norephedrine on mouse and human sperm that they were capable of stimulating the final stage of sperm maturation. Moreover, both compounds maintained the sperm in a potentially fertilizing state for longer allowing them more time to reach an egg.

On the other hand, impairment of sexuality, inability to sustain erection, loss of libido, and spermatorrhea due to khat chewing has been reported (WHO Advisory Group, 1980). Histopathological examination of testes revealed degeneration of interstitial tissue, cellular infiltration and atrophy of Sertoli and Leydig's cells in cathinone treated animals (Islam *et al*., 1990), but this result was not confirmed by medium-term administration in rabbits (Al-Mamary *et al*., 2002). Moreover, the study of El-shoura *et al* (1995) in Yemenite individuals revealed that chronic khat chewing is associated with deleterious effects on semen parameters and sperm ultrastructural morphology. They compared khat-addicts and non-khat addict subjects and found parameters including semen volume, sperm count, sperm motility and percentage of normal spermatozoa to be lower among addicts. In addition, the study showed percentage deformed spermatozoa of 65% and sperm deformation patterns such as aflagellate heads, headless flagella and multiple heads and flagella were demonstrated.

Taha *et al* (1995) reported that oral treatment of cathinone and its combination with caffeine for 15 days increased sexual arousal (motivation) in male rats as evidenced by increased mounting performance and anogenital investigatory behavior. Recently, Abdulwaheb *et al* (2007) reported that low doses of khat extract exerted enhanced sexual motivation/arousal, characterized by reduced ML and IL, while high doses of the extract
produced opposite effects on both sexual motivation/arousal and performance in male rats. In addition, concurrent administration of the low dose extract followed by ethanol was found to enhance male rat sexual motivation/arousal (Abdulwaheb et al., 2007). This is similar to amphetamine, which at low dose is reported to evoke penile erection, though its effect at high dose is inconclusive. It was suggested that alteration of both dopamine (at low dose) and/or 5-HT (at high dose) levels in the CNS could explain the biphasic sexual behavior of rats after khat administration, although the role of testosterone cannot be ruled out (Taha et al., 1995).

Endocrinological disturbances including changes in sex hormone level have been observed in khat chewers (Abdulwehab and Muche, 2007). A study showed that administration of khat extracts to adult male olive baboons significantly increased testosterone but down-regulated prolactin and cortisol levels in blood plasma compared to the basal levels before khat administration (Jason et al., 2006). In marked contradiction other study performed by Islam et al. (1990) demonstrated that cathinone causes a significant decrease in plasma testosterone of male rat. It has been also reported that khat extract lowers plasma LH and testosterone secretion, but increases cortisol levels in male rabbits (Nyongesa et al., 2008). More recently, a study on the effect of different concentrations of khat extract on testosterone levels showed that high concentrations of khat extract significantly inhibited testosterone production while low concentrations significantly stimulated testosterone production by mouse interstitial cells (Nyongesa et al., 2007).

**Effect of Khat on Female Reproductive Health**

Similar to males, in females, khat chewing at mild dose has been reported to increase sexual desire (Feyissa and John, 2008). Moreover, moderate levels of cathine and norephedrine, especially in the female reproductive tract, could have a positive effect on natural fertility (Adeoya-Osiguwa and Fraser, 2005).

On the contrary, deleterious effects on reproductive health are also observed with khat chewing in females. Khat reduced the food consumption and maternal weight gain in rats (Islam et al., 1994). Similarly, maternal daily food intake of guinea pigs was significantly
reduced during the first 10 days of feeding and maternal weight gain was slightly lower in
the khat group (Jansson et al, 1988a). Moreover, the report demonstrated that khat feeding
of the mother significantly reduced the mean birth weight of the offspring by 7% without
any effect on litter size or length of gestational period. Since low birth weight is a well-
established risk factor for both perinatal and young infant death, khat chewing during
pregnancy may be one of the factors contributing to infant mortality (Mwenda et al, 2003).

In other study performed on rats by Islam et al (1994) illustrated that the administration of
khat had no effect on fetal sex ratio. However, at a dose of 125 mg/kg body weight and
above, it produced a significant increase in fetal wastage. Khat administration in utero also
reduced the litter size and caused intrauterine growth retardation (Islam et al, 1994). In
addition, Jansson et al (1988b) reported that placental blood flow was reduced by 10% a 75
min and by 24% 180 min after khat feeding, which might contribute to the intrauterine
growth retardation. Very recently, Bedada and Engidawork (2009) reported deleterious
effect of khat extract in mice progeny following exposure of their mothers to khat during
gestation and lactation period. They found that khat exposure during pregnancy and
lactation impairs cognition, brings about emotional instability, and causes peripheral organs
toxicity in the progeny.

