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BACHELOR OF SCIENCE, BIOCHEMISTRY

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RESEARCH PROJECT PROPOSAL: HBC 305

STUDY ON THE EFFECTS ACTIVE INGREDIENT (CATHINONE) IN KHAT ALSO KNOWN AS MIRAA

BY

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DECLARATION

This dissertation is my original work and it has not been submitted to any other University for any degree award.

Signature..... Date.....13/10/2014.....

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ABSTRACT

The principal active components in khat are cathinone and cathine which are alkaloids that are structurally related to amphetamine. The pharmacological effects of cathinone are qualitatively similar to those of amphetamine, although it is less potent. These compounds act on dopaminergic , narodrenergic and serotonergic neurotransmitter systems to produce a range of physical and psychiatric affects that are quantitatively similar to the effects of other psychostimulant. The aim of this study will be to establish, a profile of behavioural alterations and also monitoring hormonal changes resulting from short- and long-term exposure to cathinone in presence and/or absence of cage enrichment over a period of 6 months. A total of 15 adult vervet monkeys (eight males and four females) will be used. They will be randomly divided into three groups. The study will be carried out in two phases: Pre-treatment period will take about 2 months. However, the first month will serve to habituate the animals to the presence of the observer as well as handling while during the second month of pre-treatment phase, blood sampling together with individual behavioral observations will be done. Treatment phase- Control animals will be administered 10 ml normal saline (0.9 % sodium chloride) via oral gavages three times a week (Mondays, Wednesdays and Fridays) for 4 months. Expected results: The behavior during the pre-treatment period will be different compared to the treatment period. The animals are expected to be more aggressive and also show withdrawal symptoms. The hormone levels of cortisol will show different levels in the two phases. The study will show that cathinone usually alters hormonal levels depending on the dosage and this results to alterations in the behavior of the khat chewers. In extent it can be linked to some psychotic behavior.

CHAPTER 1

1.0 INTODUCTION

khat comprises the leaves and fresh shoots of *Catha edulis* Forsk, a flowering evergreen shrub cultivated in East Africa and the Arabian Peninsula. In yemen khat chewing is a traditional around these places. Khat usage in Australia has increased significantly as more people from the horn of Africa have continued to settle here majorly Somali people. Khat is chewed because of its stimulant properties and by some it has been categorized as addictive. There has been intensive discussion across the board involving both the professionals and non professionals concerning khat. This has lead to khat being termed as illegal in some countries while other countries refer to khat as one of the cash crop that bring a lot of money to the economy.

In Kenya, *miraa* has semi-legal status and is classified as an addictive drug (NACADA, 2006). According to the Agricultural Act Cap 318 Laws of Kenya, *miraa* is recognized as both horticultural and a special crop under the subsection of "others". Beckerleg (2006), Kalix and Khan (1984) state that *miraa* has adverse socioeconomic and health hazards. This is also supported by Sikiru and Babu (2009) who estimated that about one-third of all wages were spent on *miraa* consumption at the expense of vital needs, indicating dependence. Further, Kalix and Khan (1984) add that family life is harmed because of neglect, dissipation of the family income and inappropriate behavior which in many cases leads to divorce. Current use of miraa in Kenya is 3.9%; it also varies by region of residence and gender. Like tobacco products, use of miraa is largely a male dominated affair. In North Eastern region, 35.8% of the male respondents reported using miraa. This was closely followed by Coast at 12.8%. Miraa usage is marginal in Nyanza and Western Kenya. In Eastern region where the bulk of the miraa comes from, only 9.4% reported being current users of the drug. It is also interesting to note that North Eastern region (7.6%) has the highest proportion of female users of miraa.

The principal active components in khat are cathinone and cathine which are alkaloids that are structurally related to amphetamine. Chewing khat releases these substances into the saliva; they are rapidly absorbed and eliminated. The pharmacological effects of cathinone are qualitatively similar to those of amphetamine, although it is less potent. These compounds act on

dopaminergic , narodrenergic and serotonergic neurotransmitter systems to produce a range of physical and psychiatric affects that are quantitatively similar to the effects of other psychostimulants. These compounds are usually absorbed through the oral mucosa and the small intestine during chewing.

