BIOCHEMICAL, HORMONAL AND TOXICOLOGICAL EFFECTS OF C*ATHA EDULIS* (KHAT) ON PREGNANCY AND FETAL DEVELOPMENT IN OLIVE BABOONS (*PAPIO ANUBIS*)

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DECLARATION

I, Muema, Emily Kamanthe declare that this thesis is my original work and has not been presented for award of degree in any other university.

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ABSTRACT

There is paucity of information on the effect of khat chewing on pregnancy outcomes and foetal development in females who engage in khat chewing. Thus, the present study was designed to utilize the olive baboons (*Papio anubis*) as animal model to determine the effect of khat on fetal toxicity, maternal blood pressure, circulating hormonal levels, blood biochemistry, organ histopathology and maternal weight. Six (6) pregnant olive baboons were randomly assigned into two groups; the control group (n = 3) that were administered with 100 ml of distilled water once a week while the treatment group (n = 3) which were given an oral dose of 5 g/kg body weight of crude khat extract for 8 weeks. Changes in dam's body weight and blood pressure were measured. Liver, kidney, heart and ovaries were collected from the dams and the foetus to determine histopathological effect of khat. An increase in the liver function enzymes, albumin, urea, creatinine and sodium was observed in the treatment group compared to the control group ($P < 0.05$). The level of sodium (Na+) electrolyte was decreased in the treatment group compared to the control group ($P < 0.001$). Significant difference in body weight gain, birth weight and estradiol levels were observed in the treatment group. However, blood pressure, progesterone, luteinizing hormone and follicle stimulating hormone did not display any difference between the groups. Upon necropsy, the organs from the dams and the foetus of the treatment group showed necrosis, periportal fibrosis with focal degenerative changes, glomerular degeneration and infiltration with inflammatory cells. The study shows for the first time using non-human primates that khat, alters liver and kidney functioning and histopathology, estradiol levels, body weight of dam and foetus confirming that its use in pregnancy is toxic to the dam and developing foetus. However, further study is indicated to determine the underlying mechanisms utilized to alter the host biochemistry and the lethal doses that can lead to abortions,

delivery of still births and foetal development. The effects of chronic consumption of khat should be evaluated during the entire period of pregnancy and at different dose rate.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

The khat tree was first described by Forskal (1736- 1763), a Swedish botanist who had travelled with his friend the geographer Karsten Niebuhr (1736-1815) on an expedition to Egypt and Yemen organized by King Friederick V of Denmark, Raman, (1983). Among the many things collected was khat, which Forskal described as *Catha edulis* in the family Celastraceae. Karsten Niebuhr was the only survivor of the five members of the expedition and in memory of his friend, he called khat (*Catha edulis)* Forskal; this was published in the botanical papers in 1775 Raman, (1983). Khat (*Catha edulis*) is a natural stimulant from the *C. edulis* plant found in the flowering evergreen tree or large shrub of the Celastraceae family, which grows mainly in Yemen, Ethiopia, Somalia, Kenya, Saudi Arabia, and at high altitude areas in South Africa and Madagascar Weir, (1985).

The chewing of khat (or qat) leaves (*Catha edulis* Forsk) is widely practiced in East Africa and parts of the Middle East, such as the Yemen where it forms a deep-rooted social and cultural function Nabil, (2012). This habit has now spread to ethnic communities in the rest of the World, including Britain such as the Somali Communities in South Wales and London Beckerleg, (2008). The pleasure derived from khat chewing is attributed to the euphoric actions of its content of -S-cathinone, a sympathomimetic amine with properties described as similar to those of amphetamine Kalix and Braenden, (1985); Kalix, (1988), (1992). Although -S-cathinone is

restricted under international convention, in the UK under the Misuse of Drugs Act 1986, khat is controlled and its possession and use are restricted in the UK Klien, (2007).

However, the world health organization concludes that khat has no therapeutic potential. The psychological effects of chronic khat use have been the subject of much debate on its influence of social structure. There is now mounting for concern over the health effects on a wide range of peripheral organs. Al-Motarreb *et al*., (2010) proposed association between khat chewing and the incidence of myocardial infarction, dilated cardiomyopathy, vascular diseases such as hypertension, cerebrovascular ischemia and thromboembolism, diabetes, sexual or reproductive dysfunction, duodenal ulcer, hepatitis, nephritis, loss or gain of weight, on both dam and foetus.

In Kenya, khat chewing is popular in Meru, Tharaka Nithi, Embu, Kitui, Isiolo, Mandera Garissa, Marsabit, Ijara, and Wajir counties but has recently spread to other parts of the country Mwenda *et al*., (2003); (2006). Its use is on the rise and females are increasingly participating in this habit in a larger scale than before Al-Motarreb *et al*., (2002); Mwenda *et al*., (2003); Khawaja *et al*., (2008). In Somalia, chewing has recently become more popular among middle-class and educated women Cox and Rampes, (2003). Men consume khat more frequently than women, though the use by women is increasing Nakajima *et al*., (2013). Although both genders may refer to the upkeep of tradition to explain khat use, men reportedly use khat for occupational and recreational purposes while women's use is often for its perceived health benefits such as relief of headache, weight loss and assisting birth and delivery Stevenson *et al*., (1996).

Biochemical and toxicological effects of Khat on pregnancy and fetal development in lower animals has been noted. Khat reduced the percentage pregnancies and increased post implantation losses in Swiss albino mice Tariq *et al*., (1986). It also causes vasoconstriction in utero-placental vascular bed which may in turn impair fetal growth through reduction of placental blood flow Jansson *et al*., (1988). Higher doses of khat significantly increase resorption, fetal wastages and intrauterine growth retardation in rats Tariq *et al*., (1990). The evidence, however, is often on limited numbers of case reports and experiments on lower animals; moreover, no data is available on non-human primates.

The baboon is close to humans in terms of phylogeny, anatomy and physiology, they are useful models for applied studies relevant to human pregnancy, implantation and reproductive physiology. The aim of the current study is to focus on the biochemical, hormonal and toxicological effects of khat on pregnancy and fetal development in olive baboons.

1.2 Justification

This study was based on the use of baboons which are phylogenetically close to humans that can yield important guidelines useful in humans. There are a number of genetic and physiological similarities between baboons and humans. Namely: Firstly, these two groups have about 95 percent genetic similarity, Valdes *et al*., (2013), secondly, the baboon serves as a good animal model for the study of human reproduction, because the menstrual cycle of 28-33days throughout the year resembles that in human, procurement of blood samples presents no problem, and, as the baboon is heavier than the rhesus monkey, larger blood samples can be taken and the follicular phase can readily be distinguished from the luteal phase by the turgescence of the sex skin. Additionally, studies using baboons offer a number of advantages such as possibility to evaluate in a dose controlled study the effects of khat on pregnancy and fetal growth and development with limited ethical restrictions, studies not possible in humans because of the generation time and examination of features not possible without fatalities.

More importantly, data from the lower animals on effect of khat on fetal growth and development are lacking. Available data is on the males which do not indicate what is expected on female animal in regard to pregnancy and fetal development. It is important to note that while the investigations in humans have provided crucial information into the effects of khat, they mostly have a retrospective background and as such they have several limitations such as the inability of retrospective analysis to permit sample collection and allow controlled parameters such as dosage as would be in a randomized controlled study. Animal models are therefore required to address this issue. Whereas studies in lower animals indicate that khat may have negative effects on body physiology, they do form a good basis of what is expected in higher

animals. Extrapolation of these results to humans is limited because of wide phylogenetic gap between humans and lower animals.

1.3 Objectives

1.3.1 General objective

To evaluate the biochemical, hormonal and toxicological effects of khat on pregnancy and fetal growth and development using the olive baboon (*Papio anubis*) as a model.

1.3.2 Specific objectives

1. To evaluate the biochemical effects of khat on liver and kidney.
2. To determine the effects of khat on maternal hormonal levels.
3. To assess the histopathological effects of khat on the fetal growth and development, maternal heart, liver and kidney.
4. To investigate the effects of khat on blood pressure, temperature and maternal and foetal weight.

CHAPTER TWO

LITERATURE REVIEW

2.1 Systematics and geographical extensions of the Celestraceae family

The Celestraceae are a large family, primarily of lianas, shrubs, and trees with a sub-cosmopolitan distribution. Within the family, the aril has undergone tremendous diversification and ranges in form from a mucilaginous pulp, Simmons *et al*., (2001). Chewing the leaves of khat (*Catha edulis*) is a social habit in East African countries. This habit is spreading to ethnic communities in the rest of the world. At present easy transportation of the khat and easing of importation restrictions has helped this habit spread to countries such as the USA where Yemeni, Somali and East African communities are living Manghi *et al*., (2009). Khat continues to have a long history of indigenous traditional use, changes in use patterns due to immigration, and governmental attempts to control its use and trade Anderson and Carrier, (2009); Gebissa, (2010); Sheikh, (2014). Khat grows at an altitude of 5,000 to 8,000 feet and is found throughout

Eastern Africa and the Middle East, but also grows in parts of Southern Africa, North Africa, and Central Asia. Wild khat trees can grow as high as 80 feet in an equatorial climate, but the farmed variety is kept at around 20 feet with constant pruning. Cultivation of khat has been confined to a narrow geographical area, ranging from Yemen in the Arabian Peninsula to the Meru highlands in Kenya. The only major centres of commercial cultivation are thus found in Yemen, Ethiopia and Kenya. In the Arabian Peninsula and Eastern Africa khat has traditionally been consumed as an appetite suppressant, and is often taken by persons involved in hard physical labour, and also in a ritual context associated with spiritual contemplation. In modern times, consumption of khat has extended as a leisure pursuit, associated with sociability.

The three main alkaloids present in Khat leaves are cathinone, cathine and norephedrine Kalix, (1992). There are also small amounts of sterols and triterpenes, together with 5% protein and ascorbic acid. Khat leaves also contains tannin and minute amount of thiamin, niacin, riboflavin, iron and amino acids. Thus, only freshly picked leaves have the full efficacy Lugman and Danowski, (1976). Taken in excess, khat causes extreme thirst, a sense of exhilaration, talkativeness, hyperactivity, wakefulness, and loss of appetite. It also can cause damage to the nervous, respiratory, circulatory, and digestive systems. Khat is reported to produce constipation and antispasmodic action Makonnen, (2000). Chewing Khat has been linked with increased oxidative stress Aleryani *et al., (*2011). It was estimated by world health organization (WHO) that 30–50% of adult females consume Khat on a regular basis WHO, (2007).

Indigenous use has persisted as a mild stimulant for enhanced energy during work, maintenance of prayers during long fasts, facilitation of social ties and as a commodity for trade, as dowry, and for dispute resolutions Anderson and Carrier, (2009); Gebissa, (2010). In areas where khat is grown it is an important and lucrative cash crop. The employment opportunity created through the cultivation of khat is very high in that large numbers of people are involved in growing, harvesting, sorting, packing, transporting, loading and unloading the commodity. The wood of the plant is commonly used for fuel and due to its resistance to termite is used in the construction of houses and fencing. It is also used for making rafters, handles of farm tools (hammers and chisels) and handles of household articles such as pots and pans, rolling pins, and to make forks, combs, spoons and for rulers.

Processed leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems, Kennedy *et al*., (1983). The root is also used for stomach ache and an infusion is taken orally to treat boils, Hill, (1965). Khat has considerable social value. It is served to welcome and entertain guests, in mourning, weddings and circumcision ceremonies and collective labour works. Khat chewing has its own associated ceremonies like smoking of incense, cigarettes and use of drinks (soft drinks, tea and milk). Khat chewing is addictive and has negative physical, economical and social connotations Ageely, (2008). Students and a number of staff in higher education institutions and high schools are using khat to increase their concentration levels and attention span Sikiru and Babu, (2009).

2.2 Phytochemical constituents of Khat

Khat contains more than forty alkaloids, glycosides, tannins, amino acids, vitamins and minerals Halbalch, (1972); Cox and Hampes, (2003); Veneiro *et al*., (2011). The environmental and climate conditions determine the chemical profile of khat leaves Al-Motarreb *et al*., (2002). In the Yemen Arab Republic, about 44 different types of khat exist originating from different geographic areas of the country Geisshusler and Brennisen (1987); Al-Motarreb *et al*., (2002).

The three main alkaloids present in khat leaves are the cathines, S-cathinone (S- -aminopropriophenone) (Fig. 2-1), norpseudoephedrine (cathine) (Fig. 2-2) and norephedrine Szendrei, (1975a), (1975b); Schorno and Steinegger, (1979), which are phenylpropylamines structurally related to amphetamine and noradrenaline. The phenylpropylamine composition varies between the plant region and country of origin Nasir, (2011). A previous comparison of khat from Kenya, Madagascar and Ethiopia showed that the highest level of cathinone occurred in khat bundles originating from trees and sold in Nairobi's street market Al-Moterrab *et al*.,

(2002). The highest concentrations of these amines were found in the young shoots already carrying leaves Nigg and Seigher, (2013). The phenylpentenylamines, merucathine, merucathinone and pseudomerucathine, were isolated from the fresh plant material cultivated in the Meru region of Northern Kenya Brenneisen *et al*., (1984); Brenneisen and Geisshusler, (1985); (1987). The third type of khat alkaloids are the cathedulines; these are a group of sesquiterpenes which are polyesters of euonyminol Samuelsson, (1992). They are identified as K1, K2, K6 and K15 from Kenyan khat, of which K2 is the most abundant. The equivalent catheduline in khat from Yemen is Y1 Crombie *et al*., (1979). It is unlikely that the cathedulines and phenylpentenylamines have significant biological activity Kalix *et al*., (1987). Other constituents present in khat leaves include small amounts of essential oils, sterols and triterpenes, and 5% protein of insignificant nutritional value Kalix and Braenden, (1985). Ascorbic acid is also present in the leaves Raman, (1983). Khat also contains tannin (7%–14% by weight in dried leaves) and minute amounts of thiamin, niacin, ribo-flavin, iron and amino acids (Lugman and Danowski, (1976). Apart from tannin, these substances are unlikely to contribute to the biological effect of khat Kalix, (1984).

The phenylalkylamines and the cathedulins are the major alkaloids. The cathedulins are based on a polyhydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol. Recently, 62 different cathedulins from fresh khat leaves were characterized Kite *et al*., (2003). The khat phenylalkylamines comprise cathinone [S-(-)-cathinone], and the two diastereoisomers cathine [1S, 2S-(+)-norpseudoephedrine or (+)-norpseudoephedrine] and norephedrine [1R,2S-(-)-norephedrine]. These compounds are structurally related to amphetamine and noradrenaline. The plant contains the (-)-enantiomer of cathinone only Kalix and Braenden, (1985). Thus, the naturally occurring S-(-)-cathinone has the same absolute configuration as S-(+)-amphetamine.

Cathinone is mainly found in the young leaves and shoots. During maturation, cathinone is metabolized to cathine [(+)-norpseudoephedrine] and (-)-norephedrine.

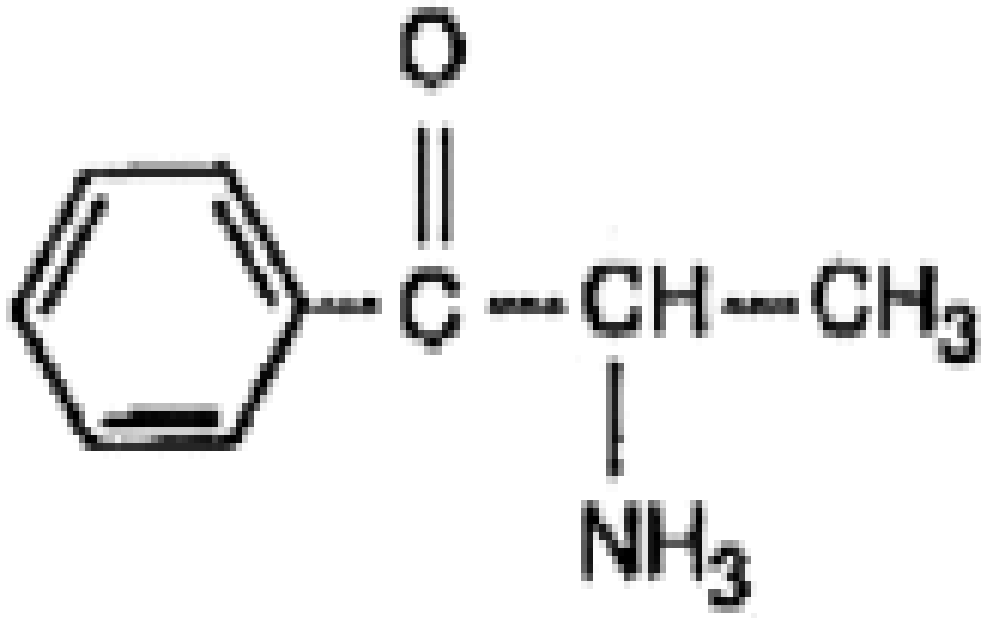

Figure 2-1: Chemical Structure of cathinone ECDD, (2006)

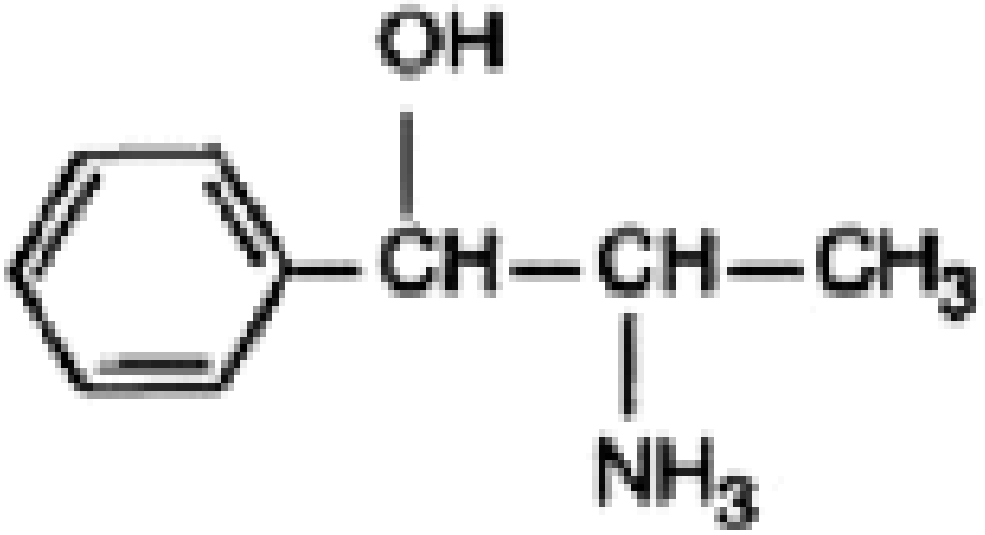

Figure 2-2: Chemical Structure of cathine ECDD, (2006)

Cathinone is unstable and undergoes decomposition reactions after harvesting and during drying or extraction of the plant material Nencini and Ahmed, (1989); Kalix and Braenden, (1985); WHO, (1980); Breinnisen and Geisshuhusler, (1987). Decomposition results into a 'dimer' (3,6-dimethyl-2,5-diphenylpyrazine) and possibly smaller fragments. Both the dimer and phenylpropanedione have been isolated from khat extracts WHO, (1980). As cathinone is presumably the main psychoactive component of khat, this explains why fresh leaves are preferred and why khat is wrapped up in banana leaves to preserve freshness.

The phenylalkylamine content of khat leaves varies within wide limits. Fresh khat from different origin contained on the average 36 mg cathinone, 120 mg cathine, and 8 mg norephedrine per 100 gram of leaves Geisshusler and Brenneisen, (1987). Toennes *et al*., (2003) found 114 mg cathinone, 83 mg cathine and 44 mg norephedrine in 100 gram of khat leaves confiscated at Frankfurt airport. Widler *et al*., (1994) found 102 mg cathinone, 86 mg cathine and 47 mg norephedrine in 100 gram of fresh leaves of khat from Kenya confiscated at Geneva Airport. Al-Motarreb *et al*. reported higher levels of cathinone in fresh leaves: 78-343 mg/100 gram Al-Motarreb *et al*., (2002). Khat leaves also contain considerable amounts of tannins (up to 10% in dried material) and flavonoids Al-Motarreb *et al*., (2002); Hassan *et al*., (2002).

2.3 Pharmacology of *Catha edulis* (khat)

2.3.1 Actions of cathinone and cathine

Cathinone is also named alpha-aminopropiophenone. It is considered to be the most active ingredient of khat. It has been isolated and synthesized and its effects have been shown to be similar to amphetamine, but with a lower potency. The general analogy between the effects of (-

)-cathinone and those of amphetamine, as well as their chemical similarity suggest that the two substances might have the same mechanism of action Graziani *et al*., (2008); Houghton, (2004); Cox and Rampes, (2003) with similar potential for abuse Kalix, (1984 a). Cathinone is estimated to be 7–10 times more potent than cathine. It is difficult to synthesize, therefore it is unsuitable for marketing as a pure substance for drug misuse, Nencini *et al.*, (1989). Cathine is also named norpseudoephedrine and phenylpropanolamine. Cathine has a milder psychostimulant action than cathinone and the effects last for only a short time, so the user must chew leaves almost continuously. It plays only a minor role in the action of khat, but it is cathine that is responsible for the unwanted systemic effects. Normally, fresh leaves contain a higher proportion of the desirable cathinone. Where the content of cathine is relatively higher, the cathine causes more unwanted systemic effects. On drying, cathinone breaks down into cathine. Therefore khat chewers prefer fresh leaves that contain a higher proportion of cathinone to cathine, so that they obtain a better stimulation with fewer systemic adverse effects.

2.3.2 Mode of action of cathinone and cathine

Many drugs of abuse are thought to exert their effects by increasing concentrations of stimulant neurotransmitters, such as dopamine, serotonin and/or noradrenaline in specific regions of the brain. Both cathinone and cathine interact with the dopaminergic pathways and prevent the reuptake of noradrenaline and dopamine Pahek *et al*., (1990). There is evidence from animal studies that cathinone causes dopamine release and this is the mechanism underlying khat use Brennesien *et al*., (2012); also the release of neurotransmitters at serotonergic (5-HT) synapses and peripheral noradrenergic sites Kalix, (1983). These biochemical properties are similar to those of amphetamine, especially its sympathomimetic properties. Brennesien *et al.*, (2012).

S-Cathinone is the most potent constituent of khat leaves as a stimulant of the central nervous system, Zelger *et al.*, (1980); Glennon *et al.*, (1984). The phenylpentenylamines, such as Merucathinone which are found in Kenyan khat are unlikely to play a significant role in the stimulating properties of khat, Kalix *et al.*, (1987). The pharmacological profile of S-cathinone has been shown both *in vivo* and *in vitro* to closely resemble that of amphetamine, Brenneisen *et al.*, (1990), and as such its mechanism of action has been linked to the release of monoamines Kalix and Braenden, (1985). Like amphetamine, the main central effects of S-cathinone are hyperactivity, euphoria, excitability, restlessness and anorexia, actions that have been attributed to the ability of amphetamine and amphetamine-like drugs to stimulate the release of 5-hydroxytryptamine (5-HT) Kalix, (1984), dopamine, Kalix, (1980) and noradrenalin, Kalix and Braenden, (1985). As well as the central stimulant actions of S-cathinone, the chewing of khat also leads to several peripheral effects that include the development of a dry mouth, blurred vision and mydriasis, Nencini *et al.*, (1984) and increases in blood pressure and heart rate Brenneisen *et al.*, (1990); Hassan *et al.*, (2000). These effects are believed to result from the ability of cathinone (like amphetamine) to act as an indirect sympathomimetic agent and to facilitate the release of catecholamines from sympathetic nerve terminals, Kohli and Goldberg, (1982); Kalix, (1983).

2.3.3 Pharmacokinetics of cathinone and cathine

The euphoric effect appears shortly after the chewing begins, suggesting absorption from the oral mucosa. The effects of cathinone occur 15–30 minutes after oral ingestion. Metabolism of cathinone is rapid, occurring mainly during first passage through the liver. Only a small fraction (about 2%) appears unchanged in the urine. Most cathinone is metabolized to norephedrine and

is excreted in this form. The rate of inactivation is about the same as the rate of absorption, which limits the cathinone blood levels attainable by chewing. Cathine has a slower onset of action, with a serum half-life in humans of about 3 hours. It is excreted unchanged in the urine within about 24 hours. There is pharmacological synergism with drinks containing methylxanthines (e.g. tea and cola), which therefore enhances the effects of khat.