Khat is genotoxic and has teratogenic effects on the fetus if regularly consumed by
pregnant mothers (Mwenda et al, 2003). The occurrence of a mutagenic effect has been
verified in germinal cells of male mice (Tariq et al., 1990) with the oral administration of
different khat doses through a 6-week period, after which the male animals were mated
with females. Results demonstrate a fertility reduction in the first week, which becomes
irreversible during the second week at high doses (200 mg/kg). A significant increase in the
postimplantation loss was also found in the early stages of the treatment.

Anecdotal reports associate khat use with loss of libido and published reports indicate khat
use to be a culprit to 50% of the divorces in Djibouti (Kalix et al, 1985). Despite these
assumptions, very limited studies have been done to investigate the sexual effects of khat.
Some of the studies are done using cathinone (Islam et al, 1990; Taha et al, 1995) and the
others produced conflicting results (Islam et al, 1990; Mwenda et al, 2003; Nyongesa et
Performing a comprehensive study using both cathinone and khat extract would at least, in part, clarify some of the confusions. In addition, the plant is widely chewed by individuals in the reproductive age, particularly young people. Since chewing khat has no acute signs and symptoms on reproductive capability, it may end-up with a terrifying adverse effect through time. Thus, given the extensive use of khat use in East Africa and Arabian Peninsula as well as the fact that khat use has become a growing concern in the developed world, initiating such a comprehensive study would add to the existing body of knowledge on khat and reproductive behavior.
2. **Study objectives**

2.1. **General Objective**

- To evaluate the effect of crude khat extract and cathinone on reproductive parameters of male rats.

2.2. **Specific Objectives**

- To study effect of administration of khat extract and cathinone on sexual behavior of male rats.
- To study the effect of khat extract and cathinone on plasma testosterone and cortisol levels of male rats.
- To evaluate dose related effects of khat extract on plasma levels of testosterone and cortisol.
- To assess the effect of khat extract and cathinone on epididymal sperm count.
- To examine effect of khat extract and cathinone on histopathology of testes and adrenal gland.
3. Materials and Methods

3.1. Chemicals

Cathinone was kindly donated by the Laboratory and Scientific Section of the United Office on Drugs and Crime (UNODC). Chloroform, diethyl ether, ethyl acetate, methanol, aqueous ammonia and Tween 80 were purchased from Sigma Aldrich, Germany and USA. Hydroxyprogesterone and estradiol valerate were purchased from a local market, which imported from Medipharm Pvt Ltd, Berlin, Germany.

3.2. Experimental Animals

A total of 30 male and 30 female Sprague Dawley rats, 8-12 weeks of age, were obtained from the Animal House of School of Pharmacy, Addis Ababa University. The animals were kept under natural lighting conditions (12 h light: 12 h dark cycle) with an average room temp of 21°C and relative humidity of approximately 50%. Six rats were housed in a single transparent cage of dimension 610 mm x 435 mm x 215 mm, male and female rats separately. The rats were fed on standard pellet diet and water ad libitum. All animals were handled according to internationally accepted guidelines and the protocol was approved by the School of Pharmacy Ethics committee.

3.3. Collection of Catha edulis

Bundles of the plant material shoots and small branches were purchased fresh at a local market from Belechie, in its natural habitat, 290 km South of Addis Ababa, Ethiopia. The fresh bundles were packed in plastic bags and transported in an icebox to the laboratory.

3.4. Preparation of Crude Extract

The extracts were prepared as described by Connor et al (2000), with slight modification. Fresh leaves were finely chopped on glass plates, weighed and placed in a flask containing reagent grade chloroform and diethyl ether in a 1:3 (v/v) ratio. For every 100 g of minced leaves, 400 ml of solvent combinations were used. The extractant were decanted, filtered with whatman no.1 filter paper, and then dried using a lyophilizer (Labconco corporation,
USA). The dried extract were kept in a refrigerator (-20 °C) until use. The yield was calculated and found to be 1.1%.

3.5. Thin Layer Chromatography of Khat Extract

Thin-layer chromatography of khat extract was done as described by Marshal (1995). The plant extract were spotted directly onto a precoated 5 by 10 cm silica gel 60 (Kieselgel F254) plate. Cathinone drug standard which was dissolved in methanol was also applied parallel to the extract spot. The plate was developed in ethyl acetate:methanol:aqueous ammonia (85:10:5) solvent combination, and then viewed under an ultraviolet lamp (254 nm). Finally, the Rf values of the spots were recorded.

3.6. Dosing of Animals

The rats were divided into five experimental groups of 6 each. The first group served as a control and the rats were given the vehicle, Tween 80 (3%, v/v) in water (CON). The second group was treated with pure cathinone, at a dose of 5 mg/Kg (CAT5) for four weeks. The rest of the groups were treated with khat extract at different doses. The extract, prepared in an aqueous solution containing Tween 80 (3% v/v), was administered orally in a once-daily regimen at three different doses 100 mg/kg (K100), 200 mg/kg (K200), and 300 mg/kg (K300) for a period of 4 weeks.