Through the years there has been a conflicting evidence regarding the extent to which cathine and cathinone pose some health problems like anxiety, depression, diabetes and also cardiovascular disease as opposed to social health determinants such as the social isolation, poverty, poor nutrition and high rates of smoking.

The components found in Catha edulis include

Alkaloids

• Phenyl-alkylamines

– Norpseudoephedrine

– Cathinone

– Cathine

– Merucathinone

– Peudomerucathinone

– Merucathine

• Cathedulins

– More than sixty different cathedulins

Terpenoids

Flavonoids

Sterols

Glycosides

Tannins

Amino acids

Minerals

CHAPTER 2

2.0 LITERATURE REVIEW

In the year 1935 the advisory committee of the league of nations on the traffic in opium and other dangerous drugs were confronted with the problem of khat, and before it were two technical studies on the subject of khat. According to those reports there was no clear approval or disapproval wether the harmful effects of khat were very severe to warrant any international intervention. Though no further actions were taken by the international narcotics control authorities the cultivation, sale, and use of khat were prohibited in British Somaliland in 1921 and again in 1939(East Afr. med. J., 1945). The problem of khat did not end here but was taken up again by the UN commission on Narcotic Drug in 1956 after having considering the need for prior investigation concerning the harmful character of khat. The then commission on Narcotic Drugs decided to postpone further reviews of any measures to be taken until the World Health Organization had studied the pharmacological aspects of the habitual chewing of khat leaves. After the study kat plant was found to have be Catha edulis Forsk, which belongs to the family Celastraceae.

According to the study done Arch toxicol in 2014, khat and the synthetic cathinone were shown to have effect on the locomotive behavior of animals but at variable level of potencies and different time course of action. The acute and repeated oral administration of Catha edulis (200mg/kg) or cathinone (15 mg/kg) increasingly enhanced the locomotor activity and aggressive behavior in male Sprague–Dawley rats (Banjaw et al. 2006). In a single khat session, approximately 100–500 g of khat leaves is slowly chewed for several hours (Feyissa and Kelly 2008). Cathinone is the main active alkaloid present in the khat plant and was found to be present at around 78– 343 mg per 100 g of fresh leaves (Arunotayanun and Gibbons 2012; Klein et al. 2012; Sakitama et al. 1995). The psychostimulant effects elicited by khat appear after approximately half an hour of chewing and last for about 3 hours (Brenneisen et al. 1990; Kalix 1996). During this time, nearly 90 % of the alkaloids are efficiently released from the leaves. The absorption of these compounds occurs in two phases: the oral mucosa being the main route, with 60 % of cathinone being efficiently absorbed, and the second route takes place in the stomach and small intestine after the juice of khat has been swallowed (Arunotayanun and Gibbons 2012;

Feyissa and Kelly 2008; Toennes et al. 2003; Toennes and Kauert 2002). After the metabolism less than 7% of cathinone appears unchanged in the urine, being mainly eliminated in the form of its metabolites cathine and norephedrine (Brenneisen et al. 1986; Toennes and Kauert 2002).

Cathinone is the β-keto analog of amphetamine, while its metabolites (cathine and norephedrine) are structurally closely related to noradrenaline. There is a chemical similarities between cathinone and amphetamine.

In fact, cathinone shares with amphetamine both CNS stimulant and sympathomimetic effects. Early studies on the pharmacological activity of the khat leaves showed that cathinone, cathine, and norephedrine are capable of inducing an amphetamine-like CNS dopamine release, with cathinone being the most potent of the three alkaloids (Kalix 1983; Kalix and Braenden 1985).

Khat chewing is characterized by a rapid onset of psychostimulant effects. Users often describe increased energy and excitement, and euphoric sensations, which historically resulted in their use to treat the symptoms of melancholia and depression. Users also experience improved sense of mental alertness, high self-esteem, and enhanced ability to focus, associate ideas, and communicate, which greatly contributes to the social character of this tradition (Alem et al. 1999; Cox and Rampes 2003; Dhaifalah and Santavy 2004). Unpleasant physical or psychological effects emerge right after users stop chewing the leaves, but symptoms like restlessness, anxiety, and hypnagogic hallucinations may be experienced also during the process of chewing (Balint et al. 2009; Cox and Rampes 2003; Granek et al. 1988).