Table 2-1: Adverse effects of Khat on various body systems reported in literature

Body system	Reported adverse effects	Reference
Cardiovascular system	Tachycardia, arrhythmias, palpitations, hypertension, vasoconstriction, ischaemia, infarction, pulmonary oedema, cerebral haemorrhage, Exacerbation of pre-existing cardiac condition	Gianni *et al*, (1986); El-Guindy, (1971)
Respiratory system	Bronchitis, tachypnoea, dyspnoea, tuberculosis	Kennedy *et al*., (1983);
Gastrointestinal system	Dry mouth, polydipsia, dental caries, periodontal disease, chronic gastritis, gastric ulcer, constipation, paralytic ileus, anorexia, weight loss, increased risk of upper gastrointestinal malignancy	Elmi, (1983); Giannini *et al*., (1986)
Hepato-billiary system	Cirrhosis	Al-Hashem *et al*., (2011)

Cont' Table 2-1: Adverse effects of Khat on various body systems reported in literature

Body system	Reported adverse effects	Reference
Genitourinary system	Spermatorrhoea, impotence, libido change, urinary retention (complicated by diuresis from increased fluid intake), Obstetric effects, Low birth weight, stillbirths, impaired lactation	Nyachieo *et al*., (2012); Mwenda *et al*., (2003); Mwenda *et al*., (2006); Al-Hashem *et al*., (2011)
Metabolic and endocrine effects	Hyperthermia, perspiration, hyperglycemia	
Central nervous system	Dizziness, impaired concentration, insomnia, headaches, migraine, mydriasis, conjunctival congestion, impaired motor coordination, fine tremor and stereotypical behaviour	Kalix, (1984)

2.3.4 Interactions between khat and other drugs

Khat can interact with therapeutic drugs. Phenylpropanolamine (a decongestant), which can display synergism with khat, is widely available in over-the-counter cold and appetite-suppressant preparations and in prescription drugs. The use of monoamine oxidase inhibitors is to be avoided in khat users, as this is likely to precipitate a dangerous level of sympathetic stimulation, possibly leading to a hypertensive crisis. It may interfere with absorption of some orally administered antibiotics, Valko *et al.*, (2007). Reactions to surgical anesthetics may be bizarre in the chronic khat user and during the postoperative period the patient may be agitated and over arouse Cox and Rampes, (2003).

2.4 Studies of effects of khat in lower animals

2.4.1 Cardiovascular effects

Cathinone has been shown to possess vasoconstrictor activity in isolated perfused hearts from guinea pigs, Al-Motarreb and Broadley, (2003). The effect was likely to be due to an indirect action by release of noradrenaline from sympathetic nerve endings or due to a direct action on alpha1-adrenoreceptors. Cathinone is able to potentiate noradrenaline-evoked contractions of the rat right ventricle, Cleary *et al.*, (2002) and inhibit the uptake of noradrenaline into ventricular slices by a mechanism involving competitive blockade of the noradrenaline transporter Cleary and Docherty, (2003). The vasoconstrictor activity of cathinone explains the increase in blood pressure seen in humans Brennesien *et al.*, (1990) and in animals Kohli and Goldberg, (1982) and might be related to the increased incidence of myocardial infarction occurring during

khat sessions, i.e. during the khat-effective period Al-Motarrreb *et al*., (2002) and associated with heavy khat chewing Al-Motarrreb *et al*., (2005).

2.4.2 Effects on the reproductive system

As shown in table 2-2 treatment of male mice with khat extract over a period of 6 weeks produced a dose-dependent reduction in fertility rate Tariq *et al*., (1990). In cathinone-treated rats, a significant decrease in sperm count and motility, and an increase in the number of abnormal sperm cells were found Islam *et al.,* (1990). Histopathological examination of the testes revealed degeneration of interstitial tissue, cellular infiltration and atrophy of sertoli and leydig cells in cathinone-treated animals. Cathinone also produced a significant decrease in plasma testosterone levels of the rats. Although both enantiomers of cathinone produced deleterious effects on male reproductive system, (-)-cathinone was found to be more toxic Islam *et al.,* (1990). In contrast, rabbits fed khat for three months had an increased rate of spermatogenesis and the Leydig cells were in good condition Al-Mamary *et al*., (2002). In male adult olive baboon, crude khat extract (equivalent to 60 g leaves and shoots) given orally once a week during 2 months produced an increase in plasma testosterone levels and a decrease in the plasma levels of prolactin and cortisol Mwenda *et al*., (2006). The testosterone results are in contrast with earlier observations in humans Kalix and Braenden, (1985) and rats Islam *et al*., (1990). In biopsies taken one month after the last khat administration, no histopathological changes were found in the testis, epididymis, liver, kidney and pituitary gland of the animals. This contrasts with results of cathinone on rabbit liver, which showed increasing chronic inflammation with peri-portal fibrosis in the tissue sections obtained from animals treated with both 20% and 30% *C. edulis* Al-Habori *et al*., (2002). The doses and administration regimens

were different and this may explain the differences. Khat given to pregnant guinea pigs reduced placental blood flow Jansson *et al*., (1988b) and produced growth retardation in the offspring Jansson *et al*., (1988a). The effects of khat are summarized in table 2-2 below.

Table 2-2: Effects of cathinone and methanolic crude extract (ME) derived from Khat on the reproductive system

Reproductive parameter	Effects	References
Sperm volume	Reduced	Dalu, (2000); Hakim, (2002)
Sperm motility	Reduced	Islam *et al.*, (1990); El-Shoura *et al*. (1995)
Sperm motility index	Reduced	El-Shoura *et al*., (1995)
Sperm count	Reduced	Islam *et al.*, (1990); El-Shoura *et al*., (1995)
Abnormal sperm	Increased	Islam *et al.*, (1990); El-Shoura *et al*., (1995)
Utero-placental blood flow	Reduced	Jansson *et al*., (1987); Jansson *et al*., (1988a)
Post implantation losses	Increased	Tariq *et al.*, (1986)
Maternal weight gain	Reduced	El-Shoura *et al*., (1995); Abdul *et al.*, (1987); Jansson *et al*., (1988b)
Placental vascular resistance	Increased	Jansson *et al*., (1987); Jansson *et al.*, (1988a); Jansson *et al*., (1988b)
Maternal blood pressure	Increased	Jansson *et al*., (1987); Jansson *et al.*, (1988a); Jansson *et al*., (1988b)
Maternal myoendometrial blood flow	Reduced	Jansson *et al*., (1987); Jansson *et al.*, (1988a); Jansson *et al*., (1988b)
Sex organ size	Reduced	Islam *et al.*, (1990)
Sex ratio	No effect	Eriksson *et al.*, (1991)
Pup size	Reduced	Jansson *et al*., (1987); Jansson *et al.*, (1988a); Jansson *et al*., (1988b)
Plasma testosterone	Reduced	El-Shoura *et al*., (1995)
Fertility	Reduced	Tariq *et al.*, (1986)
Potency	Reduced	Islam *et al.*, (1990)
Maternal milk production	Reduced	Eriksson *et al.*, (1991)

ME* methanolic crude Khat extract

2.4.3 Genotoxicity and teratogenic effects

Orally administered khat extract induced lethal mutations of dominant alleles and chromosomal aberrations in sperm cells in mice Tariq *et al*., (1990); Qureshi *et al*., (1988) and teratogenic effects in rats Islam *et al*., (1994). With the micronucleus test to determine genetic damage, an 8-fold increase in micronucleated buccal mucosa cells was seen among khat chewing individuals living in the area of the horn of Africa Kassie *et al*., (2001). Khat consumption did not lead to a detectable elevation of micronucleated bladder mucosa cells Kassie *et al*., (2001). Among heavy khat chewers, 81% of the micronuclei had a centromere signal indicating that khat is aneuploidogenic Kassie *et al*., (2001). The effect of khat, tobacco and alcohol was found to be additive. These results suggest that khat consumption, especially when accompanied by alcohol and tobacco, might be a potential cause of oral malignancy Kassie *et al*., (2001).

Khat affects blood formation in mice by inducing various chromosomal aberrations and suppression of bone marrow and also reduces the mitotic index of somatic cells Qureshi *et al*., (1988). In the same study, khat was shown to induce chromosomal aberrations in gametes of mice. Khat has a potential to affect the systemic capacity to handle free radicals Al-Qirim *et al*., (2002) but may also have some antioxidant effects owing to some of its antioxidant constituents such as flavonoids Al-Zubairi *et al*., (2003). In an *in vitro* study, an organic extract of khat was shown to inhibit *de novo* RNA, DNA and protein synthesis in mammalian cells Al-Ahdal *et al*., (1988). An extract of khat has been shown to induce caspase-dependent apoptotic cell death in human leukemia cell lines Dimba *et al*., (2004). Tests using khat extracts in mice have shown that the effects of khat are generally dose dependent and tend to affect certain systems of the body more than others Qureshi e*t al*., (1988). Therefore, it is likely that the potential adverse effects of khat are limited by the inability of khat users to consume large quantities of khat.

2.4.4 Biochemical and histopathological effects

Biochemically, khat leaves decreased plasma cholesterol, glucose and triglycerides in rabbits, Al-Habori and Al-Mamary, (2004) and increased plasma alkaline phosphatase and alanine aminotransferase in white rabbits Al-Mamary *et al.*, (2002). Histopathological signs of congestion of the central liver veins were observed with acute hepatocellular damage and regeneration in rabbits. In addition, some kidney lesions were seen with the presence of fat droplets in the upper cortical tubules, acute cellular swelling, hyaline tubules, and acute tubular necrosis. Spleen was not affected and the histoarchitecture of the testes and caudal epididymis was normal, however showing increased rate of spermatogenesis Al-Mamary *et al*., (2002). Adverse effects of khat may be summarized according to the system involved for example hepatocellular damage in the liver, nephrotocity in the kidney and myocardial infarction in the heart Cox and Rampes, (2003).

2.5 Studies of effects of khat in non-human primate reproductive system (monkeys)

Administration of 5 g/kg body weight of crude khat extract orally using a feeding tube twice a week with the baboon under anesthesia for one month resulted in significant reduction in sperm motility, sperm count, sperm chromatin integrity, testosterone levels and prolactin Nyachieo *et al*., (2013). A study by Mwenda *et al*., (2006) using baboon as the animal model indicated that khat may exert a transient effect on the male fertility by interfering with the hormonal profiles.

2.6 Studies of effects khat in humans

2.6.1 Cardiovascular effects

Khat chewing induces small and transient rises in blood pressure and heart rate Al-Motarreb *et al*., (2002); Hassan *et al*., (2005). Cathinone (0.5 mg base/kg of body weight) has similar effects coinciding with the presence of cathinone in blood plasma Brennesien *et al*., (1990); Kalix *et al*., (1990). These effects could be blocked by the beta1-adrenoreceptor blocker atenolol, but not by the alpha1-adrenoreceptor blocker indoramin, indicating mediation through stimulation of beta-1-adrenoreceptors Hassan *et al*., (2005).

In a pharmacokinetic study, diastolic and systolic blood pressures were elevated for about 3 hours after chewing Toennes *et al.,* (2003). The rise of blood pressure already started before the rise of alkaloid plasma concentrations, indicating an initial study engagement effect. The dose used was about one quarter (0.6 g/kg) of a traditional khat session dose and chewing was for 1 hour. This resulted in a mean oral dose of 45 mg cathinone. This rather low dose did not affect heart rate, pupil size and reaction to light, and it did not induce rotary nystagmus or impairment of reaction, Toennes *et al.,* (2003). All participants reported the personal feeling of being alert and energetic. An impairment of other psychophysical functions could not be objectified Toennes *et al.,* (2003). In another study, diastolic and systolic blood pressure, mean arterial blood pressure, and heart rate were raised during the 3 hours of khat chewing and during the following hour Hassan *et al.,* (2000).

2.6.2 Reproductive studies

There are limited studies on the effects of khat on human reproduction. However, the available data suggest that chronic use may cause spermatorrhea and may lead to decreased sexual functioning and impotence Halbach, (1972); Mwenda *et al*., (2003). In chronic chewers, sperm count, volume and motility were decreased El-shoura *et al*., (1995); Hakim, (2002). Deformed spermatozoa (65% of total) have been found in Yemenite daily khat users, with different patterns including head and flagella malformations in complete spermatozoa, aflagellate heads, headless flagella, and multiple heads and flagella El-shoura *et al*., (1995). In pregnant women, khat consumption may have detrimental effects on uteri-placental blood flow and as a consequence, on fetal growth and development Mwenda *et al*., (2003). Lower mean birth weights have been reported in khat-chewing mothers compared to non-using mothers indicating an association between khat chewing and decreased birth weight Abdul *et al*., (1987).

2.6.3 Toxicological aspects of khat

Khat usage affects cardiovascular, digestive, respiratory, endocrine, and genito-urinary systems. In addition, it affects the nervous system and can induce paranoid psychosis and hypomanic illness with grandiose delusions Kalix, (1988). The effects on the nervous system resemble those of amphetamine with differences being quantitative rather than qualitative Cox and Rampes, (2003); Hassan *et al*., (2002); Dhaifalah and Santary, (2004).

The main toxic effects include increased blood pressure, tachycardia, insomnia, anorexia, constipation, general malaise, irritability, migraine and impaired sexual potency in men Nencini and Ahmed, (1989). Mild depressive reactions have been reported during khat withdrawal or at

the end of a khat session Kalix and Braenden, (1985); Hassan *et al*., (2002); Pantenlis *et al*., (1989). Frequent use of high doses may evoke psychotic reactions.

Biochemically, khat leaves decreased plasma cholesterol, glucose and triglycerides in rabbits, Al-Habori and Al-Mamary, (2004) and increased plasma alkaline phosphatase and alanine aminotransferase in white rabbits, Al-Habori *et al.,* (2002). Histopathological signs of congestion of the central liver veins were observed with acute hepatocellular damage and regeneration. In addition, some kidney lesions were seen with the presence of fat droplets in the upper cortical tubules, acute cellular swelling, hyaline tubules, and acute tubular necrosis. Spleen was not affected and the histoarchitecture of the testes and cauda epididymis was normal showing, however, increased rate of spermatogenesis was observed Al-Habori and Al-Mamary, (2004); Al-Habori *et al*., (2002).

2.7 Baboon endocrinology and physiology

Previously baboons have been used as models to study normal endocrine functions during pregnancy development of fetus, Pepe and Albrecht, (1994 and 1995). Spontaneous cyclic ovulation and menstruation occur monthly in baboons throughout the year (no seasonal breeding like rhesus monkeys) with menstrual cycle lasting 33.4 ± 2.1 days and menstruation lasting 3.2 ± 1.0 days with the selection of a dominant follicle in baboon natural cycle taking place in day 5-7 of the cycle similar to humans. As the baboons age, they exhibit decreased ovarian reserve and a natural menopause as in women with variations in menstrual cycle length and hormonal levels, a characteristic not found in most other laboratory animals. In addition, reproductive diseases like endometriosis and adenomyosis occur spontaneously in baboons, D'Hooghe *et al*., (2009).

Baboon reproductive endocrinology reveal similar hormonal profiles as observed in humans with the peak estrogen serum level at the time of ovulation being 245 ± 30.5 pg/ml (range 200-300pg/ml) followed by progesterone secretion increase after the onset of LH surge and reaches a maximum level of 11.5 ± 2.3 ng/ml Stevens, (1997). Other studies have revealed the presence of estrogen receptors in the baboon ovary, as in humans, Billiar *et al*., (1992). Since baboons' exhibit perineal sex skin inflation and deflation in response to the levels of estrogen, it is easy to follow the menstrual cycle by daily inspection and recording of perineal sex skin inflation and deflation which correspond with relative precision, to the follicular and luteal phases respectively Stevens, (1997). The baboon perineal stages have been classified as: stage 7 (menstrual), stage 1 (post menstrual), stage 2 (pre-ovulation), stage 3, 4 or 5 (ovulation), stage 6 and 0 (luteal/quiescent/pre-menstrual) and stage 8 (pregnancy).

2.7.1 Baboon embryo implantation, placentation and pregnancy

The close similarities between baboon and human embryo implantation have been extensively reviewed, Hearn *et al*., (1986); Carter, (2007). In various studies, the baboon embryo takes 4 days to arrive in the uterus, similar to humans (3-4 days). The gestation period for a female baboon is 180 days (6 months) i.e. 1^{st} trimester (2 months i.e. 1^{st} and 2^{nd} months); 2^{nd} trimester (2 months i.e. 3^{rd} and 4^{th} months); and 3^{rd} trimester (2 months i.e. 5^{th} and 6^{th} months)

2.8 Liver Function tests

2.8.1 Serum Bilirubin

Bilirubin is the catabolic product of hemoglobin produced within the reticulo-endothelial system, released in unconjugated form which enters into the liver, converted to conjugated forms bilirubin mono and diglucuronides by the enzyme UDP-glucuronyltransferase Mauro *et al.* (2006). Jaundice occurs when bilirubin becomes visible within the sclera, skin, and mucous membranes at a blood concentration of around 40 μmol/l Beckingham and Ryder, (2001). Normal serum total bilirubin varies from 2 to 21 μmol/l Diana, (2007). The indirect (unconjugated) bilirubin level is less than 12 μmol/l and direct (conjugated) bilirubin less than 8 μmol/l Diana, (2007). The serum bilirubin levels more than 17 μmol/l suggest liver diseases and levels above 24 μmol/l indicate abnormal laboratory liver tests Thapa and Anuj, (2007); Wong *et al*., (2004).

The occurrence of unconjugated hyperbilirubinemia is due to over production of bilirubin, decreased hepatic uptake or conjugation or both Tiribelli and Ostrow, (1995). It is observed that genetic defect of UDP-glucuronyltransferase cause Gilbert's syndrome, Crigler-Najjar syndrome and reabsorption of large hematomas and ineffective erythropoiesis Thomsen *et al*., (1981). In viral hepatitis, hepatocellular damage, toxic or ischemic liver injury higher levels of serum conjugated bilirubin is seen Thapa and Anuy, (2007). Hyperbilirubinemia in acute viral hepatitis is directly proportional to the degree of histological injury of hepatocytes and the longer course of the disease Thapa and Anuy, (2007). It has also been observed that the decrease of conjugated serum bilirubin is a bimodal fashion when the biliary obstruction is resolved Alvarez *et al*., (1999). Parenchymal liver diseases or incomplete extrahepatic obstruction due to biliary

canaliculi give lower serum bilirubin value than those occur with malignant obstruction of common bile duct but the level remains normal in infiltrative diseases like tumours and granulomas Daniel *et al*., (2007). Raised serum bilirubin from 20.52 µmol/l to 143.64 µmol/l in acute inflammation of appendix has been observed Khan, (2006). In normal asymptomatic pregnant women total and free bilirubin concentrations were significantly lower during all three trimesters and a decreased conjugated bilirubin was observed in the second and third trimesters, Bacq *et al*., (1996). Recent study has shown that a high serum total bilirubin level may protect neurologic damage due to stroke, Parlstein *et al*., (2008).

2.8.2 Alanine amino transferase (ALT)

Alanine amino transferase (ALT) is found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body. ALT is purely cytoplasmic catalyzing the transamination reaction Mauro *et al*., (2006). Any type of liver cell injury can reasonably increases ALT levels. Normal serum ALT is 7–56 U/ L Diana, (2007). Marked elevations of ALT levels greater than 500 U/L observed most often in persons with diseases that affect primarily hepatocytes such as viral hepatitis, ischemic liver injury (shock liver) and toxin-induced liver damage. Despite the association between greatly elevated ALT levels and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage Kallei *et al*., (1964). Viral hepatitis like A, B, C, D and E may be responsible for a marked increase in aminotransferase levels Marcellin, (1999). The increase in ALT associated with hepatitis C infection tends to be more than that associated with hepatitis A or B Marcellin, (1999). Moreover in patients with acute hepatitis C serum ALT is measured periodically for about 1 to 2 years Mauro *et al*., (2006). Persistence of elevated ALT

for more than six months after an occurrence of acute hepatitis is used in the diagnosis of chronic hepatitis Sleth *et al*., (1997). Elevation in ALT levels is greater in persons with nonalcoholic steato-hepatitis than in those with uncomplicated hepatic steatosis Sleth *et al*., (1997). In a recent study the hepatic fat accumulation in childhood obesity and nonalcoholic fatty liver disease caused serum ALT elevation. Moreover increased ALT level was associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides James *et al*., (2006). Presence of bright liver and elevated plasma ALT level was independently associated with increased risk of the metabolic syndrome in adults Tzong-ttsi *et al*., (2005).

2.8.3 Aspartate amino transferase (AST)

Aspartate amino transferase (AST) catalyzes transamination reaction. AST exist in two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasmic form Mauro *et al*., (2006). AST is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney Mauro *et al*., (2006). Normal serum AST is 0 to 35U/L Diana, (2007). Elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also in chronic liver diseases like liver tissue degeneration and necrosis Thapa and Anuy, (2007). About 80% of AST activity of the liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme Thapa and Anuy, (2007). However the ratio of mitochondrial AST to total AST activity has diagnostic importance in identifying the liver cell necrotic type condition and alcoholic hepatitis, Panteghini *et al*., (1983).

2.8.4 Aspartate amino transferase (AST)/ Alanine amino transferase (ALT) ratio (AST/ALT ratio)

The ratio of AST to ALT has more clinical utility than assessing individual elevated levels. Most causes of liver cell injury are associated with an AST that is lower than the ALT. The magnitude of AST and ALT elevations vary depending on the cause of the hepatocellular injury Nyblom *et al*., (2006). AST/ALT ratio is increased (>2) in chronic liver damage and decreased (<1) in acute hepatitis. A coenzyme pyridoxal-5'-phosphate deficiency may depress serum ALT activity and consequently increases the AST/ALT ratio Cohen and Kaplan, (1979). The ratio increases in progressive liver functional impairment and found 81.3% sensitivity and 55.3% specificity in identifying cirrhotic patients, Edoardo *et al*., (2003)

2.8.5 Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) is present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. It performs lipid transportation in the intestine and calcification in bone Mauro *et al*., (2006). Normal serum ALP is 41 to 133U/L Diana, (2007). In acute viral hepatitis, ALP usually remains normal or moderately increased. Elevation of ALP with prolonged itching is related with Hepatitis A presenting cholestasis Rosalki and Mcintyre, (1999). Tumours secrete ALP into plasma and there are tumour specific isoenzymes Rosalki and Mcintyre, (1999). Hepatic and bony metastasis can also cause elevated levels of ALP, Rosalki and Mcintyre, (1999). Other diseases like infiltrative liver diseases, abscesses, granulomatous liver disease and amyloidosis may cause a rise in ALP. Mildly elevated levels of ALP may be seen in cirrhosis, hepatitis and congestive cardiac failure Rosalki and Mcintyre, (1999). Low levels of ALP occur in hypothyroidism, pernicious anaemia, zinc

deficiency and congenital hypophosphatasia Simko, (1991). ALP activity was significantly higher in the third trimester of asymptomatic normal pregnancy showing extra production from placental tissue in human, Bacq *et al*., (1996).

2.9 Renal function tests

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Over decades research and utilization of biomarkers has evolved substantially. National Institute of Health (NIH) 2001 defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention Ramachandran, (2006). As markers of renal function creatinine, urea, uric acid and electrolytes are for routine analysis whereas several studies have confirmed and consolidated the usefulness of markers such as cystatin C and β-Trace Protein Priem *et al*., (2001); Laura *et al*., (2007).

2.9.1 Creatinine

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass Yuegang, (2008). Creatinine is commonly used as a measure of kidney function. The normal creatinine clearance test valve is 110-150 ml/min in male and in female it is 100-130 ml/min Corbett, (2008). The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the normal interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules Edmund and David, (2006). Creatinine values may alter as its generation may not be simply a product of muscle mass but influenced by muscle

function, muscle composition, activity, diet and health status Banfi and Del, (2006). The elevated values are also seen in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration Edmund and David, (2006).

2.9.2 Urea

Blood urea nitrogen is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys blood urea nitrogen is filtered out of blood by glomeruli and is partially being reabsorbed with water, Corbett, (2008). In general, 7 to 20 mg/dL (2.5 to 7.1 mmol/L) is considered normal. The most frequently determined clinical indices for estimating renal function depends upon concentration of blood urea nitrogen in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased Mitchel and Kline, (2006). Blood urea nitrogen clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends on several non-renal factors, including diet and blood urea nitrogen cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract. The high BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods.