The 5 mg/kg dose of cathinone was selected following previous similar study in male rats (Islam et al, 1990). The dose for the khat extracts were chosen after considering estimated amount of khat weight taken by human and the yield value of the khat extract. Then, the human dose was converted into rat’s dose based on body surface area as described by Reagon-Shaw et al (2008).

The male rats were weighed and treated with vehicle only, khat extracts and cathinone using an oral gavage. The khat extract was weighed, mixed with Tween 80 in water (3%, v/v) to a predetermined concentration, stirred continuously while filling the syringe. Similarly, cathinone was weighed, mixed with Tween 80 in water (3%, v/v) to a predetermined concentration.
3.7. Sexual Behavioral Testing

The male rats were randomly assigned to the groups mentioned above. Sexual behavior studies were carried out in a separate room under dim red illumination according to the standard procedure described by Gauthaman et al (2003). Each male rat was placed in a rectangular plexiglass chamber (610 mm x 435 mm x 215 mm), 10 min before the introduction of a primed female, for the male rat to get acclimatized to the chamber conditions. The primed female rat was then introduced into the chamber and the following sexual behavior parameters were recorded:

- **Mount frequency (MF)**—the number of mounts without intromission from the time of introduction of the female until ejaculation.
- **Intromission frequency (IF)**—the number of intromissions from the time of introduction of the female until ejaculation.
- **Mount latency (ML)**—the time interval between the introduction of the female and the first mount by the male.
- **Intromission latency (IL)**—the interval from the time of introduction of the female to the first intromission by the male (characterized by pelvic thrusting and springing dismount).
- **Ejaculation latency (EL)**—the time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting and a slow dismount followed by a period of inactivity).
- **Postejaculatory interval (PEI)**—the time interval between ejaculation and the first intromission of the following series.
- **Intromission ratio (IR)**: is a derived parameter obtained by dividing the number of intromissions by the number of mounts plus the number of intromission.
- **Intercopulatory interval (ICI)**: average interval between successive intromissions (calculated as ejaculation latency divided by intromission frequency) (Abdulwehab et al, 2007; Gauthaman et al, 2003).

Ovariectomized rats were used for the copulatory studies. They were ovariectomized under anesthesia with ketamine hydrochloride (100 mg/Kg, sc) and diazepam (5 mg/Kg, im).
They were brought to estrus by sequential administration of estradiol valerate (10 mg/100g body weight) and progesterone (500 mg/100g body weight), via intramuscular injections, 48 and 4 h before the copulatory studies, respectively (Zanoli et al, 2008).

A baseline sexual behavior study was carried out in rats from all groups to render them sexually experienced and was then repeated following administration of the vehicle and extract to the male rats. The male rats sexual behavior tests were conducted 2 h after the onset of darkness (Gauthaman et al, 2003).

3.8. Hormonal Analysis

After four weeks of treatment, blood was drawn from the eye orbit sinus of the male rats. Then it was allowed to stand for up to an hour until it coagulates. The coagulated blood was centrifuged for 10 min at a rate of 3000 revolution per minute. The supernatant (serum) was next taken using a Pasteur pipette. The serum was stored at -20 °C until use. The serum was then thawed and vortexed. 50 μl of the serum was taken and analysed for testosterone and cortisol level using electrochemiluminescence method using Elecsys 2010 Immunoassay (Roche Diagnostics, Indianapolis, USA). The sensitivities were 120 μg/mL and 0.07 μg/dL for testosterone and cortisol, respectively.

3.9. Epididymal Sperm Count

The epididymal sperm were counted as described by Sharma et al (2009). For counting spermatozoa right epididymis of four rats of each group were homogenized and taken into 5 ml of 1% sodium citrate solution, squashed thoroughly in mortar and pestle until a milky suspension was obtained. The suspension was filtered through 80 μ mesh and the final volume made up to 10 ml. The made up volume was inclusive of washings of the filter. The suspension was thoroughly shaken and the spermatozoa were counted using a hematocytometer. The average numbers of sperms determined in every group are reported.
3.10. **Histological Examinations of Testis and Adrenal Gland**

The histological examinations of testis and adrenal gland were done as described by Yakubu and Afolayan (2009). The testis and adrenal glands were dissected out. Then the tissues were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90%, and 95%, v/v), cleaned in xylene, and embedded in paraffin wax (melting point 56 °C). Tissue sections were stained with hematoxylin and eosin. Microscopic evaluation of the thin section was undertaken and histoarchitecture was observed.

3.11. **Effect of Khat Extract and Cathinone on Weights of Tissues**

Effect of khat extract and cathinone on weights of testis and adrenal glands were studied as described by Sharma *et al* (2009). After four weeks of treatment, the body weight of the animals was recorded. The animals were then sacrificed and testis and adrenal glands were carefully removed and weight of each organ was determined. The relative weights (weight of the organ/body weight of the rat) were then calculated.