A study done in ethiopia byfaculty of medicine in addis ababa university entitled khat a contrevesial plant showed that there were a majority of toxicity associated with khat. The toxicity was brought about by the active components mainly cathine and cathinone which are almost 10 times more potent than amphetamine. They noted that an increased incidemce of cardiovascular complications occurred during or after khat session. The authors concluded that khat chewing is an independent dose-related risk factor for the development of acute myocardial infarction with a very significantly increased risk. Khat chewing has also been reported to be a significant threat for acute cerebral infarction. The dorminance/prevalence of high blood pressure was also significantly high among khat chewers than among the non chewers. Another significant cardiovascular complication that was noted among the khat chewers was the higher incidence of hemorrhoids found in chronic chewers.

Pronounced hyperthermia has been observed in rabbits treated with (–)-cathinone .This response was blocked by haloperidol and strongly inhibited by pimozide, two well known antagonists of amphetamine hyperthermia. These results are in complete accordance with our previous results. Khat chewing also affects the oral cavity and certain parts of the digestive system. Periodontal disease and gastritis have been seen but other studies have indicated no such detrimental effects of khat . No significant association could be found between khat chewing and oral leukoplakia in a study which was done here in kenya. The tannins present in the leaves are held responsible for the gastritis that has been observed in khat chewers. According to some studies done chewing khat has been found to reduce the absorption of ampicillin and to a lesser extent that of amoxicillin but the effects are minimal 2 hours after khat chewing stops . According to Raja'a et al, khat chewing appears to be a risk factor for duodenal ulcer. In some studies though not clear khat chewing has been associated with a reproduction problem called spermatorrhea. It has also been reported that about 50% of khat users usually develop oral mucosa keratosis.this pathological change is considered a pre-cancerous lesion that may develop into a more complicated oral cancer. The prevalence of this lesion and its severity increseases with the frequency and duration of khat usage.

Among many other health risks that has been associated with khat, there has also been correlation of dependence syndrome. Several authors have argued that regular khat consumption (seriously) affects the social and economic life of the user or kaht chewers. The medical problems that arise from khat chewing are somehow due to the sympathomimetic effects of the drug and partly to its effect on mental health. According to Kalix, khat chewing may induce a moderate but often persistent psychological dependence. Withdrawal symptoms after prolonged use are mild and may consist of lethargy, mild depression, slight trembling and recurrent bad dreams. There are very few reports on khat dependence, and habitual users do not show serious problems when stopping use . Tolerance is difficult to evaluate because chewing sets an upper limit to the amount of khat that can be consumed. It seems that a certain degree of tolerance is developing to the increases in blood pressure, heart rate, respiratory rate and body temperature. A real khat withdrawal syndrome has not yet been described, although interrupting prolonged use may result to mild depression, anergia and sleep disturbance.

2.1 JUSIFICATION

The long-term health impact of chewing khat is not well established. Scattered reports from various researches and also different countries like Yemen and elsewhere have indicated that chronic khat consumption may be a leading cause of cancer, cellular toxicity and other metabolic disorders. The current understanding of many khat-related topics is still poor, and thus further research is urgently needed. In the world there are different views regarding the legality of growing and also consumption of khat. Some countries have burned the consumption of khat terming it as disastrous to the health while other countries especially in east Africa still regard it as cash crop and also as a source of economic backbone in some counties in Kenya. Due to this misunderstanding and also scanty knowledge among the public there is a need to come up with a conclusive research piece that will once and for all rule out on both the negative and positive effects of khat if any so that proper decisions based on knowledge and not fallacies can be made. So far the researches which have been conducted majority have linked a number of prevalent health conditions with khat consumption among them being cancer which is currently on of the threats in health sector.

