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EFFECT OF *CATHA EDULIS* (KHAT) ON BEHAVIOUR AND ITS POTENTIAL TO INDUCE SEIZURES IN SPRAGUE DAWLEY RATS

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ABSTRACT

Background: Khat is a plant whose young shoots and leaves are habitually used in Eastern Africa and the Arabian Peninsula as a drug of recreation. Although it is used without any control in these regions, it contains two controlled substances, cathinone (schedule I) which is present in fresh khat and cathine (schedule VI) which is a degradation product of cathinone abundant in old khat.

Objective: To determine the effect of khat on locomotor behaviour and seizures in rats.

Design: Experimental study.

Setting: University of Nairobi.

Subjects: Adult male rats in groups of six were given fresh khat, old khat, methylphenidate and saline at varying doses and observed over three hours.

Results: Fresh khat at low doses and old khat at high doses stimulated locomotor activity. High doses of fresh and old khat induced stereotype behaviour and seizures.

Conclusion: The results show that khat stimulates locomotor and stereotypic behavioural activity and can induce seizures; results similar to those observed with amphetamine analogs.

INTRODUCTION

Khat refers to the young leaves and twigs of an evergreen shrub (*Catha edulis*), which is widely consumed in the Arabian Peninsula and Eastern Africa (1). In 1975 it was estimated that between five and ten million people used khat (*Catha edulis*) daily (2) and that number is likely to have increased. The availability of air transport and better storage facilities has led to the spread of khat use to other parts of the world. Depending on the region, khat is known by different names: *miraa* (Kenya) and *qat* (Ethiopia). The effects of khat in humans are characterised by moderate central nervous system stimulation resulting in a state of euphoria, increased alertness, excitement, insomnia and anorexia, often accompanied by loquacity or even logorrhoea (3). High consumption can induce

hyperactivity that may lead to maniac behaviour or a state resembling paranoid-schizophrenia (1). The leaves contain psychoactive agents, cathinone and cathine (d-norpseudoephedrine) which are controlled substances under the United States Drug Enforcement Agency. The molecular structure of cathinone is similar to amphetamines and studies have suggested that it is the active ingredient responsible for the khat effect on the central nervous system (1,3-5). Fresh khat leaves contain approximately 0.9 mg of cathinone per gram, which over a period of hours to few days is reduced to cathine (2,5,6).

Cathinone has been shown to increase locomotor activity in mice and rats starting in the first 30 minutes after administration (7). Cathine has similar effect on locomotor activity in rats and mice but the effect starts after latency of 90 to 120 minutes (8).

Surprisingly, despite its widespread use, information on adverse effects of acute and chronic use of this amphetamine-like substance is sparse. In this paper we describe the effects of varying doses of fresh khat on locomotor behaviour and seizures in Sprague Dawley rats.

MATERIALS AND METHODS

Animals: A total of 102 adult male Sprague Dawley rats (200-500g) were obtained from the Department of Biochemistry, College of Health Sciences, University of Nairobi and housed in rat cages in the animal house, Department of Medical Physiology.

The cages were cleaned and bedding changed three times per week. The rats were kept on a twelve-hour light/dark cycle and fed on standard rodent pellets and water ad libitum. Each rat was habituated to the experimental set up by transferring it to the observation chamber for 30 minutes daily for five days before the start of the experiments.

Preparation of aqueous extract of khat: Fresh khat, less than 24 hours old, was purchased on the day of the experiment from one retailer who received daily supply from Meru, Kenya. A stock solution of fresh khat was prepared on the day of experiment by blending 300 g of fresh khat in 50 ml of normal saline to give a solution of khat extract equivalent to 6g/ml. The mixture was filtered using Whatman grade 1 filter paper (Whatman Plc. USA) and the decoction used in the experiments. Old khat was prepared by leaving fresh khat stock solution at room temperature for three days in order to allow for degradation of cathinone content.

Methylphenidate: Methylphenidate (Norvatis AG. Switzerland), an amphetamine analog and established central nervous system stimulant, was used for comparison. The tablets were ground into powder and dissolved in normal saline to make stock solution of 5 mg/ml.

Behavioural observation: The observation activity chambers were 30 x 30 x 30 cm plexiglass boxes with the floor marked out in grid of 10 x 10 cm². Rats were randomly divided into treatment groups using a predetermined schedule based on doses of either fresh khat or old khat solution (0.5, 1.5, 4.5, 13.5, 40.5 and 121.5 g/kg) or methylphenidate (4.4,

13.3, 26.7 and 40mg/kg) or normal saline. There were seventeen groups and each group had six rats. Each rat was used only once to avoid the possibility of developing tolerance, drug toxicity or both.