3.12. **Statistical Analysis**

The data are expressed as mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA), with 95% confidence interval. Post hoc comparisons between individual treatment groups and controls were made with Dunnet’s t-test and level of significance was set at P<0.05. The Graphpad Prism version 2.0 (Executable Graphpad Software Inc, San Diego) was employed for all statistical analysis.
4. Results

4.1. Effect of Khat on Male Rat Sexual Behavior

The effect of khat extract and cathinone on sexual behavior was evaluated in sexually experienced male rats as shown in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>K100</th>
<th>K200</th>
<th>K300</th>
<th>CAT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML (Sec)</td>
<td>82 ± 3.95</td>
<td>27 ± 4.2*</td>
<td>42.75 ± 2.9</td>
<td>159.5 ± 25.9** +</td>
<td>73 ± 39</td>
</tr>
<tr>
<td>IL (Sec)</td>
<td>96 ± 0.24</td>
<td>35 ± 0.28** +/+</td>
<td>43 ± 0.09** +/+</td>
<td>196 ± 0.37 ** +/+</td>
<td>307 ± 0.4**</td>
</tr>
<tr>
<td>EL (Sec)</td>
<td>1310 ± 170.4</td>
<td>990 ± 45.83 +</td>
<td>1064 ± 155.4</td>
<td>461.4 ± 40.85**</td>
<td>618 ± 36.5*</td>
</tr>
<tr>
<td>PEL (Sec)</td>
<td>577.5 ± 90.48</td>
<td>387.5 ± 71.67</td>
<td>1081 ± 150.4** +/+</td>
<td>1650 ± 147.6 ** +/+</td>
<td>210 ± 30</td>
</tr>
<tr>
<td>MF</td>
<td>3 ± 0.3</td>
<td>2 ± 0.5</td>
<td>3 ± 0.8</td>
<td>9 ± 1.4 **</td>
<td>5 ± 1*</td>
</tr>
<tr>
<td>IF</td>
<td>34 ± 1.1</td>
<td>34 ± 3.7</td>
<td>14 ± 4.2 **</td>
<td>4 ± 0.6 **</td>
<td>16 ± 1*</td>
</tr>
<tr>
<td>IR (%)</td>
<td>92</td>
<td>94</td>
<td>82</td>
<td>29</td>
<td>76</td>
</tr>
<tr>
<td>ICI (Sec)</td>
<td>38.5</td>
<td>29.1</td>
<td>76</td>
<td>115.4</td>
<td>38.6</td>
</tr>
</tbody>
</table>

ML, mount latency; IL, intromission latency; EL, ejaculation latency; PEL, post-ejaculatory latency; MF, mount frequency; IF, intromission frequency; IR, Intromission ratio; and ICI, Intercopulatory interval. *: P < 0.05 and **: P < 0.01 relative to vehicle; †: P < 0.05 and ‡: P < 0.01 relative to cathinone. CON, control; K100, 100 mg/kg; K200, 200 mg/kg; K300, 300 mg/kg of khat extract; and CAT5, 5 mg/kg of cathinone.

The results illustrated that lower dose of the extract appeared to enhance sexual desire while parameters showing sexual performance were unchanged. The higher dose, however, seemed to decrease parameters showing sexual desire and performance.

Those rats treated with mild dose of the extract (K100) quickly approached the receptive female, decreasing mount latency by about 67% (P<0.05) compared to control (ML being 27±4.2 vs 82±3.95). Moderate dose (K200) also reduced the ML, although failed to reach
statistical significance. However, cathinone treatment failed to affect the rate at which the male rats approach the receptive females. By contrast, those rats treated with high dose of the extract (K300) spent longer time to approach the receptive female, increasing latency by 94.5% (P<0.01) and 118.5% (P<0.05) compared to controls and cathinone treated groups, respectively. Dose related effect was observed on ML when the rats were treated with khat extract. ML happened quickly at mild dose of the extract and was seen to slightly increase with increasing dose and reached its maximum during treatment with the high dose of the extract.

IL was affected by both khat extract and cathinone treatment. Male rats of K100 and K200 had an easier vaginal penetration, which was verified by a shorter IL compared with the vehicle treated rats. K100 rats required only six sec after the first mount to have vaginal penetration and those in K200 were able to almost simultaneously penetrate during the first mount. In contrast, K300 and cathinone treated rats had been observed to have difficulty in vaginal penetration, with delay being severe with cathinone treated rats. The delay was two-fold for K300 and three fold for cathinone treated rats. In other words, K300 rats had vaginal penetration after thirty six sec of the first mount and cathinone treated rats displayed vaginal penetration long after two hundred thirty four sec. Like ML, dose related effect was observed on IL when the rats were treated with khat extract. IL happened quickly at mild dose, fairly increased at moderate dose and very much increased at higher dose of the extract. Cathinone treated rats exhibited IL after longer period of female introduction, which was even beyond the high dose extract treatment.

The duration of time between the start of vaginal intromission and the start of intravaginal ejaculation was observed not to be affected by mild and moderate doses of khat extract, which was confirmed by EL of 990±45.83 and 1064±155.4, respectively. The higher dose of the extract, however, reduced this duration by 65% (P<0.01) compared with the control group. Similar to the higher dose, cathinone treatment caused a reduction in EL by 53% (P<0.05). The reduction was considerable (P<0.05) when compared with K100.