2.9.3 Proteinuria

Clinically the appearance of significant amount of protein in urine is one of the earliest sign of almost all renal diseases. Normally excretion in most healthy adults is between 20-150 mg of protein in urine over 24 hrs. Proteinuria more than 3.5 gm/day is taken to be diagnostic of nephrotic syndrome Sandeep *et al*., (2004). Estimation of proteinuria helps in differentiating between tubule-interstitial and glomerular diseases and also to follow the progress of renal disease and to assess the response to therapy. Panels of protein measurement including albumin, α 2-macroglobulin and IgG have been employed in differential diagnosis of prerenal and postrenal disease.

2.9.4 Electrolytes

Electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions. Potassium is used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium James and Mitchel, (2006). Hyperkalemia is the most significant and life-threatening complication of renal failure James and Mitchel, (2006).

Table 2-3: Normal values of electrolytes in the body

Parameter	Standared International units
Bicarbonate	24-30 mmol/L
Potassium	3.5-5.0 mmol/L
Sodium	135-145 mmol/L
Chloride	98-106 mmol/L

2.10 Hormonal assay

FSH stimulates the maturation of ovarian follicles in females. Ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete the steroid hormones progesterone and estrogen. Progesterone is necessary for maintenance of pregnancy, in most mammals. Estrogen regulates progesterone, protecting pregnancy and is also known to kick-start one of the major processes of fetal maturation. Without, a fetus's lungs, liver and other organs and tissues cannot mature. LH is required for continued development and function of corpora lutea. The name luteinizing hormone derives from this effect of inducing luteinization of ovarian follicles.

The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. Estradiol is a type of endogenous estrogen hormone, called E2, found in both males and females, and is the most dominant estrogen hormone found in women. During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Though estradiol is the most plentiful of the three estrogens it is also the weakest, whereas estrogen is the strongest with a potency of approximately 80 times that of estriol. Thus, estradiol is the most important estrogen in non-pregnant females who are between the menarche and menopause stages of life. However, during pregnancy this role shifts to estriol, and in postmenopausal women estrone becomes the primary form of estrogen in the body. Another type of estrogen called estetrol (E4) is produced only during pregnancy. All of the different forms of

estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase.

Changes in endocrine levels, such as progesterone, estrogen, prolactin, and oxytocin, are important for pregnancy, parturition, maternal responsiveness, and lactation in most mammals Pryce, (1996), and have been related to different maternal responses throughout the peripartum period. Progesterone is a hormone that stimulates and regulates important functions, playing a role in maintaining pregnancy, preparing the body for conception and regulating the monthly menstrual cycle. The study of primate all maternal behavior has most often focused on ultimate causes relevant to fitness and social organization rather than biological causes Coe, (1990); Keverne, (1996); Lancaster, (1971); Pryce, (1992). Finding a connection between hormone levels and expressed maternal responsiveness in primates is complicated by species differences in hormone profiles evident in pregnant and lactating females marmoset French *et al*., (1996); baboon Albrecht and Townsley, (1978); Fortman *et al*., (1993); human Fleming *et al*,., (1995), and the different methodology used to measure endocrine hormones.

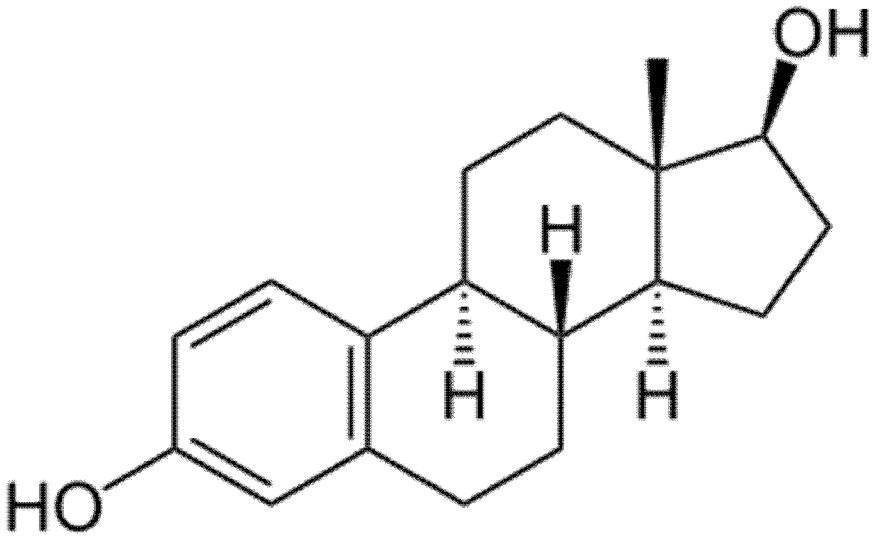

Figure 2-3: Estradiol: Note one hydroxyl group attached to the D ring. The ‘di’ refers both to this hydroxyl and the one on the A ring

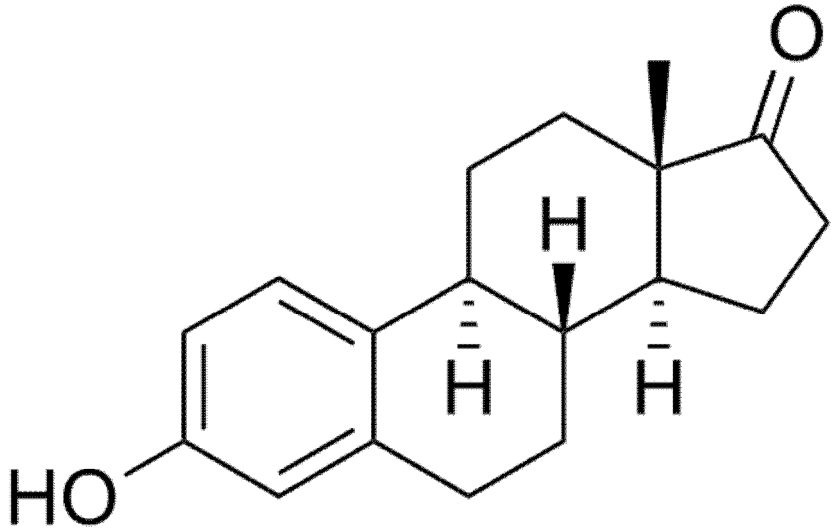

Figure 2-4: Estrone Molecule Structure: An aromatized C18 steroid with a 3-hydroxyl group and a 17-ketone. Note the ketone (=O) group attached to the D ring.

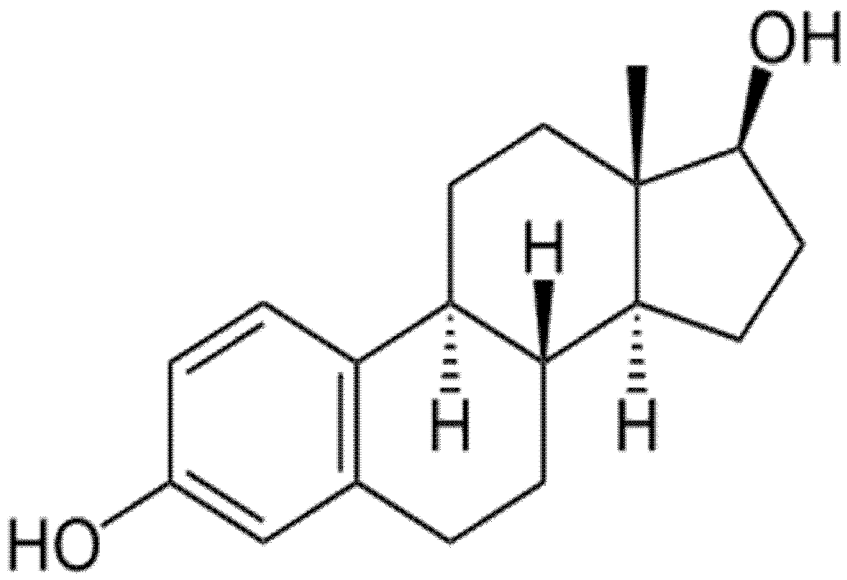

Figure 2-5: Estriol Molecule Structure: Note two hydroxyl (-OH) groups attached to the D ring.

CHAPTER THREE

THE EFFECTS OF CATHA EDULIS (KHAT) ON MATERNAL LIVER AND KIDNEY FUNCTION OF PREGNANT OLIVE BABOONS

3.1 Abstract

Khat consumption is associated with many health problems affecting the gastrointestinal system, reproductive system, cardiovascular system and other body systems. This study was conducted to evaluate the effect of khat on liver and kidney function tests during pregnancy. Six female pregnant olive baboons weighing between 11.2 to 16.5 kg were randomly assigned into two groups; treatment group that received 5 g/kg body weight of crude extract for 8 weeks during the second trimester whereas the normal controls received distilled water. The levels of the following biochemical parameters were assayed; aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, total protein, albumin, urea, creatinine and were measured on weekly basis. Sodium and potassium were also analysed. Levels of AST, ALP, ALT, urea, and creatinine were elevated in animals administered with khat compared to those in the control group ($P< 0.05$). The levels of albumin and sodium were decreased in animals in the khat group compared to those in the control group ($P < 0.007$). There were no comparable changes in the levels of total protein, potassium, bilirubin among the groups ($P > 0.005$). The findings show that khat extract regulates production of liver and kidney function parameters.

3.2 Introduction

Regular consumption of khat has serious social and economic effects as described by Penning *et al*., (2008). Khat leaves have CNS stimulation properties that are believed to improve work capacity, counteract fatigue Ageely, (2009). Improvement in modes of transport in Africa and Arabian Peninsula have allowed for wide distribution of khat to Europe, America and Asia Kassim and Croucher, (2006) leading to increased consumption.

Khat contains cathinone which is an active compound with causes increase in energy, euphoria, concentration, motivation and decrease appetite, increases metabolism and central nervous system (CNS) stimulation Nencini and Ahmed, (1989). Increase in arterial blood pressure and pulse rate caused by cathinone results in increased cardiovascular risk particularly in hypertensive patients, Al-Motarreb *et al*., (2010). Cathinone has also been associated with stomatitis gastro-oesophageal reflux, carcinoma of the mouth and oesophagitis and antibiotic absorption Valko *et al*., (2007). The toxic effect can be linked to farming practices like khat being grown in soil contaminated with heavy metals and use of pesticides and fertilisers (Anwar *et al*., 2012).

Abnormalities in liver function tests reflect not only damage to the hepatic cell, but also give clues to the site, extend and nature of the pathological process. The liver has been suspected to be particularly vulnerable to the harmful effects of khat use Halbach, (1972), and a disturbance in liver function and architecture has been described in experimental animals both on short-term Al-Mammary *et al*., (2002) and long-term Al-Habori *et al*., (2002) feeding with *Catha edulis* leaves. Khat administered chronically to animals causes an increase in liver transaminases,

leading to signs of chronic hepatic inflammation. Luqman and Danowski reported that liver cirrhosis was observed among Yemeni Khat chewers Luqman and Danowski, (1976).

Laboratory liver tests help to elucidate the alteration of markers which reflect the liver disease Gowda *et al*., (2009). The assessment of enzyme abnormalities like, the predominant pattern of enzyme alteration, the magnitude of enzyme alteration in the case of aminotransferases, isolated elevation or in conjugation with some other parameter, the rate of change and the nature of the course of alteration helps in the diagnosis of the disease Giannini *et al.,* (2005). The liver plays a major role in carbohydrate, lipid and protein homeostasis, with the processes of glycolysis, the Krebs cycle, gluconeogenesis, glycogen synthesis, glycogenolysis, lipogenesis, ketogenesis, amino acid synthesis and degradation, and protein synthesis; all taking place in the hepatocytes. Hepatocytes also metabolize and detoxify endogenous (haem) and exogenous products (drugs), which are then excreted via the biliary tree. The liver is a major organ for metabolism of foreign substances and also functionally interposed between the site of absorption and the systemic circulation. These conditions render the liver not only the most important organ for detoxification of foreign substances but also a major target of their toxicity Russmann *et al*., (2009).

Free radicals and oxidants are now seriously implicated in Khat toxicity despite the presence of different antioxidants as chemical components of Khat (Al-Qirim *et al*., 2002; Aleryani *et al*., 2011), although the decreased activity of antioxidant enzymes due to reactive oxygen species (ROS) and oxidative stress have been reported in rats (Al-Qirim *et al*., 2002;, Al-Zubairi *et al*., 2003) and human (Al-Akwa *et al*., 2009; Masoud *et al*., 2012). ROS are potentially very damaging to cells, leading to oxidation of essential cellular constituents including proteins, lipids

and DNA Paradies *et al*., (2002). The biological effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis Kumawat *et al*., (2013). These effects of ROS may lead liver and kidney cell to be damaged and their contents will appear in high amounts in the blood Al-Motarreb *et al*., (2010). It has been reported that plasma levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased in rabbits following Khat administration Al-Motarreb *et al*., (2002); Al-Habori *et al*., (2002).

The following enzymes in the liver; AST, ALT and ALP were assayed. Total protein, bilirubin and albumin were also analyzed. Protein is synthesized in the liver, its concentration in the plasma reflect the functional capacity of the liver. Its value decrease in chronic liver disease, but it is usually normal in the early stages of acute hepatitis. Bilirubin is high especially the unconjugated form in blood. The excretion of bilirubin is impaired in almost all hepatobiliary and some non-hepatic disorders, giving rise to retention of the yellow pigments in the plasma and tissues causing jaundice or icterus.

In a study by Shewamene and Engidawork, (2014**)**, administration of khat at a high dose (400 mg/kg) significantly increased serum creatinine and BUN levels, suggesting that khat use may impair renal function by reducing the ability of kidneys to handle these products. These effects perhaps may originate from changes in the renal blood flow and glomerular filtration rate induced by khat treatment Kalix and Braenden, (1985). Serum creatinine and BUN are commonly used to assess glomeruli filtration rate as well as concentrating and diluting capacity

of tubular functions of the kidneys. An increase in values of these markers may indicate development and extent of renal tubular damage, Kakadiya and Shah, (2010). On the bladder, khat chewing produced a fall in urinary flow rate, an effect that has been shown to be inhibited by the selective α_1-adrenoceptor antagonist, indoramine, and therefore attributed to activation of this receptor subtype Nasher *et al.*, (1995). Peripheral vasoconstriction following khat administration would explain the raised serum creatinine and BUN levels. Although the mechanism by which khat produces nephrotoxicity is not clearly known, it is thought to result from local decrease in blood supply, possibly from narrowing of the renal arteries Al-Motarreb and Broadley, (2003).

The use of khat at higher dose may cause oxidative stress by depleting anti-oxidative mechanisms or by enhancing pro-oxidant components of tissues, leading to renal injury. More reasonably, khat seems to be able to perturb the delicate balance between protective and damaging mechanisms of a cell that is required for optimal activity, thereby producing oxidative damage Shewamen**e** and Engidawork, (2014).

To overcome the limitations of previous human studies which were mainly retrospective, investigation on the association between khat chewing and liver and kidney functions in a prospective controlled study detailing the amount of khat given at a given time was done. Rodent's studies give useful insights to elucidate the mechanism of action of khat. There are major differences in the physiology of rodents and human that places a limitation in the interpretation of data from these experimental animals. Non-human primates are phylogenetically closely related to humans and would form useful models for further studies.

This study was undertaken to examine the effects of feeding pregnant olive baboons with crude khat extract during their second trimester on biochemical parameters of liver and kidney.

3.3 Materials and methods

3.3.1 Study animals and housing

Six adult female olive baboons were captured in the wild, Mweiga, Nyeri, Kenya and put under quarantine for ninety (90) days at the Institute of Primate Research (IPR) Nairobi, Kenya, within which they were screened for parasites by examining their stool and blood. Intradermal tuberculin-test was also carried out. Only healthy animals were selected for the study. The animals were housed in individual cages (0.6 m x 0.66 m x 0.8 m) for the duration of the study. Daily supply of commercial pellets from unga feed Kenya limited and vegetables was given; fruits were given thrice in a week while water was provided *ad libitum*. Lighting conditions with approximately 12 h: 12 h (light: dark cycle) and an average room temperature of 23^0 C with a relative humidity of approximately 60% was provided in the animal house. Cage cleaning and regular change of beddings was also observed. Complete animal health care and supervision was provided to the animals throughout the course of the study.

Healthy olive baboons (*Papio anubis*) (n=6) were maintained and used in the study at the Institute of primate research (IPR). The baboons weighed 12 kg, 13.2 kg, 13.6 kg, 11.2 kg, 16.2 kg and 16.5kg respectively. Normal cycling females were bred to proven males using the degree of perineal swelling to determine onset of ovulation. Copulation was concentrated during a short period of sexual activity (estrus) and was signaled by large sexual swellings on the female's posterior that correspond to the period around ovulation. The subjects were evaluated to

determine pregnancy beginning 18 days post mating, using a diagnostic ultrasound unit. Mating was not synchronized but was based on the estrous cycle of each baboon.

3.3.2 Ethical statement.

All study procedures followed accepted veterinary protocols for analgesia and anaesthesia that were approved by the Institutional Review Committee (IRC) at Institute of Primate Research. These protocols were guided by International Guiding Principles for Biomedical Research involving animals, and developed by the Council for International Organizations of Medical Sciences.

3.3.3 Preparation of crude khat extract

Fresh khat shoots were purchased from a specific local farm in Maua, Meru County, Kenya on weekly basis and processed as follows. 60g fresh khat twigs (leaves and shoots, peeled barks), were weighed and blended with 100 ml of distilled water to give crude khat extract. The crude extract was then filtered using a sterile dish cloth. Five grams of glucose was then added to the filtrate and administered to the animals. The dosage was selected based on the average amount that a regular khat (non addict) user can consume per week Toennes *et al.,* (2003); El-Shoura *et al.*, (1995).

3.3.4 Experimental design

The route of administration was by oral gavage. The baboons were sedated by intramuscular injection with 0.2 ml/kg body weight of a mixture of 10% Ketamine HCl (Kepro BV, Deventer, The Netherlands) and 2% xylaxine (Troy Laboratories Pty Ltd., Glendenning, N.S.W., Australia) during distilled water and khat extract administration. Ketamine produces a trance-like state with muscle rigidity and twitching when used alone. Combined with xylazine complete surgical levels of anesthesia may be attained by the ability of xylazine to soften and relax muscles. The animals were weighed and blood pressure monitored every time an animal was anesthetized to administer khat at 5 g/kg body weight or distilled water. 10 ml of blood was collected from the femoral vein weekly, over a 4-hour period under anesthesia starting on the second trimester for two months (third and fourth months of gestation). The blood samples were collected into plain tubes and kept at 4°C for 3 hours to allow blood to clot, and then centrifuged at 500 X g for 10 min to obtain the serum, which was stored at -20^0C until ready for analysis. The two groups were followed up equally for the duration of the experiment. The animals were maintained for six months of their gestation until they delivered naturally upon which they were euthanized with pentobarbital sodium 20% intravenous injection at a dose rate of 150mg/ kg body weight.

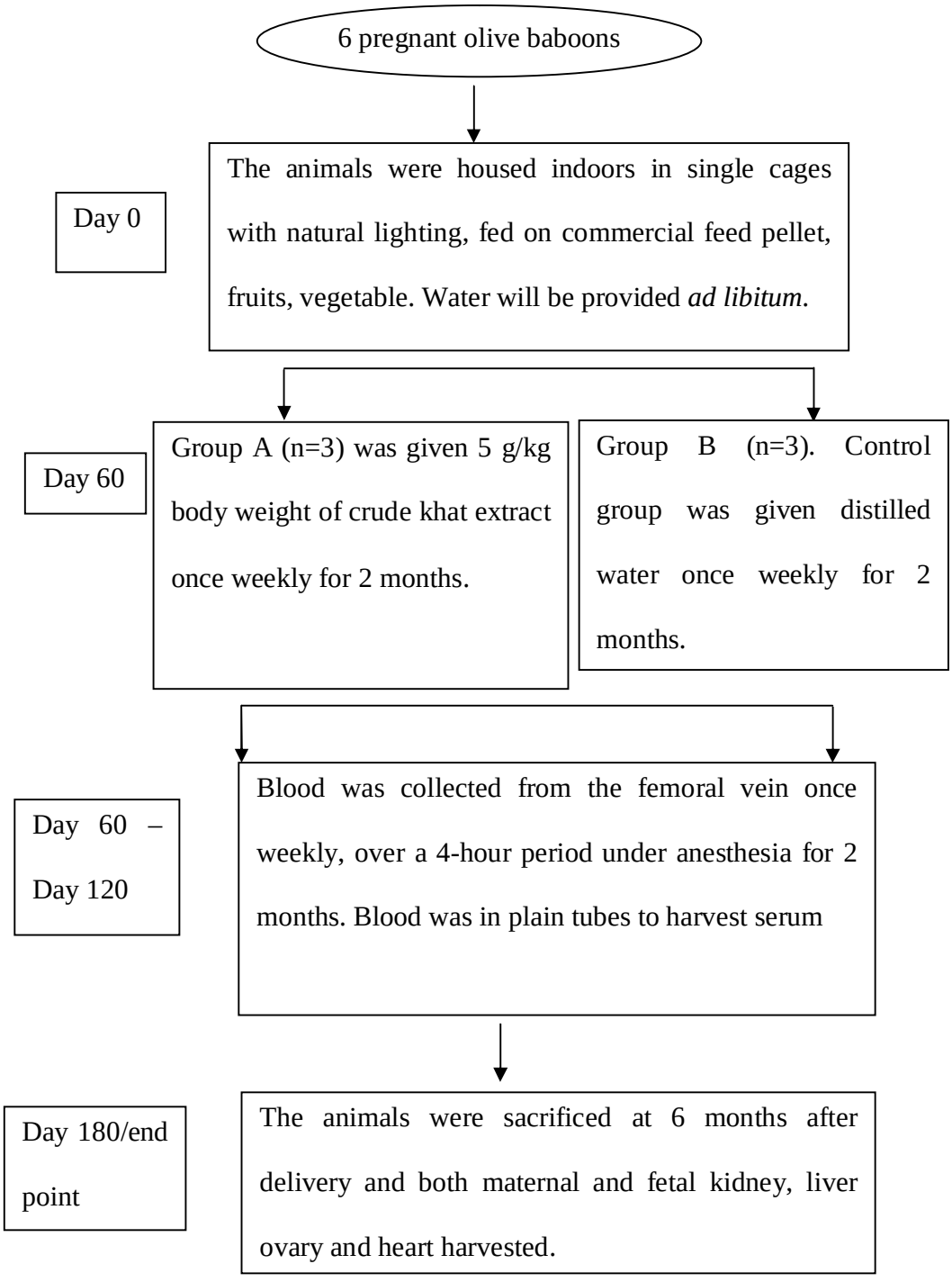

Figure 3-1: Experimental design

3.3.5 Biochemical assay

The levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), creatinine, total protein, blood urea nitrogen, potassium and sodium were measured following the manufacturer's instructions using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, as discussed in detail in specific assay below.