2.2 OBJECTIVE

The aim of this study will be to establish, a profile of behavioral alterations and also monitoring hormonal changes resulting from short- and long-term exposure to cathinone in presence and/or absence of cage enrichment over a period of 5 months. However, the first 1 month will be pre-treatment period, where animals will be observe observed in order to establish baseline levels on behaviour patterns under investigation. Cathinone will then be administered on alternate days. The administration of cathinone will take into consideration the bioavailability and plasma half-life of cathinone (Toennes and Kauert 2002). This regimen also took into account the likely development of tolerance to cathinone by the animals(monkeys).

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CHAPTER 3

3.0 METHODOLOGY

3.1 EXTRACTION OF KHAT ALKALOIDS

Khat alkaloids will be separated from the Catha edulis leaves by acid/base extraction solvents. Then the different alkaloids solvents will be separated from each other by , liquid chromatography, gas chromatography/ thin layer chromatography or HPLC and identified with GC-IRD or GC-MSD.

Approximately 200 g of fresh khat shoots from the Meru district of Kenyawere will be chopped into small pieces and dissolved in 50 ml methanol. The mixture is then sonicated at room temperature (RT) while shielding from light for 15 min, and then filtered through an 11-lm filter (grade 1, Whatman, Kent, UK). The remaining non-filtered plant residue will be re-extracted in 50 ml of fresh methanol and sonicated for 24 h. The mixture will be filtered and admixed with the initial 50 ml of filtrate. The resultant solution is then concentrated at 337 millibar in a Rotor vapor vacuum drier (Bu¨chi, Switzerland) for 4–5 h into an oily paste. The 200 g fresh plant material is expected to yield about 12.6 g of this oily paste.

The oily paste is dissolved in 40 ml of DMSO (0.315 g/ml). Aliquots (each of 200 ll) will be stored at _80_C. The quality of the extraction procedure will be verified by confirmation of the presence of khat-specific phenylpropylamines (cathinone, cathine, norephedrine) in the alkaloid fraction using differential thin layer chromatography.

Test for the comfirmation of the active compounds in the extracted materials

Color test

After the extraction and purification the following colors will develop as a confirmation test.

REAGENT	COLOUR PRODUCED
Cathine	
Marquis	No color development
Sodium nitroprusside	Rose
Cathinone	
Marquis	No color development
Sodium nitroprusside	Rose

THIN LAYER CHROMATOGRAPHY

Procedure :

. Apply for TLC according to the following condition.

Condition :

- Stationary phase : Silica gel 60 F_{254} (5X10)
- Mobile phase : ethylacetate : MeOH : ammonia (17 : 2 : 1)

or $CHCl_3$: MeOH (9 : 1)

- Detection :

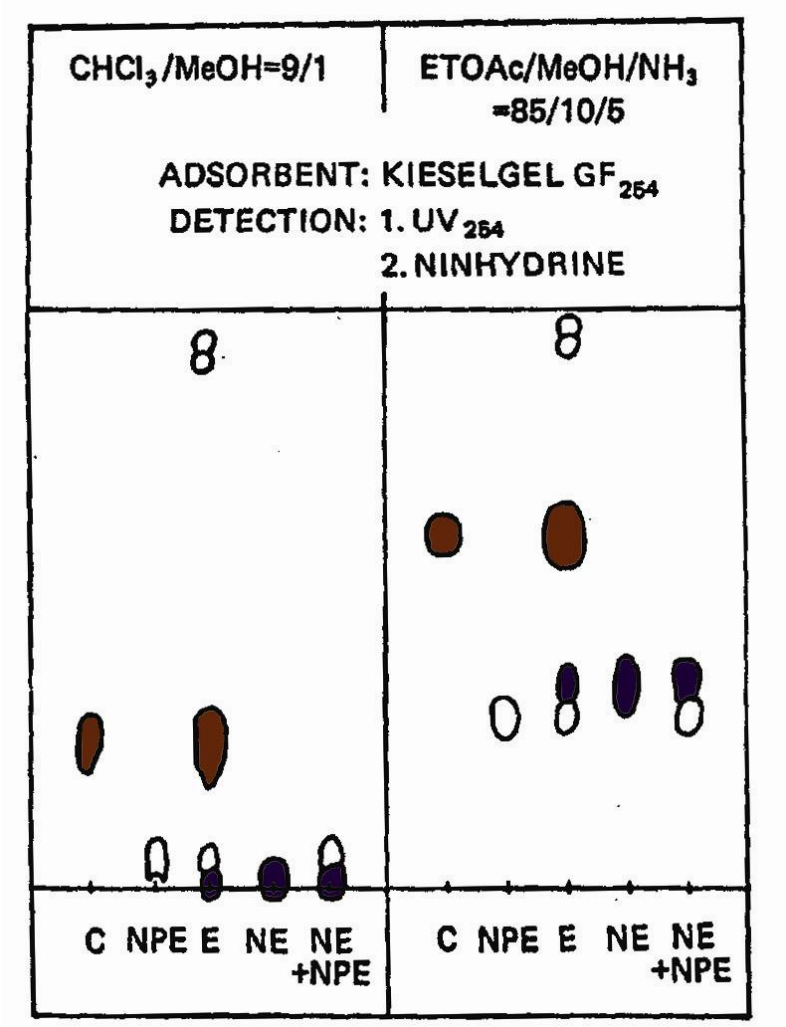
1. by UV at 254 nm
2. spray with Ninhydrine (0.1%) reagent then heat at 110^{o}C