After having intravaginal ejaculation, the rate of reapproaching receptive females appeared to be unchanged in rats of K100 when compared with control. On the contrary, rats of
K200 and K300 took very long duration to reapproach receptive females, which was demonstrated by an elongated PEL. Moderate dose of the extract caused a two fold delay to reapproach receptive females while high dose caused almost a three fold delay. The slight decrement in PEL after cathinone treatment was found to be insignificant.

K100 rats had vaginal penetration every 29 sec, which was faster than vehicle treated rats. In contrast, rats of K200 and K300 exhibited a very slow rate of vaginal penetration. Compared with vehicle treated rats, the value of ICI was doubled in K200 rats and tripled in K300 rats. Cathinone treatment was found not to affect the rate of vaginal penetration.

MF and IF were also affected by moderate and high doses of khat extract and cathinone treatment. K100 rats showed no change in MF and IF when compared with control groups. In addition, 94% of intromission trials were successful. Moderate dose of khat extract, however, reduced IF significantly (P<0.01), although MF value appeared to be unchanged. The number of vaginal penetration was observed to decline by 53% (P<0.01). Moreover, their intromission success rate was 82%. On the other hand, rats treated with high dose of khat extract demonstrated a significantly higher MF (P<0.01), meaning that most of their intromission trials were unsuccessful. Their intromission success rate was only 29%. Furthermore, these rats had very low number of vaginal penetration, in which IF was reduced by 88% (P<0.01) compared with vehicle treated rats.

4.2. Effect of Khat on Serum Levels of Testosterone and Cortisol

Fig 4 and 5 summarize effect of khat extract and pure cathinone on serum levels of testosterone and cortisol. Biphasic effect on serum testosterone level was observed when khat extract was administered at different doses. Compared with rats of CON and CAT5 groups, serum level of testosterone was doubled in K100 rats. In contrast, a significant drop in serum testosterone level was recorded in rats of K200 and K300. The moderate dose reduced the level by 18% (P<0.01) while the high dose of the extract caused a fall by 50% (P<0.01). Conversely, cathinone treatment failed to affect serum level of testosterone.

Although insignificant, mild dose of khat extract caused slight increment in serum level of cortisol. Serum cortisol raising effect was intensified with moderate dose of the extract and
reached its maximum level with further dose escalation. Unlike moderate and high dose of khat extracts, cathinone treatment was not found to produce a change in serum cortisol level.

**Fig 4: Effect of khat extract and cathinone on serum level of testosterone.** *: P < 0.05 and **: P < 0.01 relative to vehicle; +: P < 0.05 and ++: P < 0.01 relative to cathinone. CON, control; K100, 100 mg/kg; K200, 200 mg/kg; K300, 300 mg/kg of khat extract; and CAT5, 5mg/kg of cathinone.
Effect of khat extract and cathinone on serum level of cortisol

Fig 5: Effect of khat extract and cathinone on serum level of cortisol. *: P < 0.05 and **: P < 0.01 relative to vehicle; +: P < 0.05 and ++: P < 0.01 relative to cathinone. CON, control; K100, 100 mg/kg; K200, 200 mg/kg; K300, 300 mg/kg of khat extract; and CAT5, 5mg/kg of cathinone.

4.3. Effect of Khat on Sperm Count

Effect of khat extract and cathinone on epididymal sperm count is presented in Fig 6. As it is shown, mild dose of the extract caused a significant decline, by about 50% (P<0.05), in epididymal sperm count. The reduction went higher, reaching 78% (P<0.01) in rats treated with moderate dose and deeply lowered in rats treated with the highest dose of khat extract (by 89%, P<0.01). Cathinone treatment, however, had not produced a change in epididymal sperm count. Moderate and high doses of the extract resulted in a sperm count significantly lower than cathinone treated rats (P<0.05).
Effect of khat extract and cathinone on epididymal sperm count

![Bar chart showing sperm count for different groups: CON, K100, K200, K300, CAT5.](chart)

**Fig 6:** Effect of khat extract and cathinone on sperm count. *: P < 0.05 and **: P < 0.01 relative to vehicle; +: P < 0.05 and ++: P < 0.01 relative to cathinone. CON, control; K100, 100 mg/kg; K200, 200 mg/kg; K300, 300 mg/kg of khat extract; and CAT5, 5mg/kg of cathinone.

### 4.4. Morphologic Pathology Evaluation

Table 3 describes effect of khat extract and cathinone on testis and adrenal glands. As it is presented, significant difference in relative weights of testis and adrenal gland were not observed.