3.3.5.1 Alkaline phosphatase (ALP)

The biochemical assay of ALP was done using using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. The reagents and the cuvettes were warmed at 37^0C and the temperature kept constant throughout the duration of the experiment. Twenty (20) µl of the serum samples were aliquoted into tubes. One thousand (1000) µl of buffer (diethanolamine buffer) was added to the samples, mixed and incubated for 1 min at 37^0C. Two hundred and fifty (60 µl) of the substrate (p-Nitrophenyl phosphate) was added to the contents in the cuvette. The experiment was done in duplicates. The absorbance was read after one minute at 420 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.2 Alanine Aminotransferase (ALT)

The biochemical assay of ALT was done using Human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. The reagents and the cuvettes were warmed at 37^0C and the temperature kept constant throughout the duration of the experiment. One hundred (100) µl of the serum samples were aliquoted into tubes. One thousand (1000) µl of tris buffer with L-alanine was added to the samples, mixed and incubated for 5 min at 37^0C. Two hundred and fifty (60) µl of the substrate

(2-oxoglutarate, NADH and sodium azide) was added to the reaction. The experiment was done in duplicates. The absorbance was read after one minute at 340 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.3 Aspartate aminotransferase (AST)

The biochemical assay of AST was done using Human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. The reagents and the cuvettes were warmed at 37^0C and the temperature kept constant throughout the duration of the experiment. One hundred (100) µl of the serum samples were aliquoted into tubes. One thousand (1000) µl of tris buffer with L-aspartate was added to the samples, mixed and incubated for 5 min at 37^0C. Two hundred (60) µl of the substrate (2-oxoglutarate, NADH and sodium azide) was added to the cuvette contents. The experiment was done in duplicates. The absorbance was read after one minute at 340 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.4 Total Bilirubin

The biochemical assay of total bilirubin was done using Human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. One thousand (1000) µl of the Total bilirubin reagent was aliquoted into cuvettes. This was followed by addition of a drop of T-nitrite reagent, sodium Nitrite, mixed thoroughly for 5 min. One hundred (100) µl of the serum sample was added into the reaction, mixed and incubated at room temperature for 30 min. The absorbance of the samples was measured against sample blank. The experiment was done in duplicates. The absorbance was read after five minutes at 546 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.5 Direct Bilirubin

The biochemical assay of direct bilirubin was done using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. One thousand (1000) µl of the direct bilirubin reagent was aliquoted into cuvettes. This was followed by addition of a drop of D-nitrite reagent, sulphanilic acid and hydrochloric acid, mixed thoroughly for 2 min. One hundred (100) µl of the serum sample was added into the reaction, mixed and incubated at room temperature for 5 min. The experiment was done in duplicates. The absorbance of the samples was measured against sample blank. The absorbance was read after five minutes at 546 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.6 Potassium

The biochemical assay of potassium was done using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. One hundred (100) µl of the serum sample was pipetted into centrifuge tubes. 1000 µl of the precipitant (trichloroacetic acid) was added into the samples, mixed carefully and centrifuged at high speed for 5 min. Two hundred (200) µl of the supernatant was pipetted into cuvettes. A working reagent was prepared by mixing sodium tetraphenylboron (0.2 mol/l) and sodium hydroxide (0.2 mol/l) in the ratio of 1:1 and allowed to stand for 30 min prior to use. Two thousand (2000) µl of the working reagent was added to the standard (Potasium) and the Supernatant. This were mixed carefully and allowed to stand for 5 min. The experiment was done in duplicates. The absorbance was measured against one reagent blank. The absorbance was read within thirty minutes at 578 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.7 Sodium

The biochemical assay of sodium was done using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. 50 µl of the serum sample was pipetted into centrifuge tubes and 50 µl of the standard (sodium). Three thousand (3000) µl of the precipitant (trichloroacetic acid) was added into the samples and the standard, mixed carefully and allowed to stand for 5 min. The samples were then shaken intensively for 30 sec and allowed to stand for 30 min, then centrifuged at high speed for 5 min. Fifty (50) µl of the clear supernatant was pipetted into cuvettes. Three thousand (3000) µl of a colour reagent (ammonium thioglycolate) was added to the clear supernatant. The experiment was done in duplicates. The absorbance was measured against a reagent blank. The absorbance was read within thirty minutes at 410 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.8 Creatinine

The biochemical assay of creatinine was done using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. Two hundred (200) µl of serum sample was aliquoted into cuvettes. One thousand (1000) µl of the working reagent was added to the cuvettes. The experiment was done in duplicates. They were mixed properly, and absorbance read after within two minutes at 510 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.5.9 Blood urea nitrogen

The biochemical assay of blood urea nitrogen was done using human kits from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany, following the protocol briefly described below. Ten (10) µl of serum sample was aliquoted into cuvettes. One thousand (1000) µl of the enzyme reagent was added to the cuvettes. They were mixed properly and incubated for

10 minutes at 60C for five minutes. The experiment was done in duplicates. The absorbance was read within sixty minutes at 546 nm using chemistry analyzer HumaLyzer 2000® Germany.

3.3.6 Statistical analysis

The data was analyzed using graphpad statistical software. The data was expressed as mean ± standard error of mean (SEM) showing the values of the controls and the treatment group. The *P*-values were examined by unpaired student's t-tests. The results were considered significant by a *P*-value <0.05. All end points were analyzed using two -tailed test.

3.4 Results

An increase in the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the treatment group was seen compared to the control (table 3.1) The P values for the three enzymes were 0.0001, 0.007, 0.007 for ALP, AST and ALT respectively as in table 3.1 below and Fig 3-1, 3-2, 3-3. Total bilirubin and direct bilirubin had no significant difference between the treatment and the control groups (Fig 3-10, 3-11). Albumin was significantly decreased in the treatment group compared to the control group (Fig 3-8). There was no difference in total protein between the two groups (Fig 3-9). Blood urea nitrogen and Creatinine were significantly increased in the treatment group compared to the untreated control group ($p<0.02$ and $p<0.01$, Fig 3-4 and Fig 3-5 respectively). Sodium levels were decreased in the treatment group compared to the control group ($p<0.0001$, Fig 3-6). Potassium levels were $p<0.97$ showing insignificant difference between the treatment and the control group (table 3.1; Fig 3-7).

Table 3-1: Biochemical values observed in pregnant Olive baboons following oral crude Khat administration at 5 g/kg bodyweight over two month period during the second trimester (n=3).

Parameter	**Control group (n=3)**	**Treatment group (n-3)**	**P- Value**
Albumin (gm/dl)	3.336±0.399	2.065±0.083	0.0075*
Total protein(gm/dl)	6.320±0.6064	5.759±0.1606	0.386
Direct Bilirubin(mmol/I)	0.3238±0.7331	0.3175±0.074	0.9532
Total Bilirubin(mmol/l)	0.7263±0.478	0.7788±0.0586	0.499
ALP(IU/I)	190.5±10.72	936.3±95.01	0.001*
ALT(IU/I)	22.94±0.8387	26.75±0.8935	0.0078*
AST (IU/I)	22.94±0.8387	26.73±0.835	0.0078*
Creatinine(µmol/l)	0.5625±0.0232	0.8400±0.09	0.0118*
Urea(µmol/l)	39.90±1.686	49.10±3.403	0.0294*
Potasium(mmol/l)	5.524±0.5019	5.501±0.5019	0.97
Sodium(mmol/l)	162.5±5.059	81.53±3.899	0.0001*

*P-Value significant

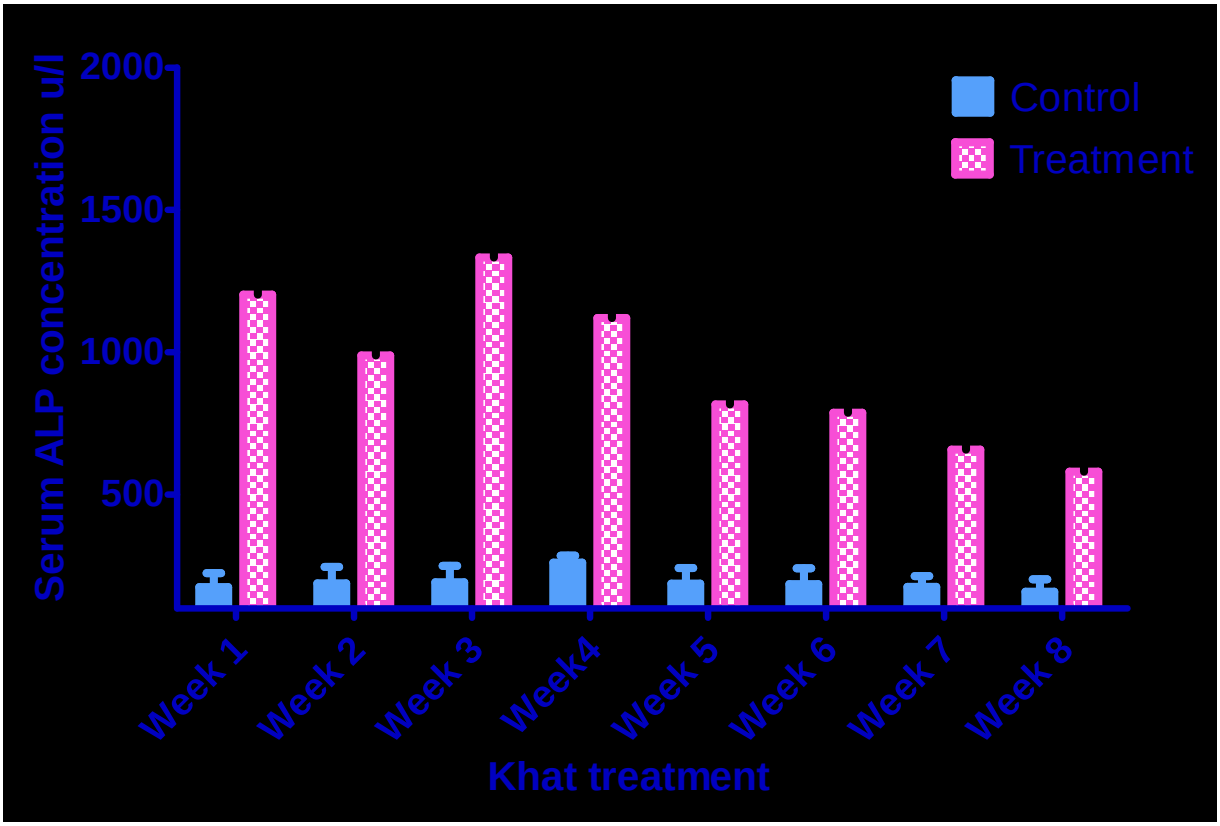

Figure 3-2: Level of Alkaline phosphatase (ALP) in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

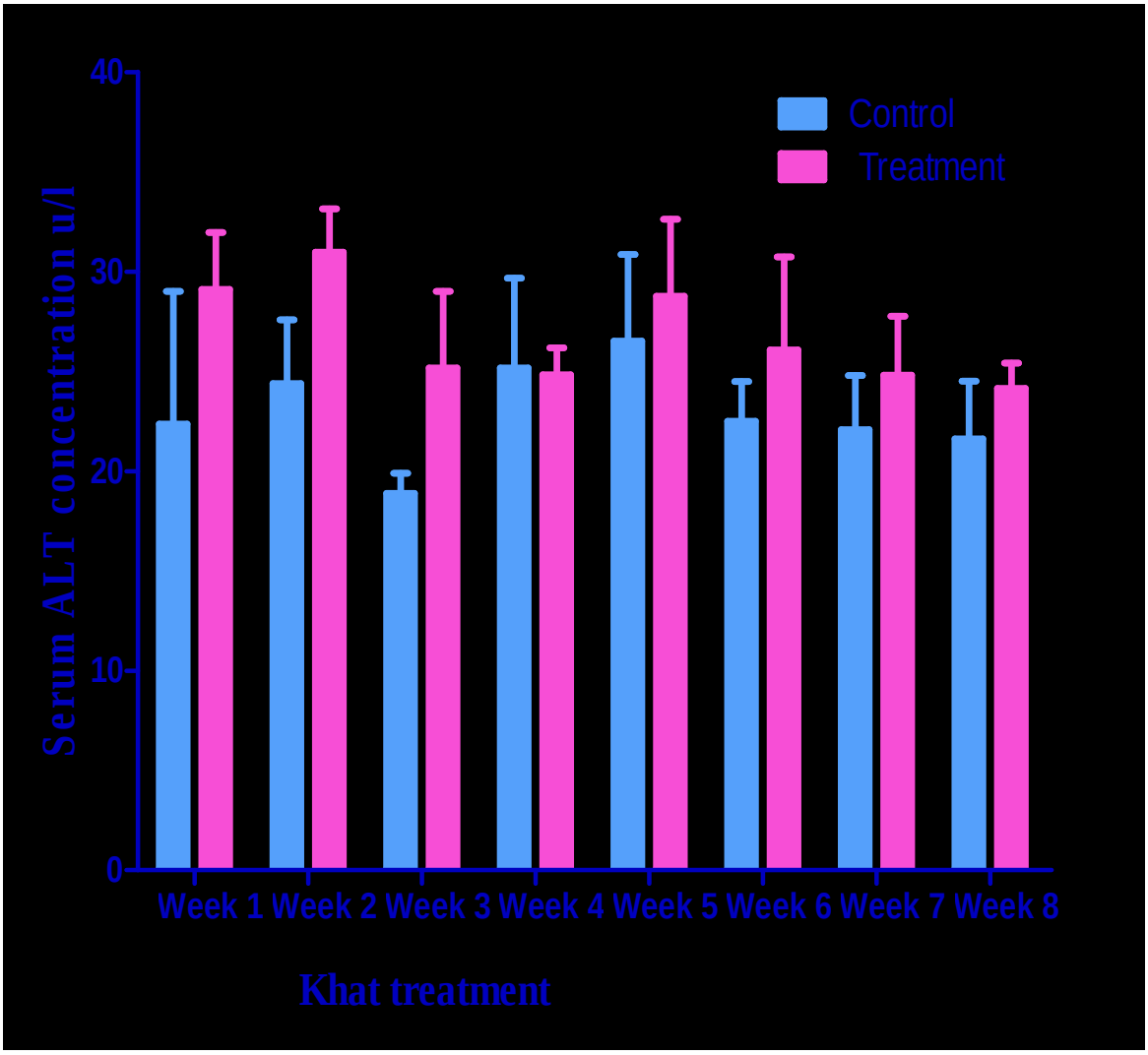

Figure 3-3: Level of Alanine aminotransferase (ALT) in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at $P< 0.05$.

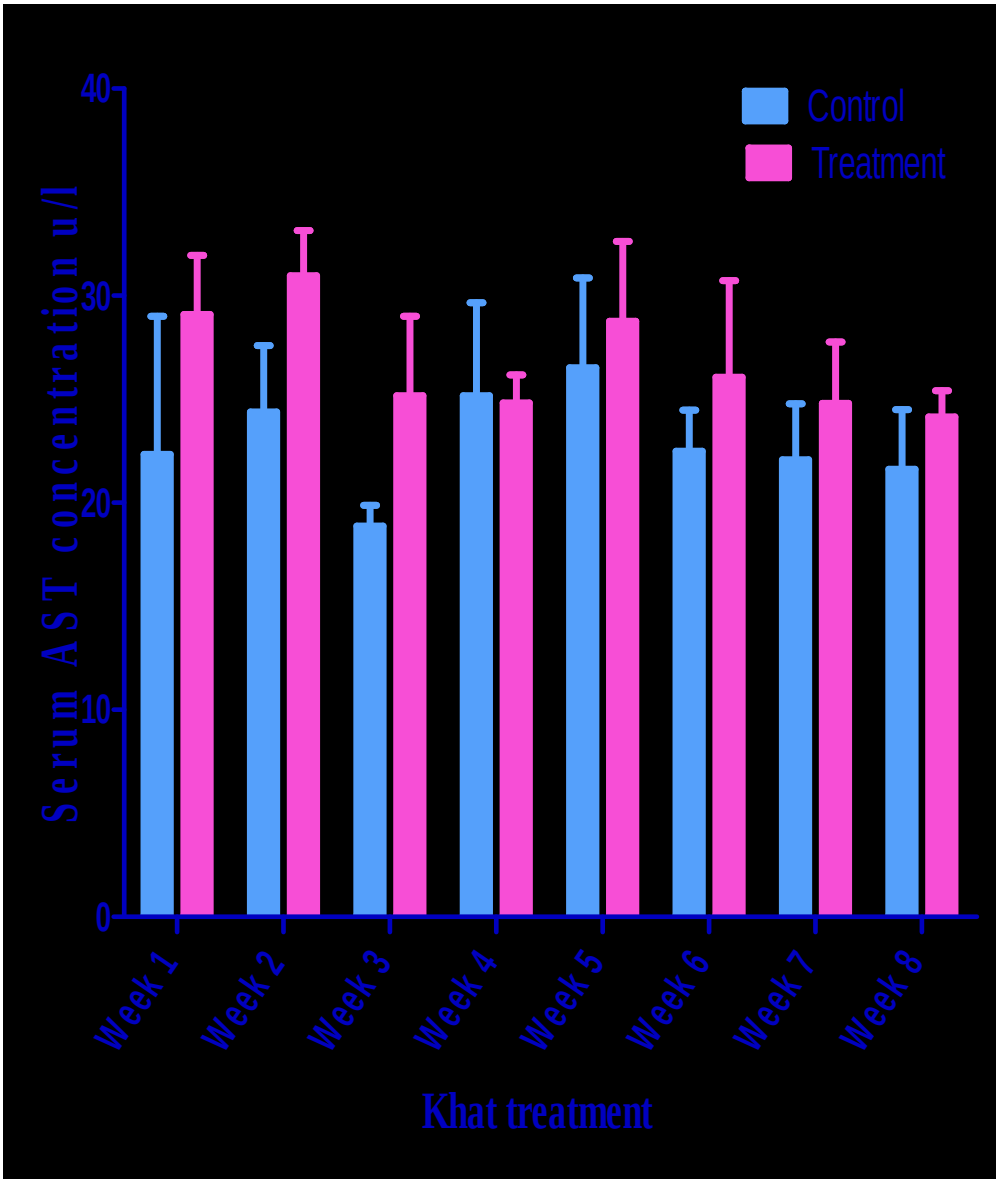

Figure 3-4: Level of Aspartate aminotransferase in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at $P< 0.05$.

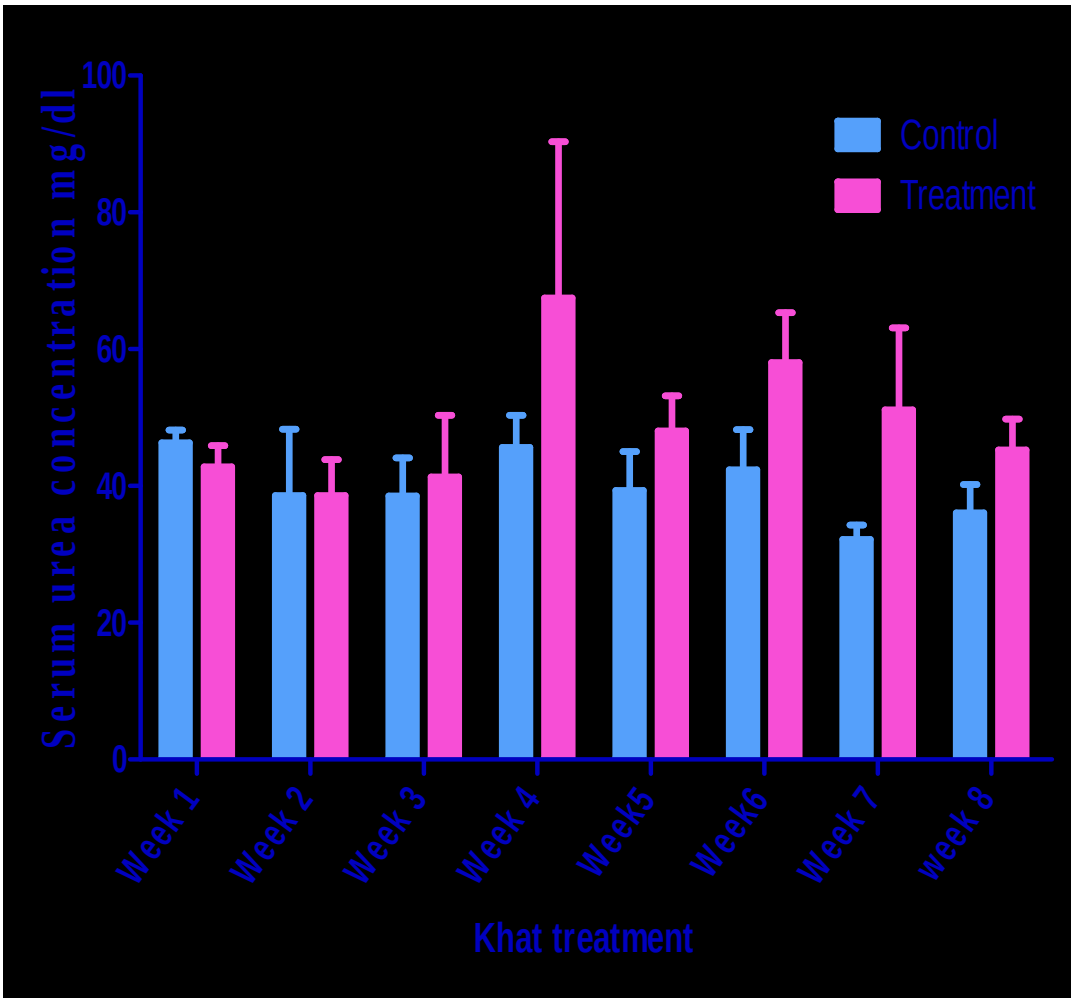

Figure 3-5: Level of urea in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

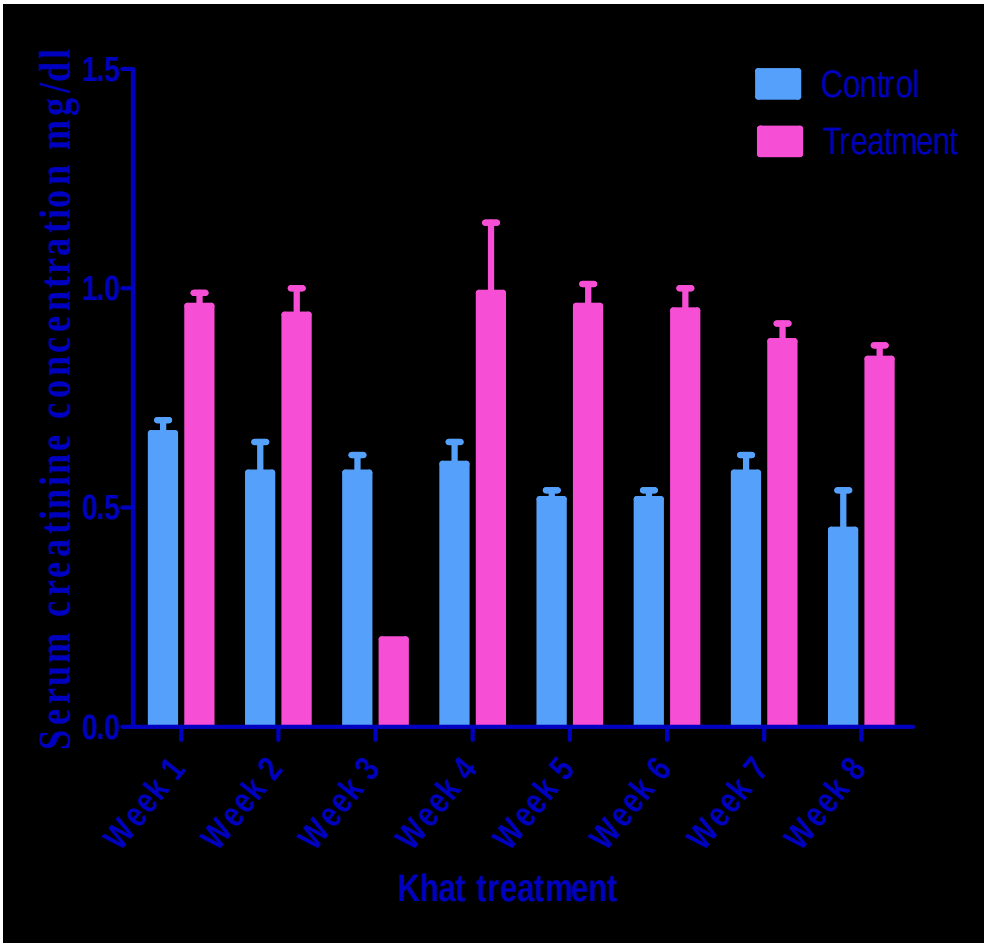

Figure 3-6: Level of creatinine in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