- References : Cathinone and cathine in MeOH .
- Result : Cathinone has high R_f value with brownish color while cathine gives violet color at low R_f value.

visualization

uv light Ninhydrin reagent

COMPOUND	RELATIVE Rf and COLOR	
	UV light	Ninhydrin spray
Cathine	0.26, brown	0.26, purple
Cathinone	0.48, brown	0.48, orange
Ephedrine	0.17, brown	0.17, purple
Phenylpropanolamine	0.26, red	0.26, orange



GAS CHROMATOGRAPHY

Method General Screen1 Instrument:	Gas chromatograph operated in split mode with FID
Column:	5% diphenyl/95% dimethylsiloxane 15 m x 0.25 mm x 0.25 μm film thickness
Carrier gas:	Helium at 1.6 mL/min
Temperatures:	Injector: 275°C Detector: 280°C Oven program: 1) 100°C initial temperature for 2.0 min 2) Ramp to 300°C at 15 degrees/min

Samples were extracted with 1N NaOH into chloroform and filtered.

COMPOUND	**RRT**
amphetamine	0.694
methamphetamine	0.865
cathinone	1.000
cathine	1.003
1S,2R-(d)-phenylpropanolamine	1.340
1S,2R-(d)-ephedrine	1.456
1S,2S-(d)-pseudoephedrine	1.470

3.2 EXPERIMENTAL DESIGN

A total of 15 adult vervet monkeys (eight males and four females) will be used. They will be randomly divided into three groups. One group will serve as control while the remaining two groups comprised of five monkeys each will be the test.
The study will be carried out in two phases:

Pre-treatment phase

Pre-treatment period will take about 2 months. However, the first month will serve to habituate the animals to the presence of the observer as well as handling while during the second month of pre-treatment phase, blood sampling
together with individual behavioral observations will be done. Nevertheless caution will be taken to avoid stress to the animals. The will be placed in plastic caged during the time of observation. During this period the monkeys will be observed three days a week: Monday, Wednesday and Friday.
The aim of behavioral observations during pre-treatment phase will be to establish baseline values of specific behaviors of interest among the monkeys. It will serve to rule out common behavior from that which is as a result of the test or any compound that will be administered to them. The femoral vein of each animal will be cannulated using a 22 G blood vessel cannula after shaving and swabbing the area with 70 % alcohol. Heparinised saline will be introduced into the cannula to prevent blood clotting inside the lumen and then will be flushed out during each time of blood sample collection. The adhesive tape will be used to anchor the cannula in situ to prevent it from dropping when the blood collection is done.