In addition, microscopic examinations of slides revealed that both crude khat extract and cathinone did not affect the histology of testis and adrenal gland of the treated male rats. There was no difference in the gross structural features of these organs from the treated animals and those that were not treated with khat or cathinone.
Table 3: Effect of khat extract and cathinone on weights of testis and adrenal glands

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>K100</th>
<th>K200</th>
<th>K300</th>
<th>CAT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average body weight of rats (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>220.75</td>
<td>191.15</td>
<td>188</td>
<td>118.8</td>
<td>159.9</td>
</tr>
<tr>
<td>Weight of testis (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>1.375</td>
<td>1.14</td>
<td>1.16</td>
<td>0.813</td>
<td>0.987</td>
</tr>
<tr>
<td>Relative</td>
<td>6.23 X 10^-3</td>
<td>5.97 X 10^-3</td>
<td>6.17 X 10^-3</td>
<td>6.84 X 10^-3</td>
<td>6.17 X 10^-3</td>
</tr>
<tr>
<td>Weight of adrenal gland (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.021</td>
<td>0.016</td>
<td>0.018</td>
<td>0.018</td>
<td>0.0165</td>
</tr>
<tr>
<td>Relative</td>
<td>9.51 X 10^-5</td>
<td>8.37 X 10^-5</td>
<td>9.6 X 10^-5</td>
<td>15.1 X 10^-5</td>
<td>10.32 X 10^-5</td>
</tr>
</tbody>
</table>

CON, control; K100, 100 mg/kg; K200, 200 mg/kg; K300, 300 mg/kg of khat extract; and CAT5, 5mg/kg of cathinone.

4.5. Thin Layer Chromatography

Fig. 7 illustrates the plate of thin layer chromatography made for khat extract. As it is shown in the figure, around four spots were observed under an ultraviolet lamp (254 nm) after the extract had been spotted on the plate. One of these spots was observed to correspond to the value of retardation factor (Rf value) of standard cathinone.
Fig 7: Thin layer chromatography of khat extract and cathinone.

Distance migrated by different components of khat extract, standard cathinone and the solvent system is depicted in Table 4. The solvent front was measured to be 9.6 cm. Spot A were found to have comparable Rf value with the standard cathinone.

Table 4: Distance migrated by different fractions of khat extract, standard cathinone and solvent.

<table>
<thead>
<tr>
<th>Distance traveled (cm)</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot A</td>
<td>3.2</td>
</tr>
<tr>
<td>Spot B</td>
<td>5.3</td>
</tr>
<tr>
<td>Spot C</td>
<td>6</td>
</tr>
<tr>
<td>Spot D</td>
<td>6.8</td>
</tr>
<tr>
<td>Standard cathinone</td>
<td>3.4</td>
</tr>
</tbody>
</table>
5. Discussion

Previously, some works have been done on the sexual effect of khat and its active constituent cathinone (Islam et al, 1990; Taha et al, 1995; Mwenda et al, 2006; Abdulwehab et al, 2007, Nyongesa et al, 2008). However, their investigation has been limited to sexual effects of either khat extract or cathinone only. In addition, most of the works did not expansively examine effects of khat extract or cathinone on sexual parameters. This work attempted to comprehensively assess effect of khat extract and pure cathinone on rat sexual behavior, hormonal levels, sperm count, and morphologic pathology.

5.1. Khat and Sexual Behavior

The present study demonstrated that the sexual desire of male rats was enhanced when they were treated with mild dose of khat extract, although sexual performance was not significantly altered. This finding is similar to the earlier observation made by Abdulwehab et al (2007), in which subchronic administration increased sexual arousal of male rats. Elevated sexual motivation was manifested by reduced mount and intromission latencies, which might be related to elevated testosterone level. Since androgens are necessary to maintain a satisfying human libido (Graziottin, 2000), enhanced sexual desire might be attributed to increased production of testosterone as observed in the present study. Deen et al (2008) reported a positive correlation between circulating testosterone and sexual desire in Camels, which goes in parallel with the current finding. The other evidence that implicates testosterone in sexual desire is the association between hypogonadism and decreased libido. Frequently, decreased libido occurs during hypogonadism and restoring androgen to the castrated animal restores sexual function (Robbins, 1996; Mikhail, 2006; Gooren, 2006; Arteaga-Silva et al, 2008).

Treatment of male rats with moderate dose of the extract reduced sexual performance, although mixed results were found in parameters that reveal sexual desire. The rats approached the receptive females quickly compared to vehicle treated rats, however, reapproached after longer period following ejaculation. MF and IF were observed to be
lower, indicating fall in sexual potency. The inhibitory effect of khat extract at moderate
dose is in agreement with previous work done on male rats by Abdulwehab et al (2007).
The highest dose of khat extract caused poor sexual desire as well as decline in
performance and this finding lends support to earlier published reports (Abdulwehab et al.,
2007). As stated above, testosterone plays an important role in maintaining male sexual
desire and the significant reduction in body amount of testosterone with increasing dose of
khat extract might have been responsible for the decline in sexual craving. In this study,
CAT5 also produced a drop in sexual desire and performance, which suggests that the
cathinone component of khat extract might have played a major role in influencing male
sexual behavior. Therefore, khat extract produced biphasic effects on sexual desire of male
rats, which increased at mild and decreased at higher doses. It was suggested that alteration
of both dopamine (at low dose) and/or 5-HT (at high dose) levels in the CNS could explain
the biphasic sexual behavior of rats after khat administration, although the role of
testosterone cannot be ruled out (Taha et al, 1995).