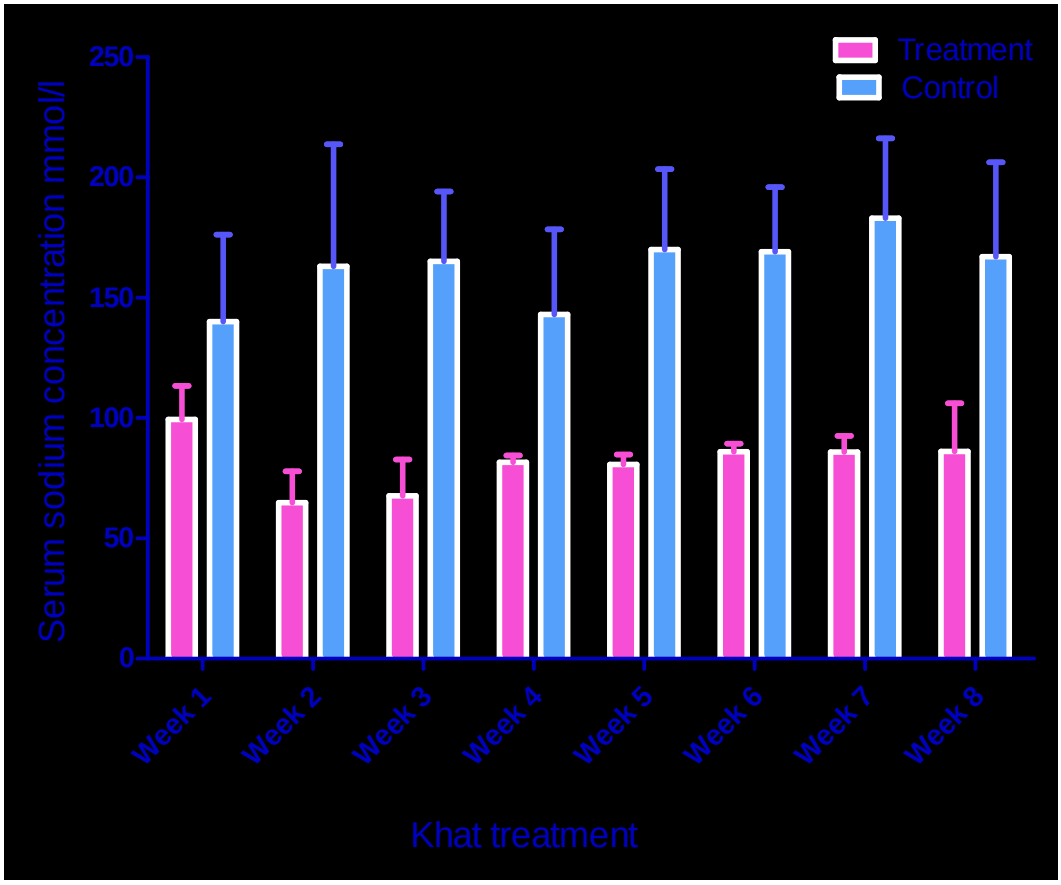

Figure 3-7: Level of Sodium in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

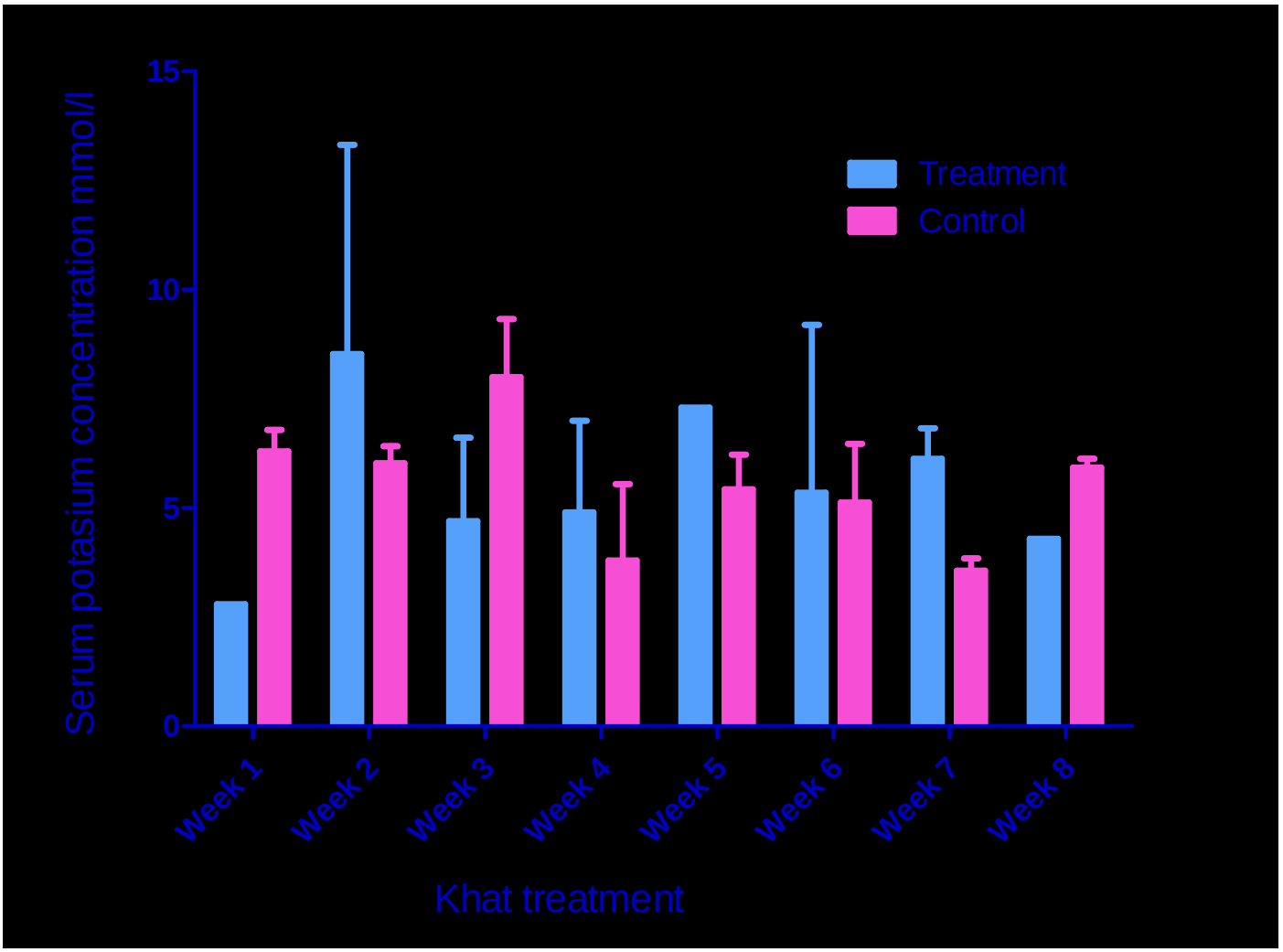

Figure 3-8: Level of Potassium in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at $P< 0.05$.

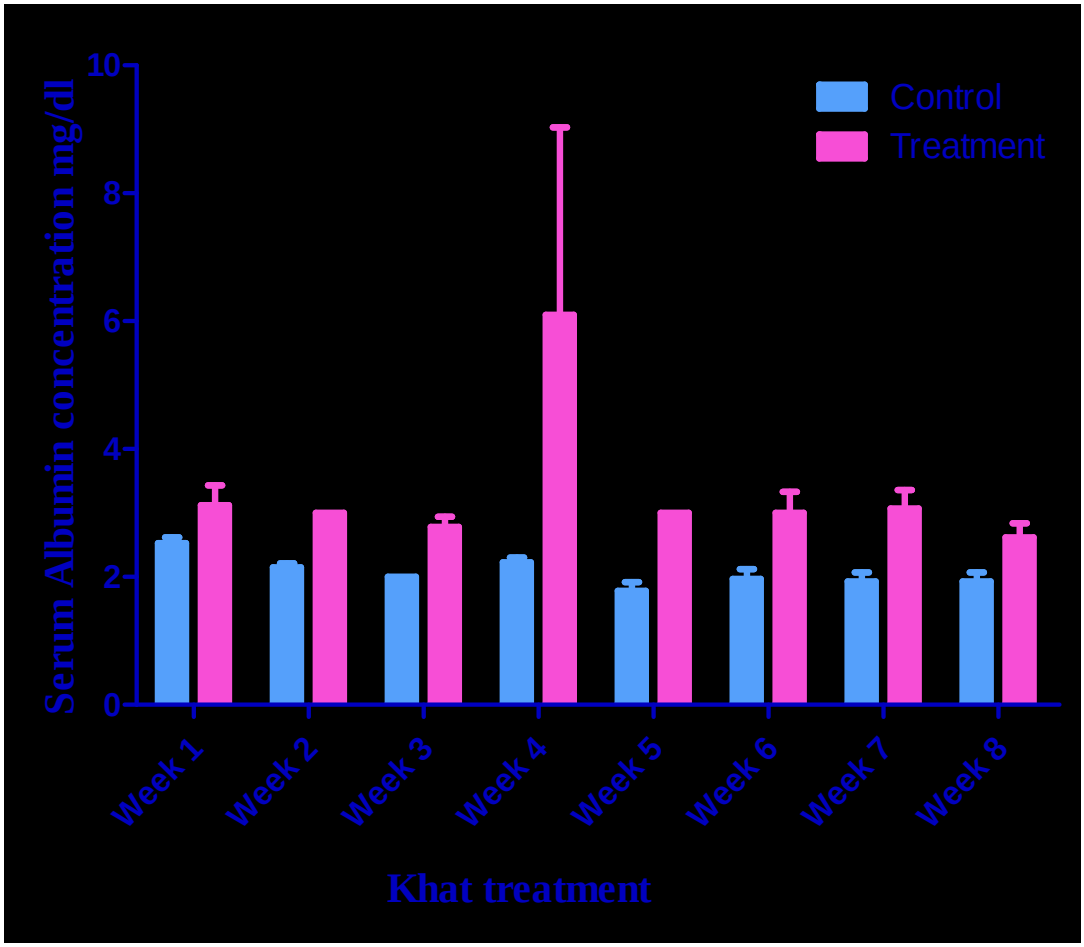

Figure 3-9: Level of albumin in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

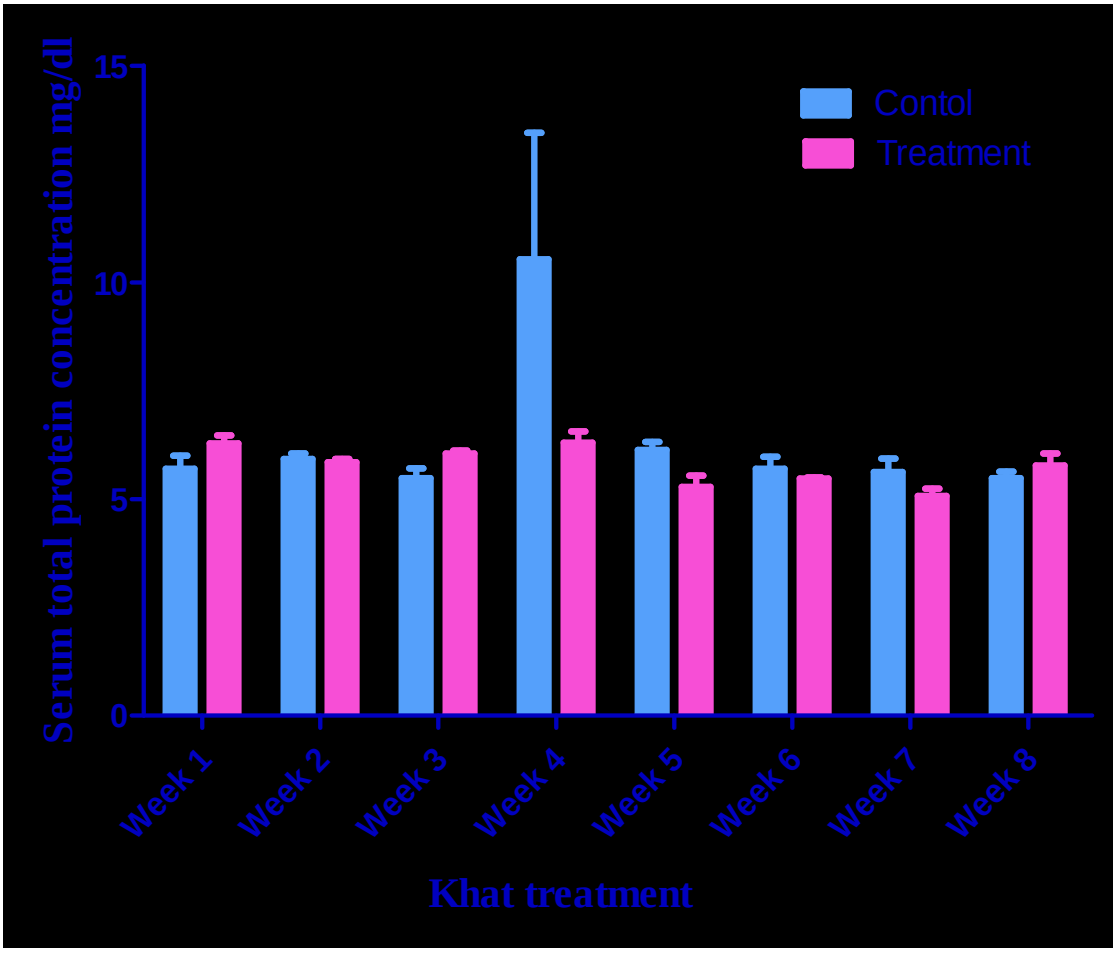

Figure 3-10: Level of Total protein in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

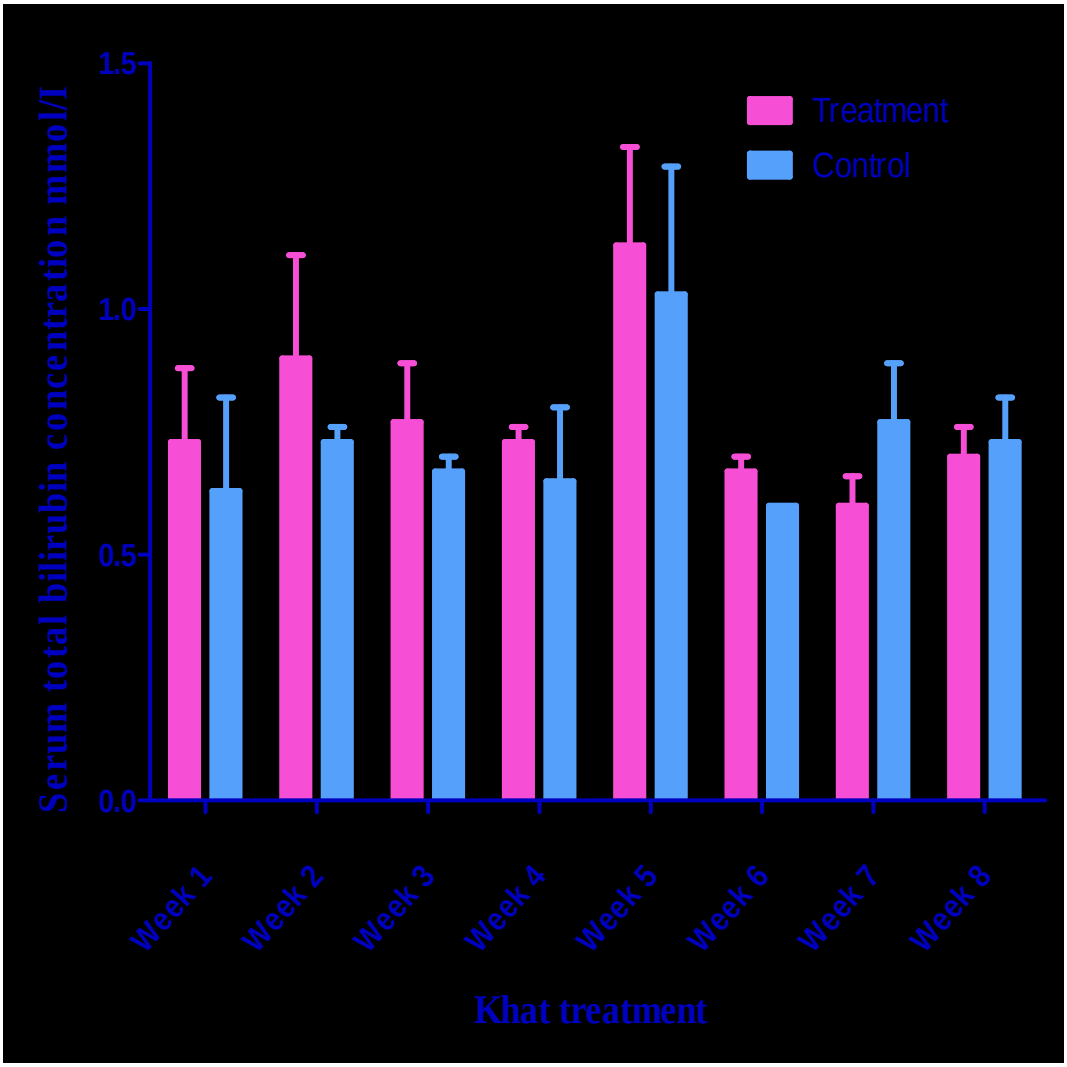

Figure 3-11: Level of Total Bilirubin in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

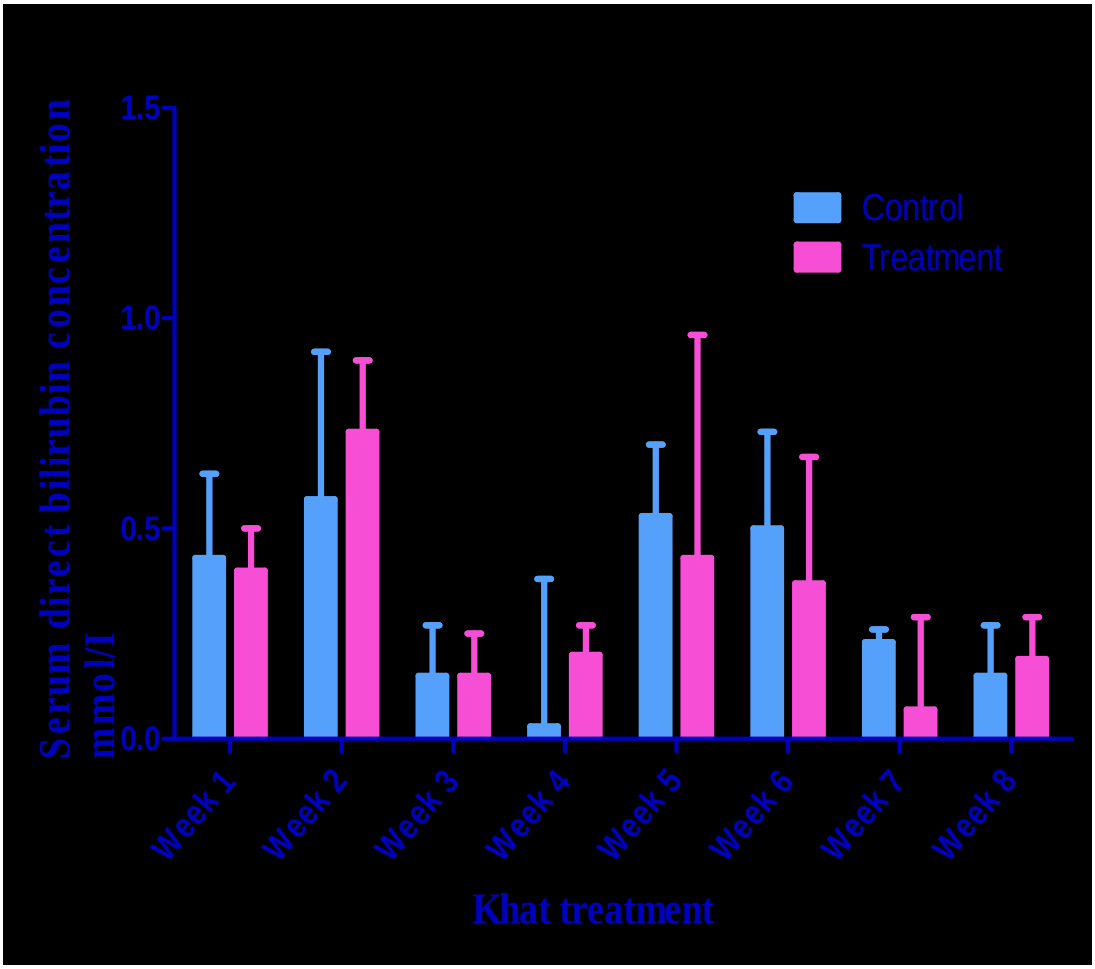

Figure 3-12: Level of Direct Bilirubin in the serum of pregnant olive baboons given Khat and pregnant olive baboons on distilled water. Results expressed as mean ± SEM. P value was considered significant at P< 0.05.

3. 5: Discussion

The present study investigated the changes in liver and kidney functions as a result of administering Khat to pregnant olive baboons. An increase in the levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), creatinine and blood urea nitrogen, whereas, the albumin and sodium content was decreased in the plasma of pregnant olive baboons treated with khat. All the treatment animals were given the same dose of crude khat extract at 5 g/kg body weight over a two month period during their second trimester of gestation (third and fourth month of gestation).

Significant difference in the liver enzymes was observed between the treatment and control animals. There was increase in ALT, AST and ALP in the treatment group indicating possible khat induced liver damage. This suggests leakage of these enzymes into extracellular fluid as a result of toxic damage of liver tissue by the extract may be compromising the membrane integrity of liver cells, the finding similar to that reported by Alam *et al*., (2014); Anwar *et al*., (2014). However, the increase of ALP was more pronounced compared to ALT and AST. ALP is present in high concentrations in a large number of tissues, such as liver, heart, kidney, skeletal muscle and pancreas Mauro, (2006). ALT is more specific since its limited to the cytosol of the hepatocytes. Similar findings to those of Al-Habori *et al*., (2002) who reported that long term feeding of Khat leaves to New Zealand white rabbits is responsible for increased liver enzyme levels. It has also been reported that Khat induces cytotoxic effects in cells, in the liver and kidney of rabbits. Liver damage due to tissue necrosis or membrane damage due to oxidative stress will lead to increase the levels of hepatic enzymes ALT, ALP and AST in the plasma, Jeschke, (2009).

The results of this study corroborated with those of Al-Hashem *et al*., (2011) who reported that the oral administration of hydroethanolic crude extract of khat shrubs in rats, for a period of one month, increased liver enzyme levels and concluded that *Catha edulis* extract had toxic effects on the liver of treated rats as evidenced by alterations in biomarkers of oxidative stress. Accordingly, Al-Mehdar *et al*., (2012) reported that the oral administration of *Catha edulis* crude extract in rats significantly increased level of liver enzymes in serum. They suggested different mechanisms may be responsible including sympathomimetic effect and induction of oxidative stress.

The alkaloids of khat including cathinone, cathine and norephedrine have peripheral sympathomimetic effect which cause increased release of norepinephrine from adrenergic nerve terminals, and also cause inhibition of its reuptake as well as inhibition of its metabolic inactivation by monoamine oxidase enzyme Hoffman and Al'Absi, (2010). Consequently, the increased norepinephrine concentration in postsynaptic space could exert hepatic vasoconstriction via stimulation of postsynaptic α1-receptors hence ischemic hepatitis may develop as a result of decrease hepatic blood flow Al-Mehdar *et al*., (2012). In drug metabolism, the oxidative phase-I and the conjugative phase-II can result in hepatotoxic metabolites. Although some parent xenobiotics are also known to induce oxidative stress in several mechanisms Chiasera, (2010). Decreased synthesis/activity of the antioxidant system in treatment group may have resulted in damaging membrane integrity of liver cells by inducing oxidative stress thereby causing the mitochondrial and cytosolic enzymes to the extracellular fluid as reported also by Al-Mehdar *et al*., (2012).

In this study, there was a significant decrease in serum albumin and no significant change in total protein of the treatment group compared to the control group, the results `agrees with those reported by Alam *et al*., (2014). Albumin is synthesized in the liver, and low serum albumin may be indicative of liver failure or diseases such as cirrhosis or chronic hepatitis. Hypoalbuminemia can also present as part of the nephrotic syndrome, in which protein is lost in the urine due to kidney damage. Low albumin levels can be an indicator of chronic malnutrition or protein losing enteropathy. It is documented that some constituents of the *Catha edulis* might be been converted to pro-oxidant metabolites decreasing the synthesis or activity of the antioxidant system in treatment group hence generation of free radicals by inhibiting synthesis of antioxidant enzymes, this may explain the damage observed in the liver.

Increase in serum urea, creatinine, and uric acid has been linked to kidney disease, Chawla *et al*., (1999). Serum creatinine and BUN are commonly used to assess glomeruli filtration rate as well as concentrating and diluting capacity of tubular functions of the kidneys. An increase in values of these markers may indicate development and or extent of renal tubular damage, Kakadiya and Shan, (2010). Specifically, increase in BUN levels may be associated with kidney disease/blockade of the urinary tract by a kidney stone, congestive heart failure, and dehydration or bleeding in the digestive tract, Gowda *et al*., (2010). In the present study, administration of khat significantly increased serum creatinine and BUN levels, suggesting that khat use may impair renal function by reducing the ability of kidneys to handle these products. These effects perhaps may originate from changes in the renal blood flow and glomerular filtration rate induced by khat treatment as suggested by Kalix and Braenden, (1985). Shewamene and Engidawork, (2014) reported that high dose of khat has a direct renotoxic potential, while Al-

Hashem *et al.,* (2011) reported that the toxic effect of khat extract on hepatic and renal functions might be related to lipid peroxidation as indicated by a significant increase in lipid peroxidation biomarkers observed in their study. By interfering with lipid metabolism, the integrity of lipid membrane will be compromised inducing the liver or kidney damage observed in the study. Waafa *et al*., (2013) has showed effects of khat on the blood glucose, lipid profiles and confirmed toxic histopathology changes on the liver of the rabbit. Additionally elsewhere, it has been reported that khat induces cytotoxicity in livers and kidneys after oral administration of khat to animals Al-Motarreb *et al.,* (2002); Dimba *et al.,* (2003); Gunaid *et al.,* (1997). Liver damage due to tissue necrosis or membrane damage by oxidative stress will lead to increased levels of hepatic enzymes in serum Jeschke, (2009).