Two research assistants, who were blind to the experimental solutions, gave by gavage the drug to each rat using a feeding tube (FTP-18-75, Instech laboratories Inc. USA) in a total volume of 15 ml. Controls received equal volume of saline. This method of drug administration simulated the human mode of khat consumption. Each rat was then placed in the activity chamber and observed for five minutes at the end of each thirty minutes period for three hours as described by Bures *et al* (9). Four behaviour modalities were measured; locomotion, rearing, circling and grooming. Locomotion was scored by the number of successive squares entered with the front paws, rearing by the number of times the rat lifted itself on its hind limbs with both forelimbs off the ground, circling when the rat traversed all four quadrants of the chambers consecutively, and grooming when a rat placed its tongue on its paws or other body parts. The five minute observation period was divided into twenty seconds intervals and each behaviour was scored: 0 when no movement occurred, 1 when movement occurred for 5 seconds, 2 when movement occurred for 10 seconds, 3 when it occurred for 15 seconds, and 4 when it occurred for 20 seconds. Overall score of the five minutes observation period represented the average for twenty seconds intervals.

Studies of seizures: Racine described a scale for classifying seizures in experimental animals on the basis of alteration in motor activity (10). He divided seizures into five classes whereby in class 1 there was mouth and facial movement, in class 2 there was head nodding, in class 3 there was forelimb clonus with lordotic posture, in class 4 there was rearing with forelimb clonus and in class 5 there was rearing with falling clonus. In this study, rats which developed at least class 2 seizures in each treatment group were recorded and probit analysis (11) was used to determine the convulsive dose (CD) of khat and methylphenidate required to produce seizures in 50% (CD₅₀) and 97% (CD₉₇) of the rats.

Statistical analysis: ANOVA was used to determine significant differences between the groups followed by Bonferoni's correction for use of Student's t-test

to test for significance between individual groups. Probit analysis for quantal events as described by Finney (11) was used to determine CD_{50} and CD_{97} . Results are expressed as mean \pm s.e.m. Significance was $p < 0.05$.

RESULTS

Behavioural response: Fresh khat produced significant differences in behaviour between the different treatment groups in locomotion, rearing and circling and ANOVA analysis showed significant differences ($F_{(6,35)} = 2.38, 2.89$ and 3.326 , respectively). There was significant increase ($p < 0.05$) in locomotion in groups given 4.5 and 13.5 g/kg fresh khat extract and in rearing and circling with 13.5 g/kg fresh khat extract. Rats given 0.5 and 1.5 g/kg of khat retreated to one corner of the observation box and remained motionless often with eyes closed. Rats given 40.5 and 121.5 g/kg of khat either developed seizures or stereotyped behaviour which interfered with locomotor activity. Figures 1 and 2 show the doses of khat, which had significant effect on behaviour.

The effect of old khat on behaviour was similar to that of fresh khat but at higher doses starting

at 13.5 g/kg (Figures 1 and 2). ANOVA analysis showed that there were significant differences in locomotion, rearing and circling between the groups ($F_{(6,35)} = 9.782, 3.541$ and 4.123 , respectively). Compared to saline, locomotion was significantly increased in the groups of rats treated with 13.5 and 40.5 g/kg old khat extract. The dose of 121.5 g/kg induced either seizures or stereotype behaviour which interfered with locomotion. Rearing and circling were significantly increased at a dose of 40.5 g/kg only.

Methylphenidate administered at 4.4, 13.3 and 26.7 mg/kg increased locomotor activity significantly as compared to saline. The dose of 40 mg/kg produced a short duration of increase in locomotor activity followed by stereotyped behaviour (Figure 3).

Time course of the effect of khat on locomotion: The maximum increase in locomotion to fresh khat extract (4.5 and 13.5 g/kg) was observed within the first sixty minutes followed by gradual decrease in activity over the remaining two hours (Figure 4). Doses which were not significantly different from saline have been omitted for clarity.