Dopamine facilitates male sexual behavior in numerous species by acting in three
integrative neural systems (the nigrostriatal tract, the mesolimbic system and the MPOA).
In contrast, 5-HT has an overall inhibitory effect on male sexual behavior because it
activates 5-HT_{1A} receptors and stimulates ejaculation and inhibits penile erection, although
stimulation of 5-HT_{2C} receptors increases erections and inhibits ejaculation (Hull et al,
1997). There is evidence from animal studies that the main active component of khat
extract cathinone, like amphetamine, causes the release of neurotransmitters at
noradrenergic, dopaminergic and serotonergic synapses (Kalix, 1996; Michael, 2005). In
addition to the two neurotransmitters, variation in plasma testosterone level might play an
important role for the biphasic response.

On the other hand, reproductive parameters that show sexual performance and desire
appeared to be affected by cathinone treatment. Parameters that reflect sexual performance,
MF, IF and EL were significantly reduced (P<0.05) by cathinone administration. Reduced
sexual motivation was evidenced by increased IL and decreased EL. This finding implies
that cathinone might be responsible for the drop in sexual desire of male rats after
administration of moderate and high doses of khat extract. However, this observation is in
contrast to earlier work of Taha et al (1995), in which cathinone treatment at 5 mg/kg for 15 days was shown to have no effect on sexual potency but enhanced sexual arousal. This discrepancy might have occurred due to the difference in the age of animals used in the studies.

5.2. Khat Extract and Hormonal Levels

Mild dose of khat extract was observed to cause significant rise in serum testosterone level, but cortisol level appeared to be not that much affected. Besides, cathinone administration did not seem to significantly alter plasma levels of testosterone and cortisol. This result is in agreement with earlier observations made by Islam et al (1990), where both (-) and (+) enantiomers of cathinone, at a dose of 5 mg/kg for 15 days, were unable to bring about change in plasma testosterone and cortisol levels of rats, although higher doses (10 mg/kg and 15 mg/kg) of cathinone were shown to reduce plasma testosterone and cortisol levels.

Moderate and high doses of khat extract resulted in reduced plasma level of testosterone and elevated level of cortisol. This finding is in contrast to earlier observation in male olive baboon (Jason et al, 2006) but in parallel with the work done in male rabbits (Nyongesa et al, 2008). Cathinone and amphetamine produce a stress like syndrome (Islam et al, 1990) and a rise in serum level of cortisol might be associated with the stress produced. The resulting elevated plasma cortisol level might have therefore resulted in reduced plasma testosterone (Jason et al, 2006) because prolonged elevations in glucocorticoids are suggested to reduce the response of LH to GnRH (Wang et al, 1986). In males, LH stimulates the Leydig cells to secrete androgens such as testosterone (Zirkin, 1998). In addition to negative effect of cortisol on LH, stress by itself affects the hypothalamo-pituitary gonadal axis thus inhibiting the secretion of GnRH from the hypothalamus resulting into a reduced release of LH and FSH from the pituitary gland (Nyongesa et al, 2008). The fact that serum cortisol level had not been affected with mild dose of khat extract reinforces the notion that dose-dependent khat induced stress is the culprit for the observed difference in testosterone level in the groups studied.
In addition, the biphasic outcome of khat extract on serum testosterone level could be attributed to modulating role of dopamine on the release of LH releasing hormone (LHRH). Although some of the evidences that implicate dopamine in LHRH release are conflicting, certain studies showed that low dose of dopamine has excitatory effect whereas higher concentration has inhibitory effect (Marcano et al, 1980). Earlier findings have shown that cathinone acts at the cathecolaminergic synapses to increase levels of dopamine in a dose dependant manner, higher dose having higher effect (Pehek et al, 1990). Thus, it is plausible to assume that mild dose of khat extract might have enhanced LHRH release but moderate and high dose decreased hormonal release, resulting in variation of testosterone level with doses of khat.

The finding of effect of khat extract on testosterone level is consistent with the work of Nyongesa et al (2007), in which khat extract produced a biphasic response in testosterone production in-vitro. They incubated isolated mouse interstitial cells with different concentrations of khat extract and found that high concentrations of the extract (30 mg/ml and 60 mg/ml) significantly inhibited testosterone production (P<0.05) while low concentrations (0.06 mg/ml, 0.6 mg/ml and 6 mg/ml) significantly stimulated (P<0.05) testosterone production by mouse interstitial cells.