In other studies on rats, administration of khat was reported to cause a reduction in the liver enzyme, alkaline phosphatase (ALP), and increased activities of acid phosphatase, lactate dehydrogenase (LDH) and increased total bilirubin Akdogan *et al*., (2003), The observed difference between lower animal (rat) and non-human primate like in case of this study and that of human suggests the probable biochemical and physiological differences which genetically are distantly related. The results in this study where baboons were used are in agreement with those observed in humans indicating baboon is better biomedical model in the study of khat effects in human. For example, in humans, it has been shown that regular khat chewing may cause kidney damage, as total serum protein levels are reduced in khat consumers.

Although, total protein results showed no statistical difference, the treatment had lower mean compared to the control group. But in the serum albumin, there was difference between the two

groups. The serum albumin level in the treatment group was lower. The decrease observed was similar to findings by Al-Hashem *et al*., (2011) and Masoud *et al*., (2014) who reported significant decrease in serum total protein and albumin of khat treated rats as compared to the control. This indicates impaired liver function causes decreased protein synthesis, either primary due to liver cell damage or secondary due to diminished protein intake and reduced absorption of amino acids Chawla, (1999). The levels of total bilirubin and direct bilirubin in this study were not significantly different between the control and the treatment groups. However, it has been observed that oral administration of *Catha edulis* hydro-ethanol extract significantly increases serum bilirubin, suggesting a direct toxic effect of the extract on liver cells leading to decreased uptake and conjugation of bilirubin and reduced secretion into bile ducts Fahaid *et al*., (2011) which may suggest similar findings in this study where liver was seen to be damaged.

3.6 Conclusion

Khat (*Catha edulis*) extract has toxic effects on liver and kidney functions. The study findings are not conclusive, further studies are indicated to establish the actual metabolisms and signalling pathways that are controlled by the chemical components available in khat.

CHAPTER FOUR

HISTOPATHOLOGICAL EFFECTS OF *CATHA EDULIS* (KHAT) ON THE LIVER, KIDNEY, HEART AND OVARY OF PREGNANT BABOONS AND THEIR FETUSES

4.1 Abstract

The pathological effect of prolonged khat (*Catha edulis)* usage has been a he subject of debate for a long period of time. This present study sought to evaluate the effect of khat usage on organ toxicity during pregnancy and foetal development. Six olive baboons were randomly assigned into two groups; treatment group which was given 5g/kg body weight of crude khat extract and control group that received distilled water for eight weeks. At end point, histological sections of liver, kidney, ovaries and heart of the both the dams and the foetus were prepared. Renal sections of treatment group both the dams and their foetuses showed histopathological changes characterized by atypical tubules, amorphous Malpighian corpuscles, and invasive infiltrative inflammatory cells. The glomerulus was degenerative and capillaries in Malpighian corpuscles were hypertrophied, some of them were destructed, and Bowman's capsules seemed dilated. Liver of the treatment group both the dams and the infants showed degenerative vacuolation and coagulative necrosis, periportal fibrosis and degenerative changes in persisting parenchyma. There were dilatation of sinusoids and mononuclear inflammatory infiltrates around the central vein and portal tracts. Massive infiltration with inflammatory cells was observed in the heart and degenerative changes of heart muscles were observed in dams and infants treated with khat. No pathological changes were observed in the ovaries. Administration of khat during pregnancy can induce organ toxicity in both the dams and foetus, and thus khat use should be discouraged and more so during pregnancy.

4.2 Introduction

The pleasure derived from khat chewing is attributed to the euphoric actions of cathinone, a sympathomimetic amine, with properties similar to those of amphetamine (Kalix, 1992). Traditionally khat chewing has been viewed as an aid to relieving fatigue and has some place in self-medication of depression Deyessa *et al*., (2008). However, the World Health Organisaton concludes that it has no therapeutic potential WHO, (2006). The psychological effects of chronic khat use have been the subject of debate on its effects on organ toxicity. In previous studies, khat usage has been shown to induce incidences of myocardial infarction, dilated cardiomyopathy, vascular diseases, cerebrovascular ischemia and thromboembolism, diabetes, sexual dysfunction, duodenal ulcer and hepatitis (Al-Motarreb *et al*., 2010). In addition, hepatic cirrhosis of unknown etiology has been noted in khat users. The presence of tannians in khat has been indicated to be the possible contributors (Chapman *et al*., 2010). However, not much data has been generated to confirm this claim due to lack of experimentation in non-human primates whose data can be easily extrapolated to humans. In guinea pigs, cathinones have been shown to have a vasoconstrictor activity in perfused hearts (Al-Motarreb *et al*., 2003). The effect is unlikely to be due to an indirect action by release of noradrenaline from sympathetic nerve endings or due to a direct action on alpha1-adrenoreceptors. Cathinone is able to potentiate noradrenaline-evoked contractions of the rat right ventricle Cleary *et al*., (2002) and inhibit the uptake of noradrenaline into ventricular slices by a mechanism involving competitive blockade of the noradrenaline transporter Brennesian *et al*., (1990). The vasoconstrictor activity of cathinone explains the increased incidence of myocardial infarction occurring during khat sessions, i.e. during the khat-effective period Al-Moterrab *et al*., (2002) and associated with heavy khat chewing Al-Motarreb *et al*., (2005). In cathinone-treated rats, histopathological examination of the testes revealed

degeneration of interstitial tissue, cellular infiltration and atrophy of sertoli and leydig cells. In male adult olive baboon, crude khat extract (equivalent to 60 g leaves and shoots) given orally once a week during 2 months produced no histopathological in the testis, epididymis, liver, kidney and pituitary gland of the animals Mwenda *et al*., (2006). Results of cathinone on rabbit liver, showed increasing chronic inflammation with peri-portal fibrosis in the tissue sections obtained from animals treated with both 20% and 30% *Catha edulis* Al-Habori *et al*., (2000). Khat leaves produced histopathological signs of congestion of the central liver veins with acute hepatocellular damage and regeneration Al-Mammary *et al*., (2002). In addition, some kidney lesions showed presence of fat droplets in the upper cortical tubules, acute cellular swelling, hyaline tubules, and acute tubular necrosis.

The hepatic enlargement observed in male and female SD-rats seemed to be due to exposure to Khat. Hepatic enlargement represents a feature of liver regeneration process which is experienced clinically after liver injury. A lot of xenobiotics are associated with hepatic enlargement due to a direct effect of xenobiotics on the size of hepatocytes Michalopoulos, (2007) or an inflammatory response Ashafa *et al*., (2009) that are associated with the histopathological changes in hepatocytes of rats. Such changes are characterized by mononuclear infiltrates, dilatation of the sinusoids, vacuolar and coagulative necrosis, and the congestive and hemorrhagic degenerative changes in hepatocytes parenchyma particularly in livers of the high dose-group of male SD-rats as well as high dose and Medium-dose of female SD-rats. The degenerative vacuolar lesions in liver probably impaired the liver function of rats and orally administered khat had a direct cytotoxic effect on liver cells.

This present study was carried out to evaluate the effect of *Catha edulis* on organ toxicity during pregnancy and foetal development

4.3 Materials and methods

4.3.1 Study animals and housing, ethical statement and Preparation of crude khat extract

These are as described in section 3.3.1, 3.3.2 and 3.3.3 respectively.

4.3.2 Experimental design

As described in section 3.3.4. At the end of the experiment, six months of pregnancy, after the delivery of the fetuses both the control and treatment animals were anaesthetized intramuscularly with a high dose of ketamine. Then, the baboons and the foetuses were dissected to collect the livers, kidneys, hearts and ovaries which were washed in normal saline and fixed in 10% neutral formalin.

4.3.5 Histological preparations

The tissues that had been stored in formalin were prepared for histological staining in order to demonstrate the microscopic structures of the tissues and cells. The following procedure was adopted in this study as described by Fisher *et al*., (2006).

4.3.5.1 Fixation

The tissues were immersed in specimen containers containing a fixative immediately after harvesting. The aim of the fixation is to preserve tissues/cell's shape, structure relationship and chemical constituents soon after removal from the body in a condition identical to the tissue/cell had before removal from the body. 10% buffered formalin was used in this study.

4.3.5.2 Dehydration

The samples were immersed in increasing concentrations of alcohol to ensure all traces of water are removed from the sample since water does not mix with paraffin. Blocks of 3mm thickness were treated successfully with 70%, 80%, 95% and 100% of alchohol for 2-4 hours each.

4.3.5.3 Clearing

Since alcohol and wax are not miscible, the alcohol was replaced by a wax solvent, toluene which clears the tissue by raising the refractive index.

4.3.5.4 Infiltration/Embedding

The tissue was then infiltrated with melted paraffin wax. The tissue after clearing was transferred to a constant temperature paraffin bath at 58-60^{0}C. Immediately after paraffin wax infiltration, the tissues were transferred from the infiltration jar to a warm embedding tray using warm forceps. Warm paraffin wax (58-60^{0}C) was dispensed about halfway the embedding tray and tissues oriented using warm forceps. The embedding tray was transferred to a cold surface briefly to allow the bottom surface to solidify and anchor the tissue. The tray was removed from the cold surface and placed on embedding mould on embedding tray. More paraffin wax was dispensed to completely fill the tray and the mold. The embedding unit was then transferred to a cold surface and paraffin wax allowed to solidify completely. The paraffin wax blocks were stored in refrigerator to prepare the tissue for sectioning.

4.3.5.5 Sectioning

Serial sections of 5 µm were cut using rotary microtome. The sections had previously been trimmed to expose the tissues. The paraffin wax block was sectioned so that a continuous ribbon was formed. The ribbon was transferred carefully and floated in a warm water bath at 43-46^{0}C

using slightly dump camel hairbrush. The ribbons were floated in the water bath until they were mounted onto clearly labeled microscope slide. 40mg of gelatin was dissolved in the water bath to allow adhesion of the section onto the slide. After mounting the sections onto a microscope slide, the slides were placed in a vertical rack and allowed to drain. The slides were stored at 45-50^{0}C in a dust free oven overnight.

4.3.5.6 Staining

The slides were removed from the drying oven and allowed to cool to room temperature.

4.3.5.7 Haematoxylin and Eosin staining technique

Haematoxylin and Eosin stain allows general view of various structures of the processed tissues. The following table 4.1 shows step by step procedure that was used.

Table 4-1: Staining schedule of liver, kidney, heart and ovary tissues of treatment and control groups from olive baboons and their fetuses.

Reagent	**Time**
Xylene	5 minutes
100% ethanol	5 minutes
95% ethanol	5 minutes
80% ethanol	3 minutes
Water wash	1 minute
Harris haematoxylin	15 minutes
Water wash	1 minute
5% acid alcohol	2 dips
Water wash	1 minute
2-3% lithium carbonate	45 seconds
Water wash	1 minute
Eosin	3 minute
95% ethanol	14 dips
95% ethanol	14 dips
100% ethanol	14 dips
Xylene	5 minutes

NB. The slides then remained in xylene overnight as they awaited mounting.

4.3.5.8 Mounting

A mountant was placed on a clean dust free cover slip. Using a clean pair of forceps the cover slip was placed on the stained section, tilted into position and allowed to dry.

4.4 Results

Microscopic examination of liver sections of controls group showed uniform hepatocytes, intact cytoplasm, prominent nuclei of cells, and uncongested central vein. In addition, no necrotic lesions, fatty changes, or inflammatory signs were observed in the control animals (Fig 4-1). In contrast, liver of the treatment group both the dams and the feotuses showed degenerative vacuolation and coagulative necrosis and degenerative changes in persisting parenchyma with congestion and hemorrhage. There were dilatation of sinusoids and mononuclear inflammatory infiltrates around the central vein and portal tracts (Fig 4-2; Fig 4-5).

Microscopic examination of renal sections of control group demonstrated typical and normal histological features of tubules, glomerular capillaries, and Bowman's capsule (Fig 4-4). In contrast, renal sections of treatment group both the dams and their infants showed histopathological changes. Such changes were characterized by atypical tubules, amorphous Malpighian corpuscles, and invasive infiltrative inflammatory cells. The glomerulus was degenerative and capillaries in Malpighian corpuscles were hypertrophied, some of them were destructed, and Bowman's capsules seemed dilated (Fig 4-5; Fig 4-6).

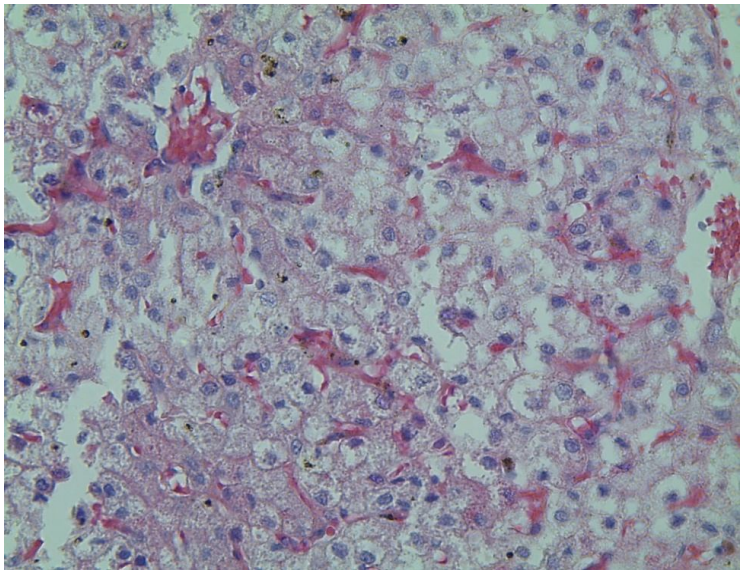

Figure 4-1: A micrograph of hematoxylin and eosin stained liver section for control baboons showing normal liver morphology. (H & E stain 20x).

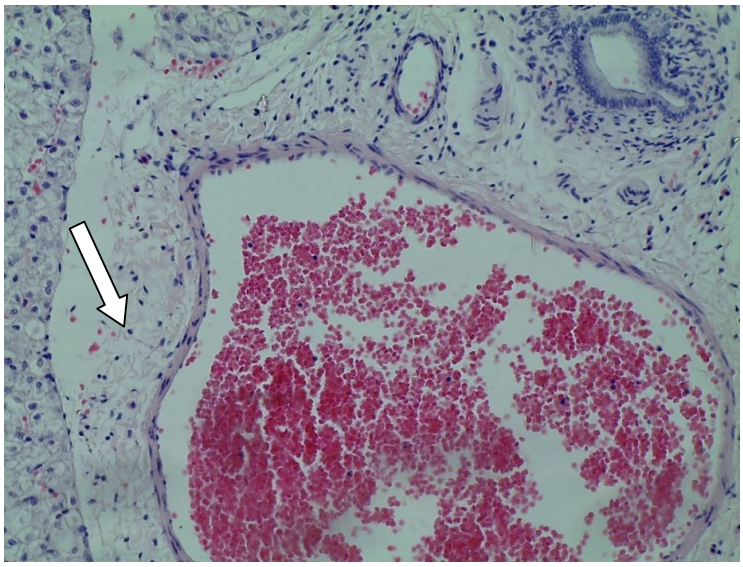

Figure 4-2: A micrograph of hematoxylin and eosin stained liver section for khat treated baboons showing perivascular cuffing with periportal fibrosis. (H & E stain 20x).

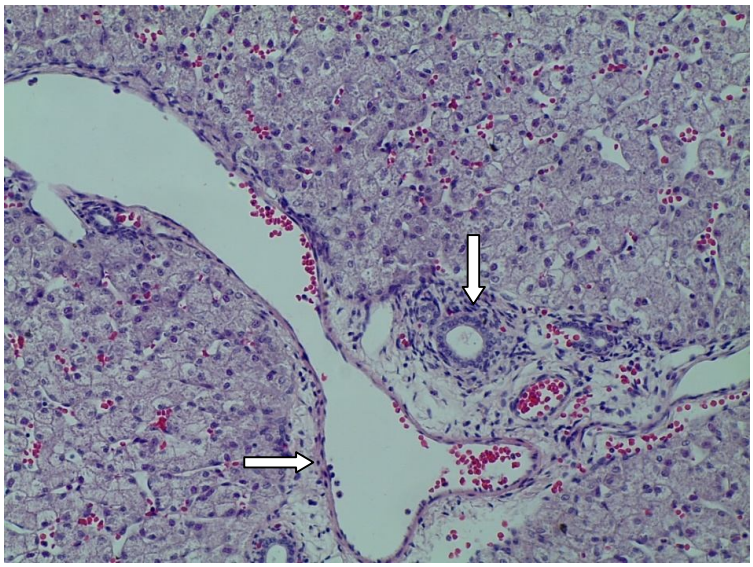

Figure 4-3: A micrograph of hematoxylin and eosin stained liver section of infant from a khat treated baboons showing perivascular cuffing with periportal fibrosis. (H & E stain 20x).

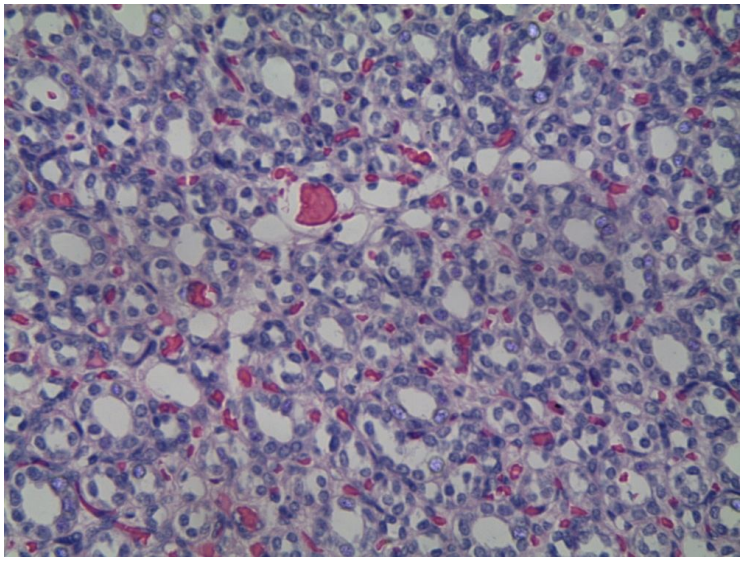

Figure 4-4: A micrograph of hematoxylin and eosin stained kidney section for control baboons showing normal kidney morphology. (H & E stain 20x).

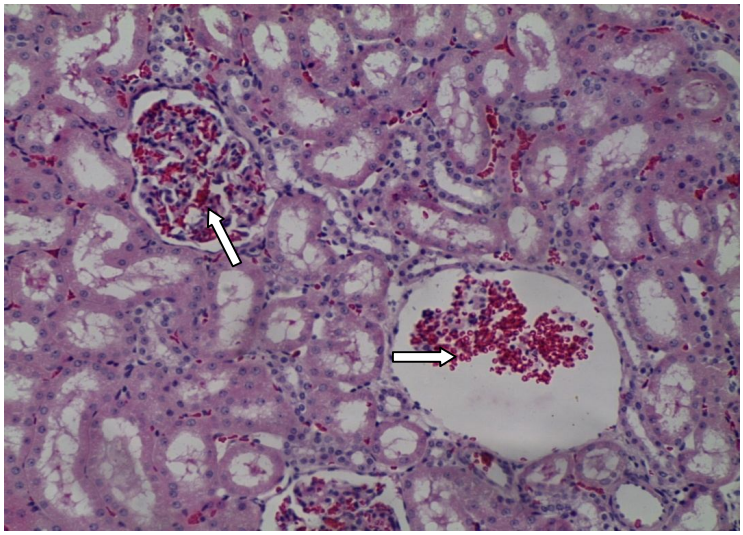

Figure 4-5: A micrograph of hematoxylin and eosin stained kidney section for khat treated baboons showing degenerative glomerulus with red blood cell infiltration. (H & E stain 20x).

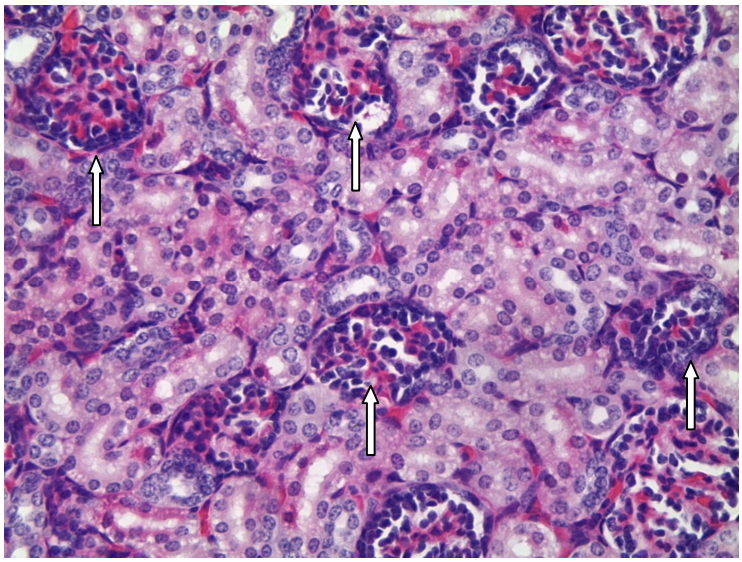

Figure 4-6: A micrograph of hematoxylin and eosin stained kidney section of infant from a khat treated baboons showing necrotic degeneration of the glomerulus. (H & E stain 20x).

The heart tissue of the control group did not show any pathological lession or histopathological change (Fig 4-7). The treatment group both the dams and their foetuses had various changes which was attributed to khat. The changes observed included massive infiltration with inflammatory cells and degenerative changes of the cardiac muscles was also observed (Fig 4-8; Fig 4-9). There were no histopathological changes that were observed in the ovary of both control and treatment groups (Fig 4-10; Fig 4-11).

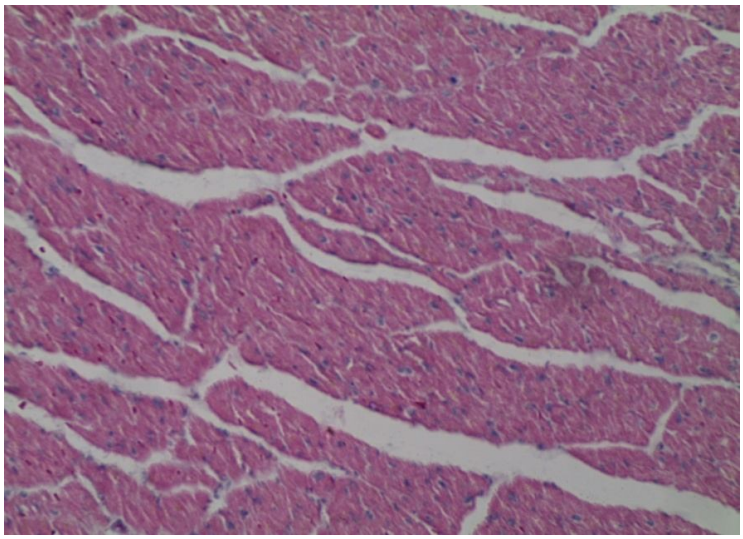

Figure 4-7: A micrograph of hematoxylin and eosin stained heart section for control baboons showing normal liver morphology**.** (H & E stain 20x).