Figure 1
Locomotion due to khat and methylphenidate

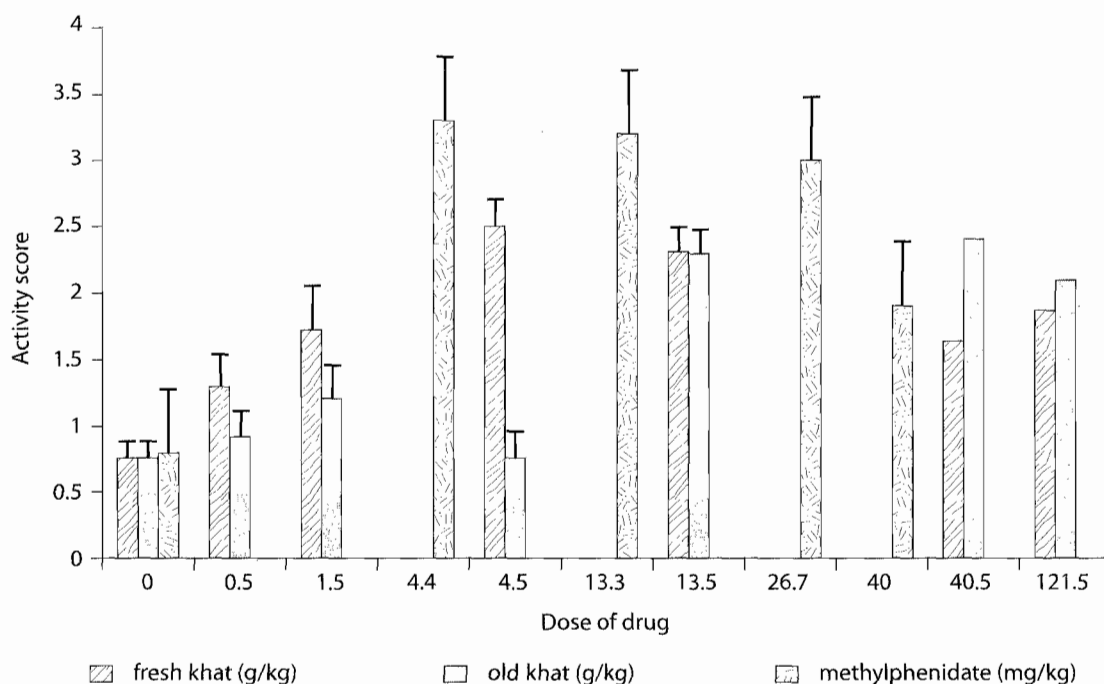


Figure 2
Effect of khat on rearing and circling

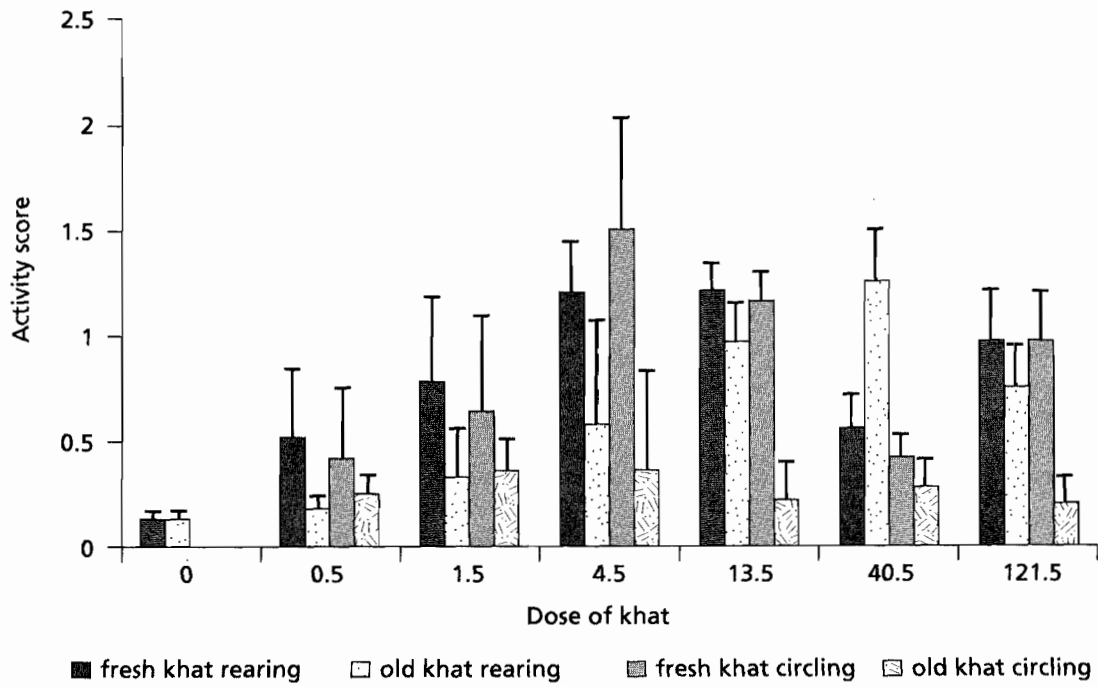


Figure 3
Percentage of animals that developed seizures when given khat or methylphenidate