Treatment phase
Control animals will be administered 10 ml normal saline (0.9 % sodium chloride) via oral gavage three times a week (Mondays, Wednesdays and Fridays) for 4 months. Test animals, grouped into two groups will be treated with respective doses (0.8, 1.6, 3.2 and 6.4 mg/kg body weight) of cathinone via oral gavage three times a week for 4 months. The doses are chosen

based on previous studies in humans and rats which indicated optimum effect of cathinone on various body parameters to be within this dose range.

Behavior studies

All behaviors will be observed from 1000 h to 1200 h of each observation day.. A video camera (Type- Image video camera CAM) connected to a video camera recorder (VCP-C10, Toshiba PTE Ltd., Singapore) will be placed at a strategic position within the animal house and three different observers will have been habituated to the animals scored individual behavioral scores. Inter-rator reliability, which will be obtained by dividing the number of times behavior will be scored by the observers by the number of times each

of the observers observed. Behavioral observations will then be collected on all focal subjects using a one zero sampling technique (Martin and Bateson 1993) for 15 individual behavioral categories that will be condensed into 5 composite behavior scores: aggression, anxiety, abnormal behavior, withdrawal and appetitive behavior. Behavior will be scored if it occurred one or more times during a 3- min session. Three x-3 min sessions will be done for each of the behaviors (aggression, withdrawal, anxiety and abnormal behavior) per experimental day. For appetitive behavior, three x-8 min sessions will be done per day. The definitions for aggression and anxiety (Melega et al. 2008), appetitive and withdrawal (Rapkin et al. 1995) and abnormal behavior (Castner and Goldman-Rakic 1999) for individual behavioral

categories will be applied.

3.3 BIOCHEMICAL ANALYSIS

Blood sampling

Food will be restricted prior to use of anaesthetics. Blood samples will be collected from the femoral vein following anaesthesia with ketamine hydrochloride at 10 mg/kg body weight intramuscular. The femoral vein of each animal will be cannulated under pre-treatment phase above and, thereafter, 1.5 ml blood samples will be collected every 20 min for 2.5 h, 10 min following cathinone administration. The blood samples will be allowed to clot, centrifuged and serum stored at -20 °C until assayed. Sampling will be done at 1,000 h on Mondays and Wednesdays of every week.

Hormonal analysis

Hormonal assays for serum cortisol will also be done by use of enzyme immunoassay technique using the kits from Nova Tec Immunodiagnostica GMBH, Germany. The technique uses the principle of competition of hormone in sample with enzyme conjugated hormone for limited binding sites on the specific antibody. Validation of the assay method for use in the monkey will follow that of Eley et al. (1989).

Cortisol enzyme immunoassay

Twenty microliters of standards and samples will be dispensed into their respectivemicrotiter strip wells pre-coated with anticortisol IgG and 200 µl cortisol—HPR conjugate containing horse-radish peroxidise- labelled cortisol, added to each well except for substrate blank. The plate will then be incubated for 1 h at 37 °C and, thereafter, the contents of each well aspirated and washed twice with 300 µl distilled water. After washing, 100 µl of TMB substrate solution containing tetramethylbenzidine/hydrogen peroxide system will be added and incubation done for exactly 15 min at 22 °C. The reaction was stopped by addition of 100 µl of 0.15 M sulphuric acid solution into all wells. The absorbance of the specimen will be measured at 450 nm within 30 min after addition of stop solution.

CHAPTER 4

4.0 EXPECTED RESULTS

Behavioral observations

Behavior associated with aggression

The results will show a significant effect of cathinone on ‘yawn’, ‘bouncing off cage walls’ and ‘head jerk’ as indicators of aggression. The individual scores for ‘yawn’ will be expected to be more observable during absence of cage enrichment.Groups with cathinone treatment will show greater indicators of aggression than the control group. Vigorous shaking of cage walls will be moderated b cathinone groups

Behavioural symptoms of anxiety

Scores for pacing will be expected to change changed as days goes by. There will be greater scores of pacing among cathinone groups in comparison to control group. Among the group administered with cathinone the behavior associated with anxiety will be expected to vary depending on the dose administered. This symptoms will be showing after a number of days.