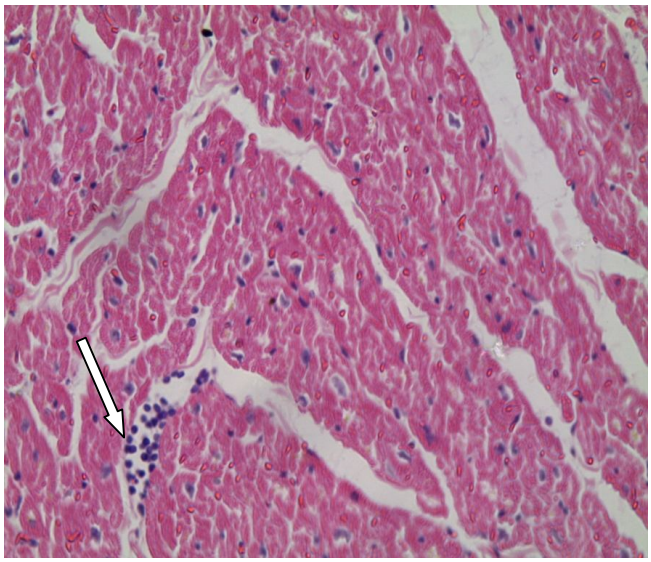

Figure 4-8: A micrograph of hematoxylin and eosin stained heart section of khat treated baboons showing infiltration with inflammatory cell into heart muscle. (H & E stain 20x).

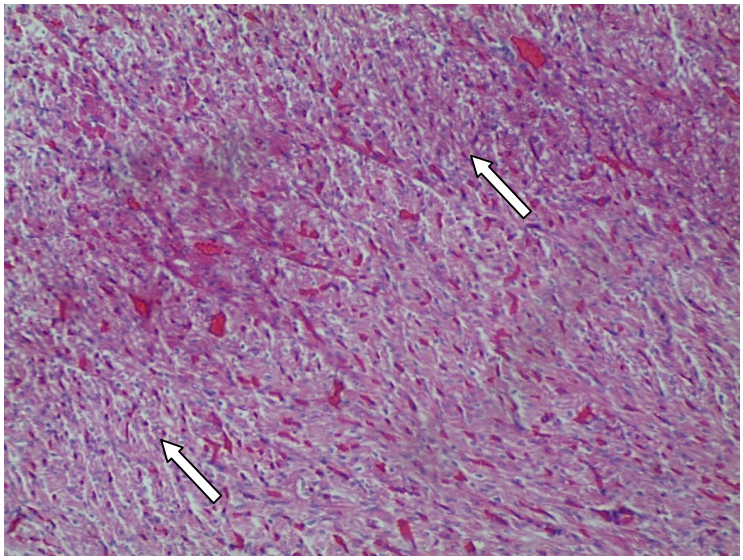

Figure 4-9: A micrograph of hematoxylin and eosin stained heart section of infant from a khat treated baboons showing degenerative changes of the muscles. (H & E stain 20x).

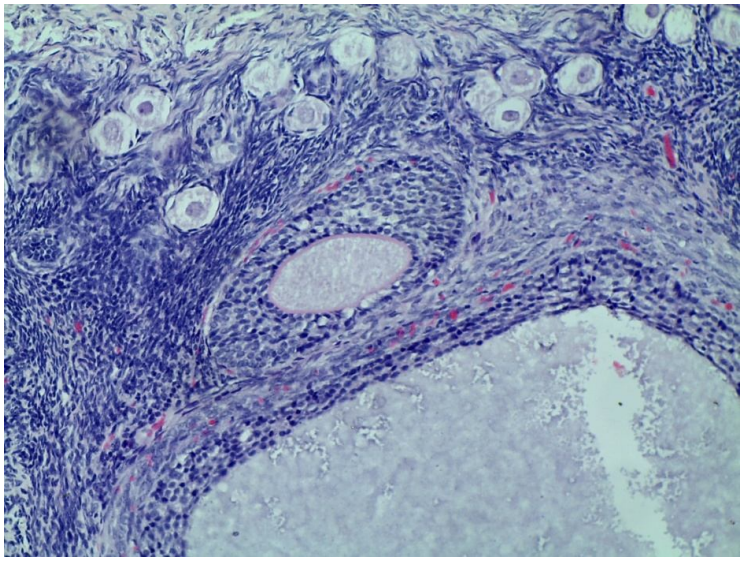

Figure 4-10: A micrograph of hematoxylin and eosin stained liver section for control baboons showing normal ovarian morphology. (H & E stain 20x).

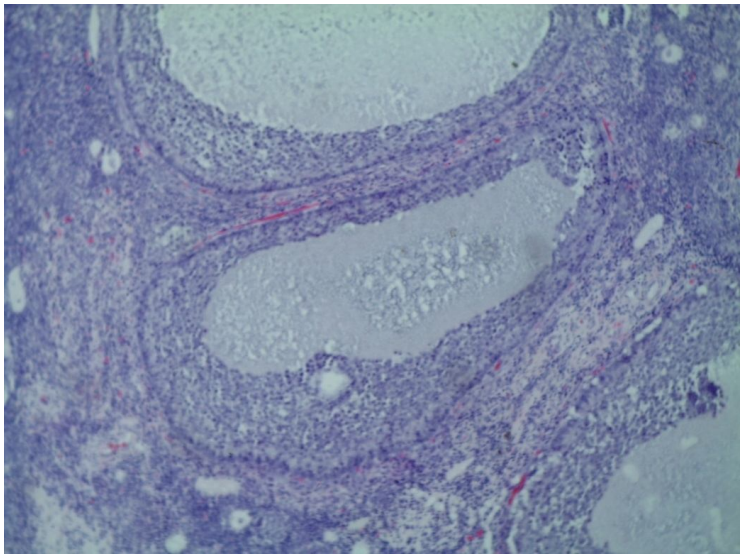

Figure 4-11: A micrograph of hematoxylin and eosin stained ovary section of khat treated baboons showing normal ovarian morphology. (H & E stain 20x).

4.5 Discussion

The histopathology results confirmed that khat has toxicological potential of inducing cytotoxic effects in liver and kidney cells. The finding corroborates what was previously reported by Al-Mammary *et al*., (2002); Carvalho, (2003). Microscopic examination of hepatic and renal sections confirmed the cytotoxic changes. The changes in liver parenchyma were in the form of necrosis with hemorrhage, mononuclear infiltrates of inflammatory cells, and vacuolar degeneration. Histopathological changes in renal sections indicated destructive changes in kidney structures characterized by invasive infiltrates of inflammatory cells and degenerative changes in the glomerulus. The histopathological study on liver and kidney findings were consistent with those reported by Al-Mamary *et al*., (2002) and Al-Habori *et al*., (2002). Cathinone is known to exert different forms of hepatotoxicity *in-vivo* and *in-vitro* when tested on hepatocytes Vitcheva *et al*., (2009). Dimba *et al.,* (2004) has documented that apoptosis occurrence perhaps through a mechanism involving activation of capase-1, capase-3 and capase-8. This suggests the same mechanism may be the one causing lesions observed in this study.

Massive infiltration with inflammatory cells was observed in the heart and degenerative changes of heart muscles were observed in dams and infants of animals treated with khat. Coronary vasoconstriction resulting in reduction of local blood supply associated with Khat use, may explain ischemia and necrosis of cells in sub endocardium a finding that was not observed in this study. This suggests Khat-induced minor cardiac injuries could be attributed to coronary vasoconstriction resulting in reduction of local blood supply, which eventually leads to ischemia and necrosis of cells of the sub endocardium. There have been reports of histological changes such as area of ischemia when sub-chronic administration of 400 mg/kg to rats, however at lower doses no lesions are observed Admassie and Engidawork, (2011). Again, the absence of

pathological changes may be attributed to physiological difference between lower animals and non-human primates.

No pathological changes were observed in the ovaries of both the treatment group and control group.

4.6 Conclusion

Administration of khat during pregnancy induced organ toxicity in both the dams and foetus, and thus khat use should be discouraged and more so during pregnancy.

CHAPTER FIVE

EFFECTS OF KHAT ON MATERNAL HORMONAL LEVELS OF PREGNANT OLIVE BABOONS

5.1 Abstract

Khat use is a common practice in men; nowadays it is also common in women, even during pregnancy. Fresh khat is thought to increase sexual motivation or libido which is more frequently observed in females than males. Khat chewing during pregnancy is on the increase among women of reproductive age, and questions have been raised on the potential effects of khat on maternal fertility and fetal development. This study was carried out to investigate the effects of khat on reproductive hormones which included follicle stimulating hormone, luteinizing hormone, estradiol and progesterone during pregnancy. Six female pregnant olive baboons weighing between 11.2 to 16.5 kg were randomly assigned into two groups; treatment group that received 5 g/kg body weight of crude extract for 8 weeks during the second trimester whereas the normal controls received distilled water and and the levels of hormones assayed. Follicle stimulating hormone and luteinizing hormones were not detected both in the treatment group and control group. Progesterone showed no significant difference between the control group and treatment group ($P > 0.05$). Estraidiol showed significant difference between the treatment group and the control group. Estradiol was greatly increased in the treatment group ($P < 0.0001$). Khat has effect on the estrogen as shown in this study. Khat could be a major factor of disorders that are caused by hormonal imbalances.

5.2 Introduction

Fresh khat is thought to increase sexual motivation or libido which is more frequently observed in females than males Aziz *et al*., (2009). Khat chewing during pregnancy is on the increase among women of reproductive age, and questions have been raised on the potential effects of khat on maternal fertility and fetal development.

Khat extract enhanced sexual motivation, increased vaginal secretions and up-regulated estradiol level in female rats Aziz *et al*., (2009). Ovarian hormone profiles for captive baboons closely resemble steroid hormones for human pregnancies (Tulchinsky *et al*., 1972). The relatively linear increase for estrogens with advancing gestation is a common feature of most primate species (baboons: Albrecht and Townsley, (1978); macaques: Bosu *et al*., (1973); chimpanzees: Reyes *et al*., (1975); Townsley, (1974); humans: Tulchinsky *et al*., (1972). However, published values from baboons as well as humans exhibit an exponential increase in estrogen production in the weeks just before parturition.

Serum progesterone concentrations in captive baboons were reported to rise until approximately day 60 and then level off from mid-gestation to term Albrecht *et al*., (1980); Albrecht and Townsley, (1976). Among primate species, baboons appear to be intermediate between rhesus macaques, in which no increase in progesterone occurs during pregnancy, and hominoids (apes and humans) in which a marked elevation occurs throughout pregnancy Albrecht and Pepe, (1990).

Reproductive hormones often have multiple roles and operate via negative feedback systems. Luteinizing hormone is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells and serves to regulate the function of the gonads. In males, LH stimulates the production and secretion of testosterone from the testes via leydig cells. In females, LH stimulates the production of oestrogens and progesterone from the ovary via theca interna cells and luteal cells. Concentrations of LH increase during ovulation and with the formation of the corpora lutea with progesterone secretion. The secretion of LH is regulated via the secretion of Gonadotropin releasing hormone.

Follicle stimulating hormone is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells. FSH secretion is regulated by GnRH from the hypothalamus. The target tissues of FSH in males are the sertoli cells within the testes and in the female the granulosa cells of the ovary. FSH stimulates the maturation of germ cells within the testes and ovaries. In the female it also stimulates follicular development and oestradiol synthesis.

Estradiol is a steroid hormone and is part of the oestrogens group of hormones and is the principle oestrogen in females. Estrone and estriol are chemically similar to estradiol but are found in lower concentrations and have a lower estrogenic activity. Production of oestrogens occurs in the ovary via granulosa cells, the placenta and the *Zona reticularis* of the adrenal cortex. In males it is produced in sertoli cells found in the testes. Estradiol is synthesised from cholestrol.

Progesterone is a steroid hormone that along with oestrogens is based on a cholesterol molecule produced by the corpus luteum and the placenta using cholesterol as the base molecule. Progesterone is produced by the corpus luteum as well as by the feto-placental unit and in the zona reticularis of the adrenal cortex (to a lesser extent). During pregnancy the plasma concentration of progesterone is maintained at an elevated level. Progesterone also inhibits secretion of FSH and LH (negative feedback at hypothalamic level by inhibiting GnRH) and thus also prevents the ovulation of follicles during the luteal phase and during pregnancy. In most domestic species the corpus luteum persists for the entire length of gestation. It is speculated that progesterone produced first during gestation by the corpus luteum and later by the placenta is vital to the maintanence of pregnancy Pepe and Albrecht, (1995). It is well established that the fetal adrenal gland in primates is important in the synthesis and secretion of androgen precursors essential to the production of estrogen by the placenta Albrecht and Pepe, (1990).

This study was carried out to investigate the effects of khat on various reproductive hormones which included follicle stimulating hormone, luteinizing hormone, estradiol and progesterone during pregnancy.

5.3 Materials and methods

5.3.1 Study animals and housing, ethical statement, preparation of crude khat extract experimental design

These were as described in section 3.3.1, 3.3.2, 3.3.3 and 3.3.4 respectively.

5.3.2 Hormonal assay

Hormonal assays for serum Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Estrogen (E2) and progesterone (P4) were done by use of enzyme immunoassay technique using commercial kits from Human Gesellschaft fur Biochemica und Diagnostic mbH, Germany according to the manufacturer's instructions. In all hormone assay procedures, assays were done in duplicate. The optical density of specimen was measured using HumaReader HS (Gessellschaft für Biochem und Diagnostica, mbH, Germany).

5.3.2.1 Follicle Stimulating Hormone

All reagents were placed in room temperature. A template was prepared to show the order of the samples. The calibrators were placed in the wells in duplicates in accordance with the template. 50µl of the calibrators was placed in the first twelve wells followed by 50µl of the serum in duplicate covered with microtiter strips and incubated for 60minutes at 60C. This was followed by three washing steps using 300µl of the washing solution as directed by the manufacturer. 100µl of the substrate reagent was added and incubated at 60c for 15 minutes. The reaction was stopped using 50µl of a stop solution and mixed carefully. The absorbance was read using

HumaReader HS (Gessellschaft für Biochem und Diagnostica, mbH, Germany) ELISA reader at 450 nm.

5.3.2.2 Luteinizing Hormone

All reagents were placed in room temperature. A template was prepared to show the order of the samples. The calibrators were placed in the wells in duplicates in accordance with the template. 50µl of the calibrators was placed in the first twelve wells followed by 50µl of the serum in duplicate in the subsequent wells. 100µl of the enzyme conjugate was added in each of the wells, rocked gently, covered with microtiter strips and incubated for 60minutes at 60C. This was followed by three washing steps using 300µl of the washing solution as directed by the manufacturer. 100µl of the substrate reagent was added and incubated at 60c for 15 minutes. The reaction was stopped using 50µl of a stop solution and mixed carefully. The absorbance was read using HumaReader HS (Gessellschaft für Biochem und Diagnostica, mbH, Germany) ELISA reader at 450 nm.

5.3.2.3 Estradiol

All reagents were placed at room temperature. A template was prepared to show the order of the samples. The calibrators were placed in the wells in duplicates in accordance with the template. 50µl of the calibrators was placed in the first twelve wells followed by 50µl of the serum in duplicate in the subsequent wells. 100µl of the enzyme conjugate was added in each of the wells, rocked gently, covered with microtiter strips and incubated for 60 minutes at 60C. This was followed by three washing steps using 300µl of the washing solution as directed by the manufacturer. 100µl of the substrate reagent was added and incubated at 60c for 15 minutes. The reaction was stopped using 50µl of a stop solution and mixed carefully. The absorbance was read

using a HumaReader HS (Gessellschaft für Biochem und Diagnostica, mbH, Germany) ELISA reader at 450 nm.

5.3.2.4 Progesterone

All reagents were placed in room temperature. A template was prepared to show the order of the samples. The calibrators were placed in the wells in duplicates in accordance with the template. 50µl of the calibrators was placed in the first twelve wells followed by 50µl of the serum in duplicate in the subsequent wells. 100µl of the enzyme conjugate was added in each of the wells, rocked gently, covered with microtiter strips and incubated for 60 minutes at 60C. This was followed by three washing steps using 300µl of the washing solution as directed by the manufacturer. 100µl of the substrate reagent was added and incubated at 60c for 15 minutes. The reaction was stopped using 50µl of a stop solution and mixed carefully. The absorbance was read using HumaReader HS (Gessellschaft für Biochem und Diagnostica, mbH, Germany) ELISA reader at 450 nm.

5.3.3 Statistical analysis

The data was analyzed using graphpad statistical software. The data was expressed as mean ± Standard error of mean (SEM) showing the values of the controls and the treatment group. The *P*-values were examined by unpaired student's t-tests. The results were considered significant by a *P*-value <0.05. All end points were analyzed using two -tailed test.

5.4 Results

The levels of FSH and LH were non detectable in both groups. Progesterone showed no significant difference between the control group and treatment group (*P*> 0.05) (Fig 5-1).

Estradiol showed significant difference between the treatment group and the control group. Estradiol was greatly increased in the treatment group ($p < 0.0001$) (Fig 5-2) suggesting that the effects observed were primarily due to khat treatment.

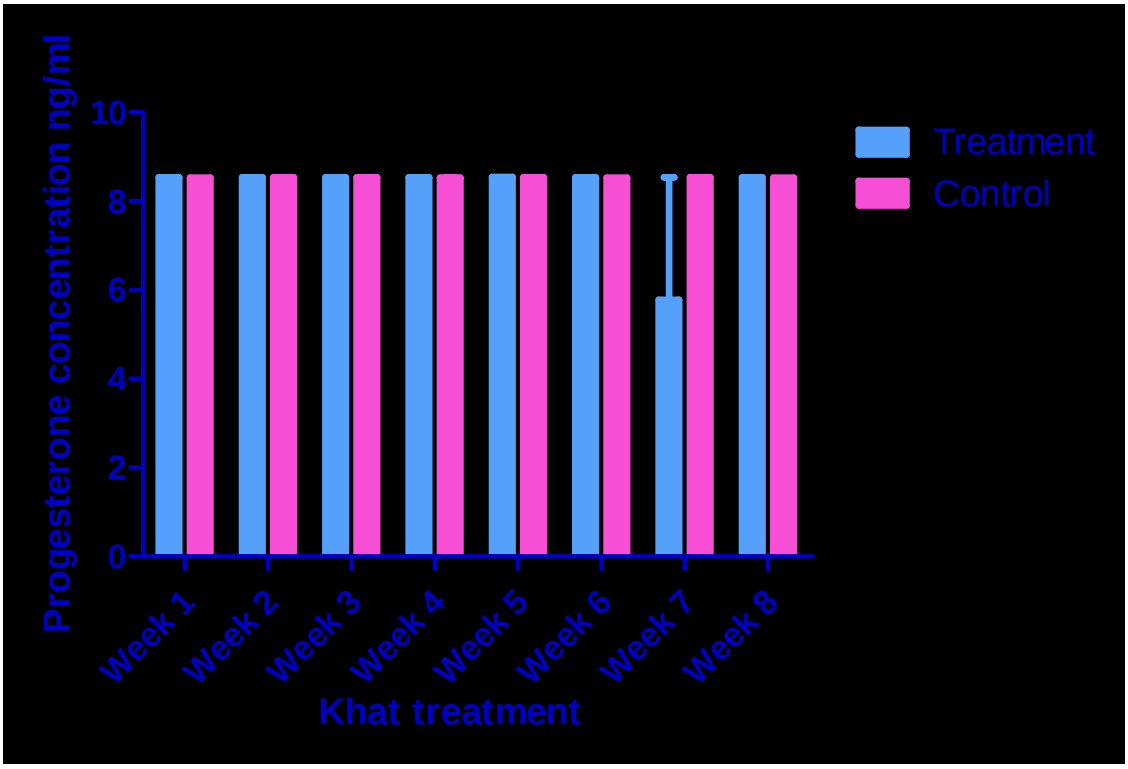

Figure 5-1: The progesterone levels of pregnant olive baboons given Khat and pregnant olive baboons on distilled water.

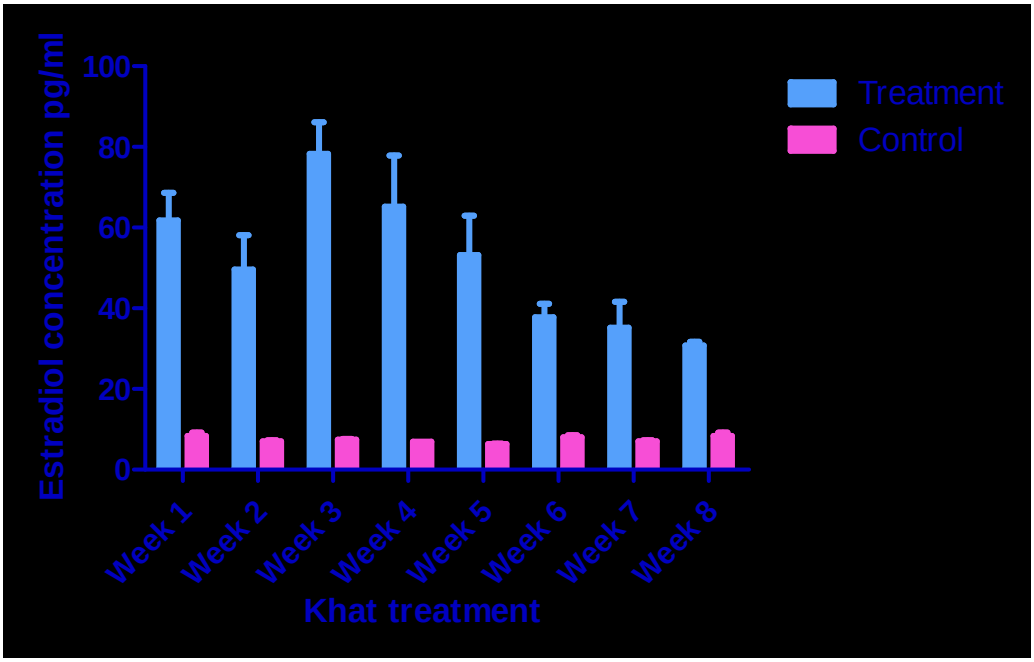

Figure 5-2: The Estradiol levels of pregnant olive baboons given Khat and pregnant olive baboons on distilled water.

5.5 Discussion

The aim of this study was to investigate the effects of khat on various reproductive hormones during pregnancy. The hormone analysed in this study included follicle stimulating hormone lutenizing hormone, progesterone and estradiol. Elevated levels of estradiol were observed in the treatment group compared to the control group. This indicates that khat has effect on the estrogen causing its upregulation. Continued upsurge of estradiol may induce abortion. Similar elevation of estrogen was observed in men khat chewers in a study by Al-Ghamdi, (2012).

The characteristics of the menstrual cycle in baboons are very similar to those of women, except, that of the baboon is slightly longer and there is a lower luteal phase concentration of oestrogen. The duration of pregnancy in baboons is about two-thirds that of humans but patterns of oestrogen and progesterone secretion are virtually identical. The principal oestrogen produced by the pregnant baboon is oestrone, while oestriol is the most abundant in human pregnancies Stevens, (1997). In females, ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete the steroid hormones progesterone and estrogen. Progesterone first produced by the corpus luteum and later by the placenta is necessary for maintenance of pregnancy, and, in most mammals, LH is required for continued development and function of corpora lutea Pepe and Albrecht, (1995). FSH stimulates the maturation of ovarian follicles.

Hence during pregnancy its expected estrogen and progesterone levels to be high while the level of FSH and LH to be low since the ovary is dormant during pregnancy. Also during pregnancy progesterone and estrogen maintain a negative feedback mechanism to the hypothalamus that

inhibits release of gonadotropin realizing hormone that is responsible for the stimulation of the anterior pituitary to release FSH and LH. This explains the absence of these two hormones both in the control group and treatment group. Estrogen plays a critically important physiologic role in the maintenance of primate pregnancy Albrecht *et al.,* (2000). Moreover, high levels of estrogen may induce abortion if a surge occurs before parturition period.

Khat may cause hormonal imbalance, as demonstrated by a decrease in serum estrogen and alteration of the estrogen: progesterone ratio in rodents Seyfe *et al*., (2013). A decrease in estradiol might results in degeneration of the early embryos and post-implantation embryonic resorptions in the uteri Juneja *et al*., (1996); Hom *et al*., (1998).

5.6 Conclusion

Khat has effect on the estradiol as shown in this study. Khat could be a major factor of disorders that are caused by hormonal imbalances.

CHAPTER SIX

EFFECTS OF KHAT ON MATERNAL WEIGHT, BLOOD PRESSURE AND TEMPERATURE OF PREGNANT OLIVE BABOONS

6.1 Abstract

Khat use has increased steadily over the last 50 years and has become a significant problem with both social and medical ramifications. This study assessed the effect of khat on blood pressure, weight and temperature on pregnant olive baboons. Six olive baboons were allocated randomly to two groups; one group was treated with crude khat extract at 5 g/kg body weight while the other was given 100 ml of distilled water. Blood pressure, weight and temperature were measured once weekly for 8 weeks during the second trimester. The animals treated with khat delivered infants with lower birth weight than the control group (0.6 ±0.05; 0.9 ± 0.05 respectively). The dams treated with khat had lower weight gain compared to the control group (13.14 ±0.09; 14.19 ± 0.13 respectively). There was no significant difference in blood pressure and temperature observed between the treatment group and the control group. Khat has effect on the maternal weight gain during pregnancy and fetal birth weight. Khat could be considered to negatively affect pregnancy and its outcome.