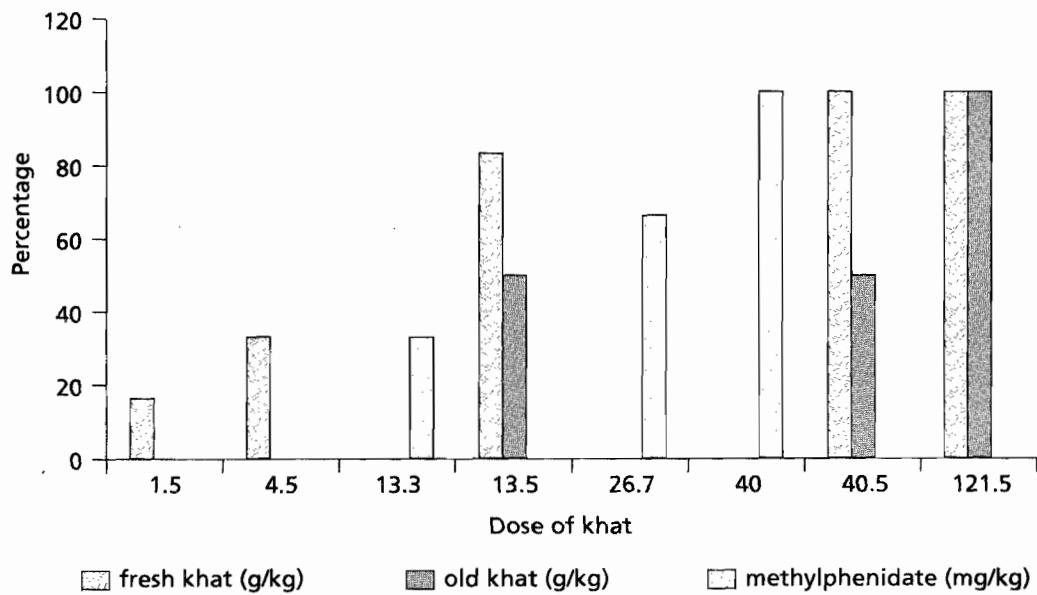


Figure 4
Time course of effect of fresh khat on locomotor activity in rats