5.3. Khat and Epididymal Sperm Count

Epididymal sperm count has been observed to be significantly reduced by the highest dose of khat extract. The decrement in sperm count could not be attributed to local tissue damage in the testis because histological examinations revealed no gross deterioration. Reduced testosterone level might be the cause for the low sperm count because testosterone in conjunction with GnRH and other factors are known to influence the germ cell fate (Sofikitis et al, 2008). Low intratesticular testosterone level has been reported by Sofikitis et al (2008) to induce germ cell death. A recent study has suggested that the Bcl-2-modifying factor (Bmf) is likely to play an important role in germ cell death in response to reduced intratesticular testosterone profiles because Bcl-xl and Bcl-2 in the testis are altered following long-term anti-androgen treatment for prostate cancer (Sofikitis et al, 2008). In addition, testosterone abandonment results in changes in the adhesion of
spermatids to the Sertoli cells, which in turn, precludes further maturation of these cells (Zirkin, 1998). It is also evident that testosterone is crucial in the maintenance of elongated spermatid adhesion in the testis (Yan et al, 2009).

Similar to the high dose of the extract, spermatogenesis was found to be diminished when rats were treated with moderate dose of khat extract, which could be attributed to reduced testosterone level (Sofikitis et al, 2008). Despite the fact that plasma testosterone was high, spermatogenesis was reduced by mild dose of khat extract. Like the high dose, both mild and moderate doses of the extract were not found to produce local tissue damage in testis, which rule out involvement of tissue damage in reduction of epididymal sperm count. This observation might suggest that there appears to be a need to maintain optimum testosterone level for spermatogenesis, as both low and high levels were associated with a decrease in spermatogenesis. Evidence for this notion comes from the observation that very high levels of testosterone suppress spermatogenesis (McLachlan et al, 2002).

When rats were treated with cathinone, sperm count was found to be unaffected. Since serum testosterone level was found to be unchanged at the same dose of cathinone, it supports the idea that testosterone might be implicated in the production of sperm. Islam et al (1990) also reported the same result, in which administration of both (-) and (+) enantiomers of cathinone (5 mg/kg), for 15 days, did not affect epididymal sperm count. However, they found that the higher doses (10 mg/kg and 15 mg/kg) of cathinone to reduce epididymal sperm count (Islam et al, 1990).

5.4. **Khat Effect on Tissues**

Microscopic examination of slides did not reveal histological damage to testis and adrenal glands of rats treated with mild, moderate, and high doses of khat extract, as well as pure cathinone. This observation is consistent with the work of Mwenda et al (2006) in which administration of khat extract to male adult baboons produced no histological change in the testis. Furthermore, significant difference in relative weights of testis and adrenal gland were not observed, which might suggest absence of tissue damage.
Scarce availability of cathinone limited the study in a single dose of cathinone. The other limitation of the work was difficulty of analyzing cathinone content of the extract. However, the TLC analysis clearly showed that the khat extract indeed contains cathinone. Although it was not possible to quantify cathinone level, one could use estimates made by Geisshijsler and Brenneisen (1987) to have a rough idea about the cathinone content of the extract. According to their analysis, 100 g of fresh leave is shown to contain 36 mg cathinone. Based on this finding, the cathinone level of the khat extract given to 150 g average weight of rats was estimated to be 0.49, 0.98 and 1.47 mg for K100, K200 and K300 rats, respectively. On the other hand, 0.75 mg of cathinone was given to rats treated with pure cathinone, which was somewhere in between the mild and moderate dose of the extract. From this one could deduce the following; i) K100 enhanced sexual desire but cathinone at a dose of 5 mg/kg, K200 and K300 reduced sexual desire. This might point to the fact that if cathinone is responsible to enhance sexual desire, a dose less than 5 mg/kg appears to be effective, ii) In parallel to sexual desire, serum testosterone level was raised in K100 rats whereas the level was dropped in K200 and K300 rats. Although cathinone treatment tended to increase serum testosterone level, the rise was not significant. This might suggest that doses of cathinone greater than 5 mg/kg decrease testosterone production, iii) K100, K200 and K300 resulted in a decline of epididymal sperm count. However, cathinone administration was found not to change sperm count. This might indicate that optimum testosterone level is required to maintain spermatogenesis.
6. Conclusion and Recommendation

6.1. Conclusion

The results of the present study show that khat has biphasic effects on male rat sexual behavior, high dose of the extract diminishing sexual desire and performance whereas low dose of the extract enhancing sexual desire. This could be related to amount of testosterone produced by the rats. Khat also decreased epididymal sperm count without affecting the endocrine glands. Moreover, effect of khat on reproductive behavior of male rats seems to depend on the level of its cathinone content.

6.2. Recommendation

Based on findings of the present study, some further works are suggested as shown below.

- In the present study, effect of khat extract and cathinone was examined on serum levels of testosterone and cortisol in male rats. It is recommended also to examine effect of khat extract and cathinone on other hormones such as prolactin, LH and FSH.
- 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.
- 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.
- 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 behaviors should be comprehensively studied.
7. References


Foster & Smith Educational Staff. Rat reproduction: mating, gestation, birthing, and growth - Pp 1-3.