Abnormal behavior

The results will be expected to show a dose-dependent increase in stereotypical responses in cathinone subjects compared to controls. Although there is expected to be a general increase in ‘response independent of stimuli’ among all subjects over experimental period, these changes will tend to be pronounced with increase in cathinone dose. Individual observation for ‘fine motor stereotypy’ will be expected to show a significant increase across observation. Groups that will be administered with cathinone dose will be expected to shou abnormal behavior compared to the control group.

Withdrawal behavior

The duration of time spent by focal subjects in withdrawal to the corner of the cage will be expected to increase over observational period as the day goes by. Cathinone administered groups will be expected to show longer duration of withdrawals than the control group due to the effects of cathinone. During the pre-treatment there was no observable withdrawal among all the subjects and this is an indication of the effects of cathinone.

Hormone analysis results

Cortisol

The results will be expected to show dose-dependent decrease in cortisol levels. During the pre-treatment period the cortisol levels will be higher when compared tp the treatment phase. As cathinone dose increased cortisol levels will be expected to reduce.

4.1Discussion

Aggression

During pre-treatment phase neither presence nor absence of cage enrichment will influence behavioral patterns. However, following cathinone treatment behavioral changes become apparent with increasing dose levels and over time period. All individual behavioral categories defining aggression will increase in both presence and absence of cage enrichment. Earlier studies on khat extracts and cathinone have reported stereotyped behavior, self-administration and anorectic effects in animal species (Gordon et al. 1993) similar to that evoked by [S-(+)-amphetamine] (Goudie 1985). Furthermore, both khat extract and cathinone have been shown to enhance baseline aggressive behaviour in isolated rats (Banjaw et al. 2005). These behaviours are partly influenced by disorders in the hypothalamic dopaminergic system (Ishikawa et al. 2007) and partly due to dopaminergic and serotonergic activity in the mesolimbic system (Eisch and Harburg 2006). Further evidence has shown involvement of both khat extract and cathinone in depletion of serotonin and its metabolite 5- hydroxyindole-acetic acid in both anterior and posterior striatum (Banjaw et al. 2005). In the present study, the use of cathinone in vervet monkey model has been stimulated to appear as in human khat ‘addicts’. The results will show a dose-dependent increase in aggressive behaviour which is in agreement with earlier findings where chronicity of use or multiple acute high doses of cathinone exposure in humans showed a wide range of dose-dependent behavioural changes including aggression, hyperactivity, various forms of psychotic illness, anxiety, paranoia and social withdrawal (Berman et al. 2009).

Anxiety

The result of the study will show that anxiety scores for ‘pacing’, ‘scratch’ and ‘self-directed’ steadily increase in a dose-dependent manner in absence and presence of cage enrichment and over observational period. Cathinone subjects will exhibit more anxiety levels during cage enrichment. Stereotypical behaviours have been demonstrated in vervet monkeys following

maternal separation and these measures are reminiscent of anxiety symptoms in humans (Marais et al.2006) Studies on cathinone and amphetamine in animals have shown their sympathomimetic effects on dopaminergic (Pehek et al. 1990) and serotonergic (Kalix 1984) synapses as well as peripherally via noradrenergic storage sites (Kalix 1983). Centrally acting neurotransmitters such as dopamine and serotonin have been shown to have modulatory effects on specific behaviours (Spoont 1992). The receptors for both dopamine and serotonin appear overlapping in the limbic and cortical regions of the brain (Goldman-Rakic et al. 1990), and this explains the inhibitory effect of serotonin on facilitatory effects of dopamine. Noradrenaline has also been shown to influence specific behaviours when interacting with other biogenic amines in the brain of different animal models and in human case studies. For example, reduced levels of serotonin are found in patients with anxiety disorders (Charney et al. 1990) and reduction in both serotonin and noradrenaline is associated with major depression (Sulser 1989). Recent studies showed that anxiolytic mechanism may include some interaction of noradrenaline and serotonin, rather than increasing synaptic levels of serotonin (Marais et al. 2006). Studies on cathinone have demonstrated maintenance of drug-seeking behaviour in rats habituated to amphetamine and monkeys trained to lever-press for cocaine injection (Yanagita 1979). Similar studies in humans by use of amphetamine showed drug-related effects of insomnia, irritability, anxiety, sadness and nightmares (Efron et al. 1997).