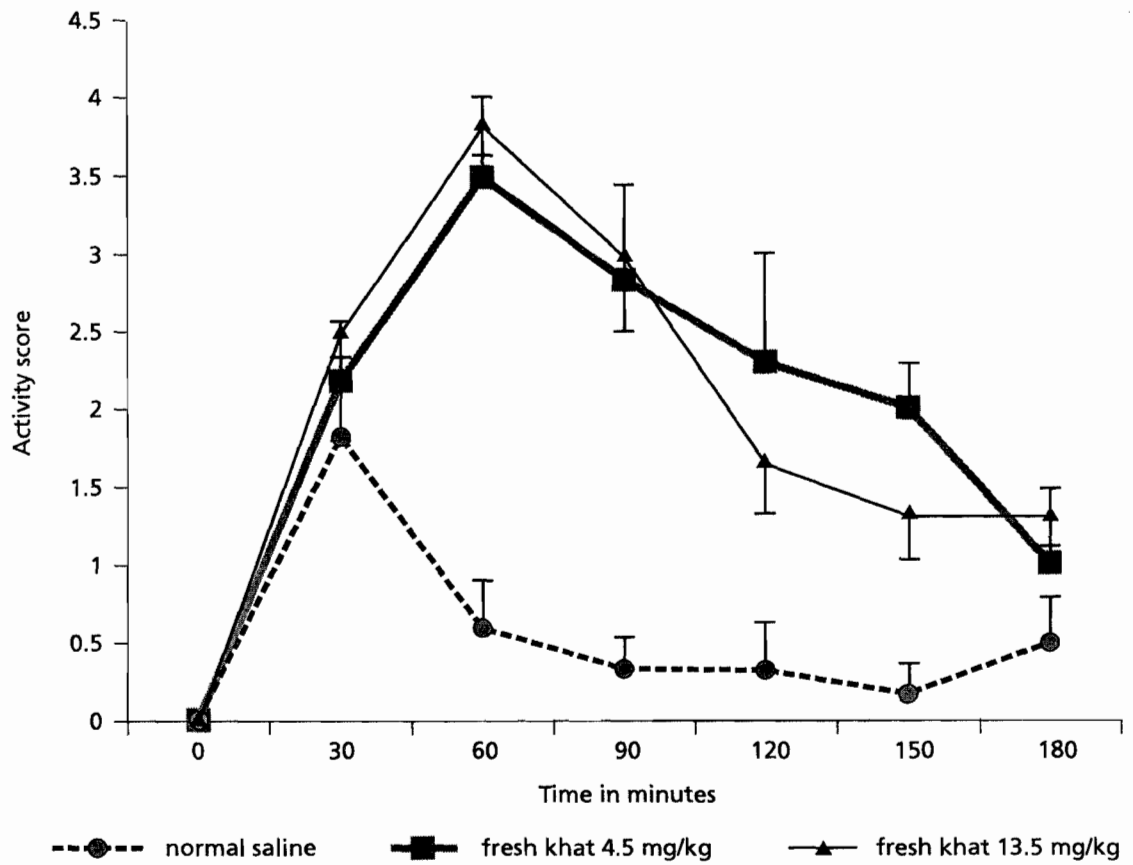
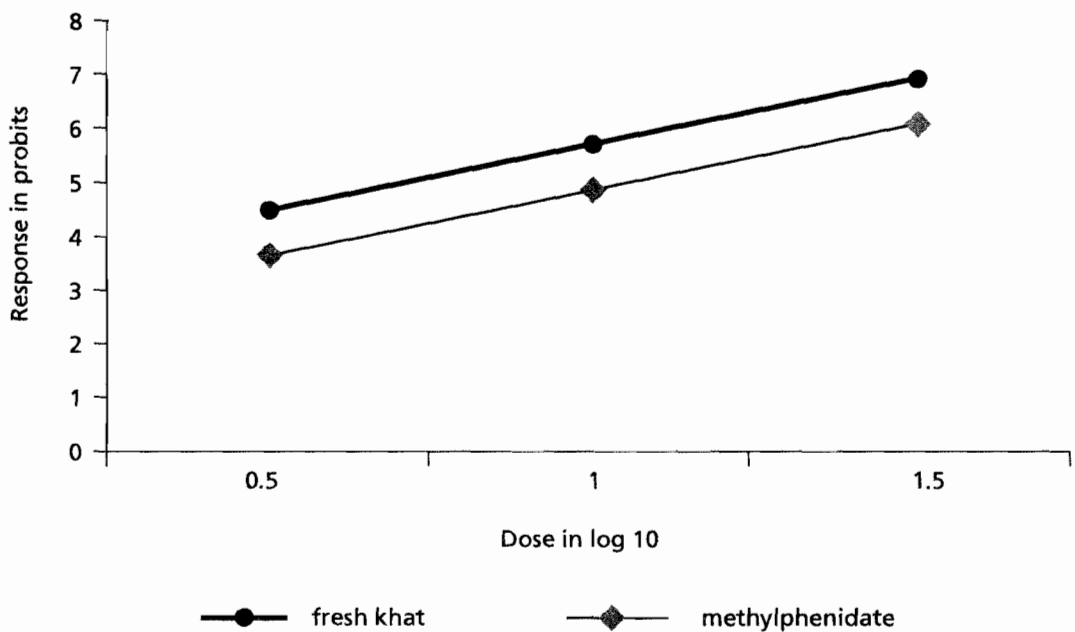
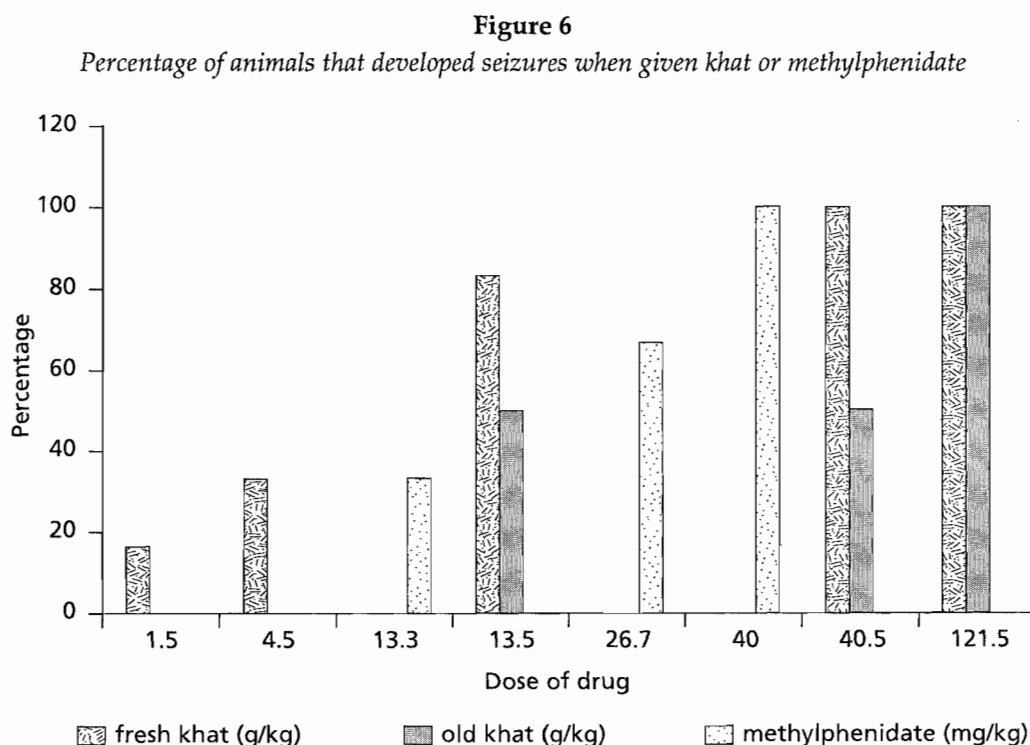