6.1 Introduction

Hypertension is a growing public health problem, with remarkable contribution to cardiovascular diseases (CVD) morbidity. Elevated blood pressure has many risk factors that are of behavioral, dietary or genetic origin. Among the main modifiable risk factors of hypertension are overweight and obesity, cigarette smoking, physical inactivity, unhealthy diet, stress, dietary salt intake, and alcohol use WHO, (2002). Khat chewing induces small and transient rises in blood pressure and heart rate. Regular and repeated intake of Khat has recently been reported to be associated with increased risk of acute myocardial infarction Al- Motarreb *et al*., (2005) and high blood pressure Tesfaye *et al*., (2008).

Cathinone is structurally and functionally closely similar to amphetamine and releases catecholamines from pre-synaptic storage sites resulting in CNS stimulation and a variety of peripheral sympathomimetic effects such as tachycardia and hypertension Hughes, (1973). It has been reported that the effects of a portion of khat are very similar to those of about 5-mg amphetamine. There may be arrhythmias and moderate increase in blood pressure which can become chronic upon long term use Halbach, (1972). There is exaggerated cardiovascular response to physical effort under the effect of khat Galkin and Mironychew, (1964) which has been associated with acute cardiovascular problems particularly in elderly people.

No data is available regarding the effects of khat on maternal weight gain during pregnancy, blood pressure and temperature in a randomized controlled experiment in non human primates. However, is important to note that while the investigations in humans have provided crucial information into the effects of khat they mostly have a retrospective background and as such they

have several limitations such as the limitations of retrospective analysis to permit sample collection and allow controlled parameters such as dosage as would be in a randomized controlled experiment. In these animals, it is possible to evaluate in a dose controlled study the effects of khat on pregnancy and fetal growth and development with limited ethical restrictions, studies not possible in humans because of the generation time. This study assessed the effect of khat on blood pressure, weight and temperature on pregnant olive baboons.

6.2 Materials and methods

6.2.1 Study animals and housing, ethical statement Preparation of crude khat extract and experimental design

These are as described in section 3.3.1, 3.3.2, 3.3.3 and 3.3.4 respectively.

Blood pressure measurements were taken using an electronic sphygmomanometer (VWR international, Leuven, Belgium). During blood pressure measurements, a baboon was anesthetized and an inflated cuff placed smoothly around a shaved arm at roughlythe same vertical height as the heart while the baboon was lying in the recumbent positon and the arm supported horizontally. The cuff was inflated until tight, and then slowly the pressure in the cuff was released until stable reading were achieved and recorded as systolic and diastolic pressures.

The body temperature was taken using a digital thermometer at the rectum (veterinary digital thermometer, china). Body weight was determined by placing the baboon on a weighing scale (Salter Brecknell VD 1000).

6.2.2 Statistical analysis

The data was analyzed using graphpad statistical software. The data was expressed as mean ± Standard error of mean (SEM) showing the values of the controls and the treatment group. The p-values were examined by unpaired student's t-tests. The results were considered significant by a p-value <0.05. All end points were analyzed using two-tailed test.

6.3 Results

The results indicated there was effect of khat on the weight of the treatment group compared to the control group ($P< 0.0001$) (table 6-1). All the animals gave birth within the expected gestation period. The animals treated with khat delivered infants with lower birth weight than the control group ($P< 0.02$) (table 6-1; Fig 6-1). The infants born of khat treated dams had lower birth weight compared to the control (Fig 6-2). No significant difference in temperature was observed between the treatment group and the control group. There was no significant difference of blood pressure between the treatment group and the control group (Fig 6-3; Fig 6-4). It should be noted that even if the difference was not significant, the treatment group had both the systolic and diastolic pressure slightly increased than the control group though within the normal ranges.

Table 6-1: Physiological effects of *Catha edulis* extract administration during second trimester

Parameter	**Control group (n=3)**	**Treatment group (n=3)**	**P-Value**
Diastolic pressure (mm Hg)	35.33±2.433	40.40±1.173	0.109
Systolic pressure (mm Hg)	72.98±2.893	78.00±2.664	0.248
Weight dams (Kg)	14.99±0.1342	13.14±0.099	0.0001
Weight Infants (Kg)	0.9 ±0.05	0.60±0.05	0.02
Temperature (^{0}C)	36.52±0.1600	36.56±0.0905	0.7953

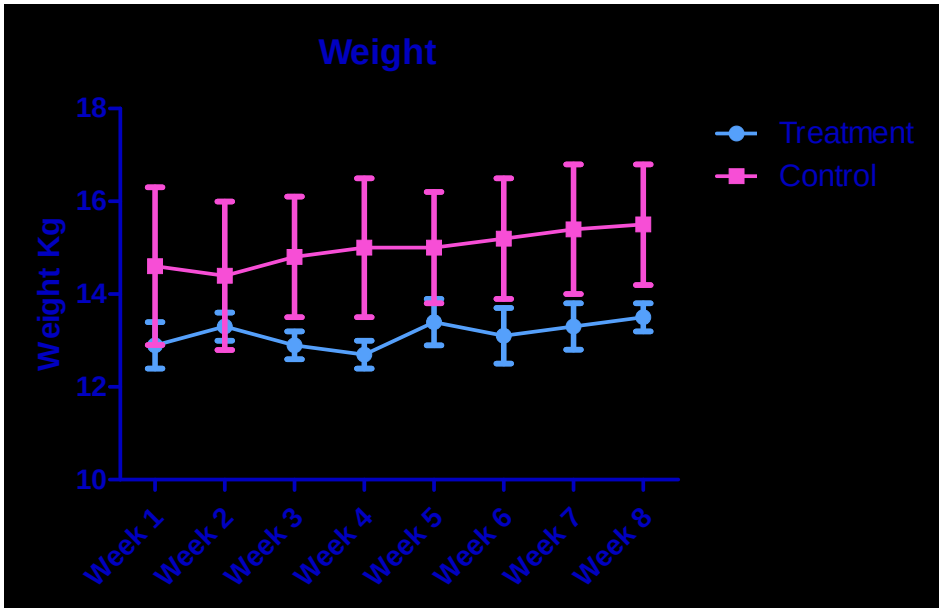

Figure 6-1: The weight of pregnant olive baboons given Khat and pregnant olive baboons on distilled water.

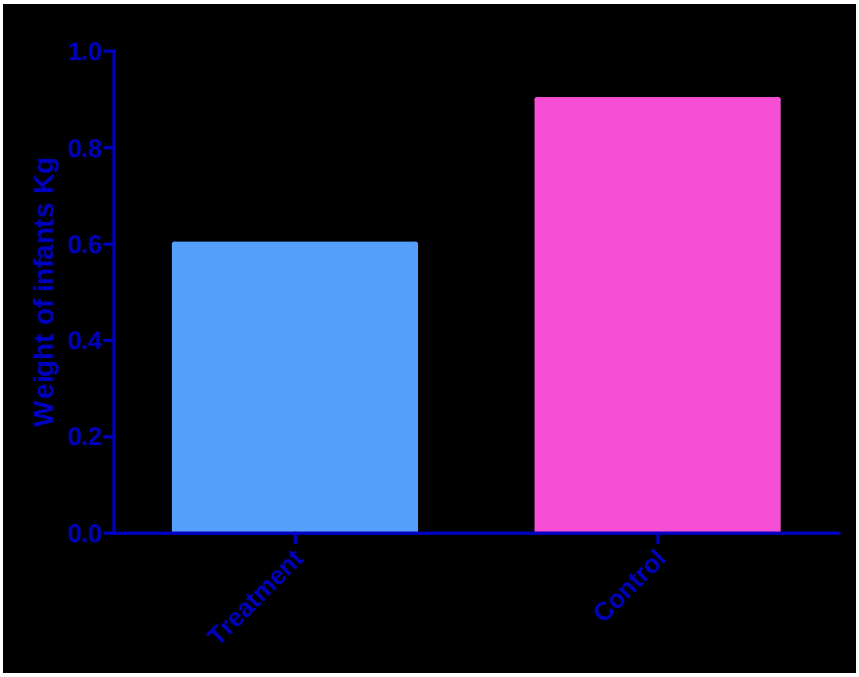

Figure 6-2: The weight of infants born of the khat treated group and the control group

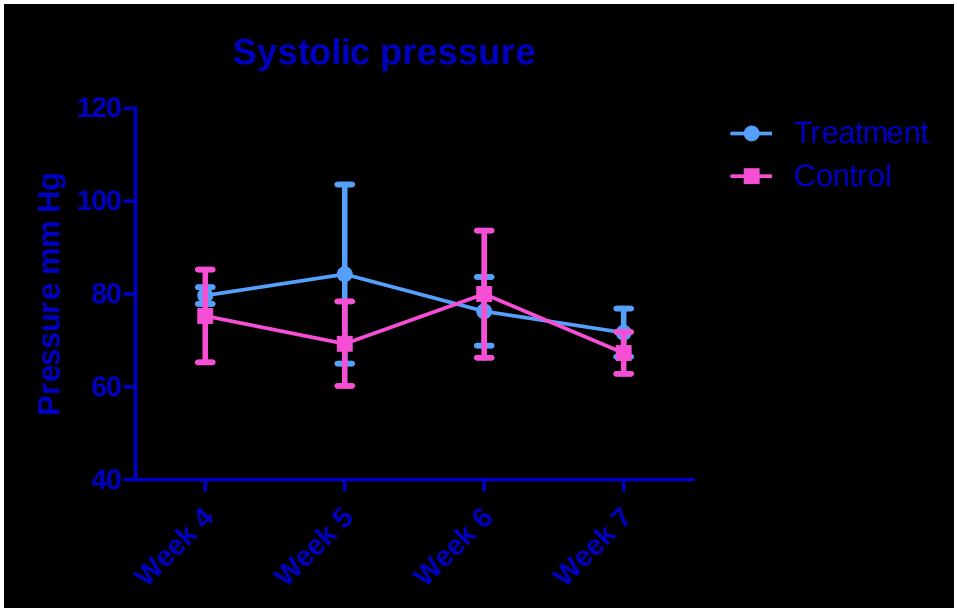

Figure 6-3: The Systolic pressure of pregnant olive baboons given Khat and pregnant olive baboons on distilled water.

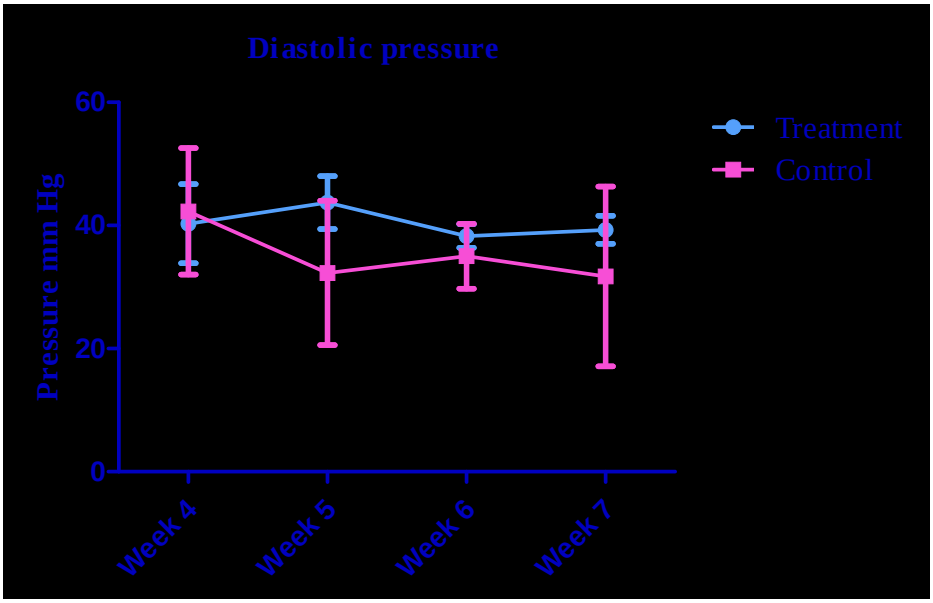

Figure 6-4: The Diastolic pressure of pregnant olive baboons given Khat and pregnant olive baboons on distilled water.

6.4 Discussion

The aim of this study was to establish the effect of khat on blood pressure, body temperature and body weight in pregnant olive baboons during their second trimester. The results showed that khat has effect on body weight and birth weight of the fetuses. Previous investigations of the effects of khat have indicated various physiological and metabolic effects. Maternal weight gain was significantly low in the treatment group and these could have been attributed to the anorexic effect of khat leading to low food intake. The reduced weight gain observed in animals treated with Khat extract might be attributed to the presence of tannins and other polyphenolic compounds, since tannins and polyphenolic compounds in khat could inhibit the digestive enzymes and consequently, the nutrients absorption would be imunpaired Al-Mammary *et al*., (2001).

The results are corroborated by those of Jansson *et al*., (1988), who reported that weight gain was lower in the pregnant guinea pig treated with khat compared to weight gain in control group which was accompanied by low birth weight. Studies of full-term human newborns have shown that khat use by the mother is associated with lower birth weight Abdul *et al.*, (1988). In humans, Murray *et al*., (2008) has shown that khat has an anorectic. Similary in rats' purified cathinone has demonstrated that acute administration induces a reduction in food intake Knoll, (1979); Zelger and Carlini, (1980). The reduction in food intake results in weight loss as established by Zelger and Carlini, (1980). Khat-associated reduction in body weight could be attributed to kidney damage, as it was shown to cause mild-to moderate injury in the histopathologic evaluation, but it could also be ascribed to khat-induced delay in intestinal

absorption that contributes to some degree of malnutrition Gunaid *et al*., (1999); Makonnen, (2000) or increased plasma leptin level that leads to loss of appetite Al-Dubai *et al.,* (2006).

Khat chewing has an important effect on carbohydrate metabolism through increased cortisol levels leading to reduced insulin secretion and insulin resistance through induced up-regulation of resistin expression Shojima *et al*., (2002); Miscra *et al*., (2008) and cathinone –induced catecholamine secretion which increase blood glucose by activation of glycogenolysis in skeletal muscles and the liver, a β2- adrenoceptor-mediated response Al-Motarreb *et al*., (2010). This activity of cathinone will lead to weight loss as observed in this study and in agreement with previous studies.

A study by Mahmood and Lindequist, (2008) reported reduction in weight in rats fed with khat indicating that the plant could be involved in metabolic processes. Lower mean birth weights have been reported in khat-chewing mothers compared to non-using mothers indicating an association between khat chewing and decreased birth weight Abdul *et al*., (1987). Possibly tannins and the inorganic ions present in khat may contribute to delayed absorption of glucose thereby reducing its levels. Khat induced delay in gastric emptying Heymann *et al*., (1995) may play a role in reducing the blood sugar after food intake. Accelerated gastric emptying increases the food reaching the distal intestine where it stimulates the glucagon like peptide-1 producing cells and subsequently increase in glucagon like peptide-1 secretion which stimulates beta cells to release insulin Miholic *et al*., (1991). Moreover, khat effects mimics those of amphetamine-like sympathetic action, hence make it possible to suppress the release of insulin through α-receptor stimulation.

Khat chewing leads to significant decrease in zinc serum levels in a study by Mohamed and Hatem, (2012). Zinc acts as an antioxidant by protecting the sulfhydryl groups of proteins and enzymes against free radical attack in the body Disilvestro, (2000) and is necessary for the synthesis of insulin hexamer Meyer and Spence, (2009), therefore its levels may have affected the level of insulin present to facilitate metabolism of the food ingested by the baboons resulting to the reduced weight gain.

The fetuses born of khat treated baboons had lower birth weight compared to those born of control group. During pregnancy, several studies documented a number of negative reproductive health consequences and adverse pregnancy outcomes of Khat chewing, including lower libido, sexual impotence, and inhibition of utero-placental blood flow, leading to a teratogenic effect and the impairment of fetal growth Mwenda *et al*., (2003). Khat chewing during pregnancy is thought to influence fetal development. Lower birth weight was also found to be associated with Khat chewing Hassan *et al*., (2005)

Low birth weight children have varying degrees of social and medical risk. They have a broad spectrum of growth, health, and developmental outcomes. While the vast majority of low birth weight children have normal outcomes, as a group they generally have higher rates of subnormal growth, illnesses, and neurodevelopment problems. During pregnancy and the first 12 months after birth, specialized brain cells are forming connections with each other, creating the networks that underlie thinking, learning, and feeling. These problems increase as the child's birth weight decreases. With the exception of a small minority of low birth weight children with mental retardation and/or cerebral palsy, the developmental sequelae for most low birth weight infants include mild problems in cognition, attention, and neuromotor functioning.

The low birth weight infants born of dams treated with khat may be more predisposed to complications like respiratory distress, sleep apnea, heart problems, jaundice, anemia, chronic lung disorders. Adverse sociodemographic factors negatively affect developmental outcomes across the continuum of low birth weight and appear to have far greater effects on long-term cognitive outcomes than most of the biological risk factors. In addition, the cognitive defects associated with social or environmental risks become more pronounced as the child ages. Low birth weight infants run the risk of developing many complications. Respiratory distress, sleep apnea, heart problems, jaundice, anemia, chronic lung disorders, and infections are just some of the obstacles that low birth weight babies may face. Although several complications associated with low birth weight may decrease or disappear with time, a few of them are permanent.

This study demonstrated no difference in blood pressure of the treatment group compared to the control group. Similarly, we did not observe adverse changes in temperature. However, Kalix, 1980 showed that khat induces hyperthermia.

6.5 Conclusion

Khat has effect on the maternal weight gain during pregnancy and fetal birth weight. Khat affects the feed intake of the subject, since high levels of energy are required for the maintenance of pregnancy as has been shown in previous studies in lower animals. Khat should be considered negatively affect pregnancy.

CHAPTER SEVEN

GENERAL DISCUSSION CONCLUSION AND RECOMMENDATION

7.1 Discussion

The study examined the effects of khat on biochemistry, hormones, and organ toxicity during pregnancy and foetal development and it established that khat, impairs liver and kidney function, hormonal profiles and foetal outcomes. The study findings show that Khat has alters food intake that results in weight loss. An indication that khat has an effect on intestinal absorption (Aziz *et al*., 2011) and induction of anorexia as documented by Halbach, (1972); Sireeratowong *et al*., (2008). This study has demonstrated for the first time that khat induces weight loss in pregnancy and alters pregnancy outcome resulting in birth of infants with low birth weight. The same effects of khat have been documented in guinea pigs fed on khat for 10 days (Wabe, 2011; Jansson *et al*., 1988). Khat (either as an extract or whole plant) influences pregnancy outcomes probably by interfering with food absorption Dhaifalah and Santavy, (2004), inducing anorexia and altering host feeding habits.

In guinea pigs, placental insufficiency has been observed and it may be resulting in vasoconstrictive effect of norpseudoephedrine which reduce the placental blood flow impairing fetal growth (Al Harazi and Frass, 2009). Similarly, in rats, Shewamene and Engidawork, (2014) showed that subacute khat exposure is associated with weight loss which can be attributed to kidney damage, malabsorption, or affecting plasma leptin level.

The birth weight observed may be linked as a well-established risk factor for both perinatal and young infant death and khat chewing during pregnancy may contribute to infant mortality

(Jansson *et al*., 1988b). High blood pressure has been registered in pregnant women chewing khat (Abdul-Ghani *et al*., (1987). On the contrary, this study established that khat did not have any effect on both the systolic and diastolic pressures. However, the cathinone in khat has been shown to elevate diastolic blood pressure (Workineh *et al*., 2010) and heart rate (Toennes *et al*., 2003).

The treatment groups showed an elevation in liver transaminases which indicates that khat plays a role in inducing hepatocellular damage as established by (Alsalahi *et al*., (2012) and Al-Habori *et al* in New Zealand white rabbits. The hepatocellular damage observed may be coupled with imunpaired liver function, decreased protein synthesis; diminished protein intake and reduced absorption of amino acids due to presence of elevate levels of total protein and albumin in serum (Fahaid *et al.*, 2011; Yi-Wen *et al*., 2011). The study further established that khat has effect on kidney function due to the presence of elevated levels of creatinine and blood urea nitrogen. This can be attributed to impairment in tubular reabsorption, renal blood flow and glomerular filtration rate Carvalho, (2003); Fahaid *et al*., (2011). Renal impairement is evident by the elevated of sodium and potassium. Elevation in the levels of the electrolytes implies that khat has chemical components that act directly or via mediation to induce overhydration, -induce secretion of vasopressin Herny et al., (1998).

Pathologicals findings showed that khat causes destruction of kidney structures characterized with infiltration of inflammatory cells, and glomerulus and periportal fibrosis. The pathological changes occurred in the both the maternal and foetal organs. The liver and kidney pathological changes observed in this study are consistent with those observed by Al-Mamary *et al*., (2002) and Al-Habori *et al*., (2002) in rabbits. The present study shows that khat plays a role in inducing liver and kidney cytotoxicities that includes coagulative necrosis, hemorrhage, mononuclear cell

infiltrations and vacuolar degeneration. However, the mechanism in how khat liver and kidney toxicity is uncertain but generation of free radicals and oxidants can be implicated in inducing the organotoxicites as shown by Al-Zubairi *et al*., (2003). Hepatic and renal toxicity are related to lipid peroxidation and oxidative stress in the liver and kidneys Fahaid *et al*., (2011).

The non-detectable of FSH and LH in pregnant dams shows that the ovaries are dormant during pregnancy. There were no differences between the levels of progesterone in the control and treatment group animals. This implies that khat did not have influence on progesterone secretion, since progesterone is first produced by the corpus luteum and later by the placenta where it has function of maintaining the pregnancy. Low levels of FSH and LH shows that the hormones are controlled via negative feedback mechanism that inhibits release of gonadotropin realizing hormone responsible for the FSH and LH release. This may be the probable explanation for the diminished levels of FSH and LH hormones in both groups. Conversely, the increase in estrogen levels of the animals in the treatment group opposed to those in the control group shows that the hormone plays a critically role of maintaining pregnancy in baboons (Albrecht *et al.,* 2000). Khat and its components may be acting as a modulator for the hormone but the hormone upsurge can lead to abortions and stillbirths whom we did not observed.

7.2 Conclusion

Our study for the first time has shown the effects of khat on biochemical, hormonal, weight and toxicological effects on pregnancy and fetal development in olive baboons. These results have shown for the first time in non-human primates that;

1. Khat has effects on liver and kidney function. The study has revealed that khat (*Catha edulis*) extract has toxic effects on biochemical components that could be an indicator for liver and the kidney toxicity.
2. Khat has effect on the maternal weight gain during pregnancy and fetal birth weight. Khat should be considered as negative factor for maternal weight gain and fetal birth weight.
3. This study has been able to demonstrate that khat could affect the level of estrogen during pregnancy. Estrogen up-regulation could be detrimental during pregnancy as it can induce abortion and therefore khat use during pregnancy should be discouraged.

7.3 Recommendation

Our study strongly suggests the use of olive baboons which are phylogenetically closely related to humans may be an appropriate model to study medical implications on the use of khat.

1. Further studies should be done to establish the effect of khat at different dose rates.
2. Further study should be done to establish the effects of khat during the entire pregnancy period.
3. From the results observed khat has effect on maternal and fetal weight hence sensitization to pregnant women can be enhanced to encourage them to abstain from khat use during pregnancy.

7.4 Limitation of the study

1. This study did not consider the metabolic rate of the olive baboons as a factor to the pharmacokinetics of the drug in the body and this may have led to the discrepancy in the results observed.

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Appendix 1 Ethical approval

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NATIONAL MUSEUMS OF KENYA
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INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **IRC/06/13**

Dear **Dr. Atunga Nyachieo,**

It is my pleasure to inform you that your proposal entitled *"The effects of Khat on pregnancy and fetal development"* in collaboration with *'Dr. Daniel Chai'* has been reviewed by the Institutional Scientific and Review Committee (IRC). The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes. The committee is guided by the Institutional guidelines (e.g. S.O.Ps) as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved at a meeting of 29th May 2013 and you are bound by the IPR Intellectual Property Policy.

Signed ..... Chairman IRC: DR. HASTINGS OZWARA

Signed ..... Secretary IRC: DR. ATUNGA NYACHIEO

Date:

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
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APPROVED....29/5/2013...