Abnormal behaviour

Abnormal behavioural scores observed in the present study

showed a steady increase in cathinone-treated animals in a dose-dependent manner and over experimental period. These behaviours were more apparent following cage enrichment. A study by Zelger et al. (1980) reported stereotypical behavior and hyper-locomotion in rats treated with cathinone, and these paradigms characterize abnormal behaviour (Melega et al. 2008). The results of the present study on scores for ‘fine motor’ and ‘whole body stereotypy’ confirm these earlier findings in other animals and, probably, a similar mechanism of action of these drugs is involved in influencing these behaviours.

Withdrawal/isolation

The durational behaviours of withdrawal and appetite loss in cathinone–treated animals will show an increase in a dose dependent manner and over the experimental period. Previous studies in humans and experimental animals reported changes affiliative and anorectic behaviours

following khat use (Gordon et al. 1993). Studies have shown social withdrawal as a common side effect of [S-(+)-amphetamine] in children with attention deficiency disorder (Clinical Practice Guideline 2001). On the other hand, anorectic behaviour is characterized by development of tolerance (Zelger and Carlini 1980). It is possible, that while cage enrichment appears to influence the expected observed behaviours, the effect may be primarily due to cathinone exposure. Cathinone increases levels of dopamine, serotonin and noradrenaline in the brain via catecholaminergic synapses (Calcagnetti and Schechter 1993). Serotonin (5-HT) is a shortacting widespread neurotransimitter which acts on a number of receptor sub-types found at high density in the limbic system and raphe nuclei as well as in the hypothalamus (Blundell 1984). Agonists at the 5-HT2c receptor show the most consistent inhibition of food intake and the 5-HT2c-knockout mouse is hyperphagic and obese (Tecott et al. 1995). The expected effects of cathinone on appetitve/consummatory behaviour in the study point at the possible involvement of the hypothalamus. The arcuate nucleus is involved in central appetite regulation by use of Agouti-related protein (AgRP) (Neary et al. 2004).

4.2 Conclusion

The study will show that cathinone usually alters hormonal levels depending on the dosage and this results to alterations in the behavior of the khat chewers. In extent it can be linked to some psychotic behaviors.

CHAPETR 5

5.0 RESEARCH PROGRAM WORKPLAN

Pre treatment period:

Will take 2 months whereby the animals behaviors will be observed to establish a base line for the interpretation of the test. At first the site for the cages will be established and then the monkeys will be acquired.

Treatment period:

Will take a duration of 4 months. During this time cathinone will be administered to the test group.

CHAPTER 6

6.0 BUDGET

Items/ Nature of Expenditure	Quantity(number)	Cost per item	Total cost
Monkey	12	600,000	7,200,000
Cages	12	20,000	240,000
Food (vegetables, fruits, monkey chow) commercial multivitamins.			600,000
Beddings for the cages	12	5,000	60,000
⇨ Bench top Freezer ⇨ Incubator ⇨ Latex Gloves ⇨ Blood Lancets ⇨ Ethanol ⇨ Pasteur pipettes ⇨ B-D Vacutainers ⇨ Injection Needles and Syringes ⇨ Sonicator ⇨ Rotor vapour vacuum ⇨ Video camera ⇨ Camera recorder ⇨ Immunodiagnostic kits	1 1 60 60 60 30 10 boxes(100/box) 1 2 30 20 15 kits	80,000 150,000 500 500 500 1800 2000 100 200,000 150,000 20,000 20,000 50,000	80,000 150,00 30,000 30,000 108,000 3,000 200,000 300,000 600,000 400,000 750,000
Other Costs: ⇨ Transportation ⇨ Stationery ⇨ Miscellaneous	1 van for hire Bulk	3000 per month 12,000 10,000	18,000 72,000 60,000
Research Assistant	3	80,000	1,440,000

Physician Graduate students' Accounting Officer	1 5 1	80,000 50,000 60,000	480,000 1,500,000 360,000

CHAPTER 7

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CHAPTER 8

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