Figure 5
Probit analysis of seizures caused by fresh khat extract and methylphenidate





Studies of seizures: The number of animals that developed seizures was proportional to the dose of fresh khat and methylphenidate. Figure 5 shows the dose response curves for fresh khat extract and methylphenidate using equations developed using probit analysis. The equation for the graph of fresh khat was $Y = 5.13 + 2.4(x - 0.77)$ and for methylphenidate $Y = 5.57 + 2.4(x - 1.3)$. The graphs satisfied the test of linearity (statistical validity) with $X^2 = 1.52$. The CD_{50} dose for fresh khat extract was 5.01 g/kg and the CD_{97} was 31.6 g/kg. The CD_{97} for methylphenidate was found to be 56.3 mg/kg. The number of rats that developed seizures in each group was directly proportional to the amount of drug given (Figure 6). Although old khat induced seizures, the effective doses were higher than those for fresh khat.

DISCUSSION

Administration of cathinone to rats and mice has been shown to elevate locomotor activity several folds (12,13). In humans khat has been shown to cause motor stimulation within 3 hours of administration (14). Studies with cocaine and amphetamine analogs have shown increased locomotor activity and distinct features of seizures (15). In this study, 4.5–13.5 g/kg of fresh khat increased locomotor activity while old

khat had similar effect but at doses above 13.5 g/kg. The lack of appearance of behavioural changes with old khat at lower doses is probably due to the low levels of cathinone remaining in old khat (1,2,5). The increased locomotor activity observed at the higher doses of old khat extract may be due to cathinone remaining in the extract or due to other psychoactive constituents of khat like cathine and cathinoids.

High doses of fresh khat extract led to stereotyped behaviour in rats characterised by restricted locomotion, exploration, sniffing, licking and gnawing. Similar effects have been observed when rats and mice are treated with amphetamine and amphetamine analogs (16). The initiation of exploratory behaviour, which typically develops into stereotyped behaviour is commonly observed in all species of mammals given moderately high dose of amphetamines (7,16). The observation of similar behaviour in rats following administration of fresh khat extract supports the view that khat acts as a psychostimulant with effects similar to those of amphetamines.

Seizures induced by high doses of fresh khat extract were similar to those induced by other psychostimulants (15). Although khat induced seizures have not been reported in humans, desynchronisation of the electroencephalogram has been reported in aviation students who had

used khat which suggested that khat may increase neuronal excitability (17). The dose of fresh khat extract that induced seizures was higher than the amount that increased locomotor activity.

In conclusion, low to moderate doses of fresh khat extract increased locomotor activity in rats and at high doses induced seizure activity. An average user of khat consumes one to two bundles per day (18). Though the bundle weight varies from one place to another, it ranges between 200-400g. Therefore on the average, 600 g of khat are consumed daily which is about 8.5 g/kg.

This range of fresh khat extract increased locomotor activity in this study and produced seizures in some of the rats. Higher doses were required to produce seizures in all the rats in the groups and reflect the possibility of seizures developing in high dose "binge" session users.

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