Original article

Behavioural changes and development of tolerance to repeated administration of khat (*Catha edulis* Forsk) in mice

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Abstract: Khat use is showing an increase in many countries, including Ethiopia. Khat leaves are chewed for their euphoric effect, to guard off tiredness, and with the assumption that chewing khat facilitates learning. Although most research done so far employed cathinone, the major amphetamine-like CNS stimulant alkaloid contained in khat leaves, it is known that there are more than 20 pharmacologically active compounds in the leaves. Moreover, results from experiments where cathinone alone was given parenterally to experimental animals may not wholly reflect the behavioural toxicity observed after administering khat in a dosage similar to that used traditionally. This work was undertaken to study how repeated ingestion of khat affects memory processes and whether tolerance or sensitization develops in the process.

The oral adminstration of an aqueous suspension of khat leaves for one week to mice produced a significant impairment of memory between days two and five of repeated dosing in an active avoidance test ($p<0.001$; Studient's paired t=test). Results from the passive avoidance test showed a similar pattern of impairment of memory. Likewise, repeated administration of khat suspension produced a significant decrease in locomotor activity on days three and four of repeated dosing ($p<.001$ $p<0.01$, respectively). The decrease in locomotor activity was not accompanied by stereotyped behaviour, possibly indicating that, at the dose employed, khat leaves may be devoid of a pronounced 5-HT-releasing action. These findings, together with that from the rotarod test, suggest that acute tolerance, rather than sensitization, develops during sub-acute khat administration. The disruption in memory processes and performance caused by khat is short-lived and values returned to baseline levels within a week. It is inferred that people, such as students and drivers who have started to use khat, could display memory impairment, which in turn may adversely affect their performance. [*Ethiop. J. Health Dev.* 1998;12(3):253-260]

Introduction

Young khat leaves (*Catha edulis* Forsk) are chewed, brewed or macerated to extract the alkaloids contained in them that stimulate the central nervous system (CNS). The alkaloids to produce euphoria and excitement accompanied by loquacity and insomnia (1). The cultivation and consumption of khat is showing a tremendous increase in many East African countries, including Ethiopia.

Although the United Nations Narcotics Laboratory has isolated more than 20 different compounds from khat leaves, the most important stimulants are cathinone and cathine (2,3,4). Evidence in favour of the pharmacological similarity between cathinone and amphetamine is

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overwhelming (3, 5, 6, 7, 8).This pharmacological similarity brought the use of khat leaves in East Africa and the Arabian Peninsula under the scrutiny of the League of Nations in 1935, and of the United Nations in 1964 and 1971 (1, 2).

Literature review shows that most pharmacological studies of khatamines that have been carried

out to date have been made with cathinone and, to a lesser extent, with cathine. The majority of studies with cathinone were performed after a single administration to animals and, on the basis of these single-dose experiments, far-reaching conclusions have been drawn concerning the actions of khat. The pharmacology of khat and its compounds has been reviewed by the World Health Organisation and by Kalix and Braenden (2,4).

While cathinone might well be the most potent CNS stimulant of the plant kingdom (3) and it is reputed to exert a reinforcing effect more similar to that of cocaine than to amphetamine (8, 9), it is rarely, if ever, used by people on a regular basis for its stimulant effect.

People use whole khat leaves for many years regularly as opposed to single administration. It is for this reason that we investigated the behavioural effects of freshly prepared suspensions of whole khat leaves in mice. The aqueous suspension dosage form employed in the present work simulates the traditional way of khat use in most countries. In addition to its recreational and social use, khat chewing is widely practised by young people, mostly students, who feel that the extract from the leaves has a positive influence on the learning process. There is no report in the literature that justifies the claimed use. Therefore, this work includes experiments on the influence of khat on memory in mice, which might be extrapolated to humans.

Tolerance develops to the sympathomimetic effects of khat as demonstrated by the nonsignificant changes in blood pressure and heart rate in human subjects that chew khat frequently (10). However, there are limited and conflicting reports as to whether tolerance develops to its behavioural effects. In experiments with cathinone, Halbach (6) reported the absence of tolerance, while Schechter et al. (11) have found that cathinone produces tolerance that is similar to that caused by amphetamine. The question of tolerance vis-à-vis khat needs to be resolved because, if tolerance occurs to a CNS-stimulating agent like khat, it would necessitate increasing the dose with time and this could lead to increased toxicity and economic burden on the user.

A great deal of experimental evidence indicates that repeated administration of a stimulant drug sensitizes laboratory animals to the stimulant effect of the drug (12, 13). Moreover, this behavioural augmentation has also been observed in human subjects and suggestions have been made that such sensitization may play a role in stimulant-induced psychosis (14, 15). Considering the wide-spread nature of chronic khat ingestion in East Africa and the Arabian Peninsula, the study of the behavioural toxicity of khat assumes great importance. The objectives of this work were to investigate whether sub-acute ingestion of khat disrupts memory processes and whether tolerance or sensitisation develops during this period.

Methods

Animals : All tests were carried out on three months old male albino mice weighing 30 - 35 g. The mice were bred in our laboratory and belonged to the same colony. Animals were caged in groups of four and kept in a room with alternating 12-hour light and darkness. Food was withheld overnight before the experiments, but water was provided *ad libitum* .

Khat suspension: Fresh young khat leaves bought from the local market were used for all experiments. The leaves were finely chopped; 20 gm of the chopped material crushed in mortar and pestle, and tap water was added to give a final thick suspension of 50 ml. The suspension was strained through a wire gauze (mesh size of No. 72; Test Sieve BS 410, Gallenkamp). As there could be differences in potencies of khat bought on different days, all test animals were administered khat suspension of the same batch. Straining was necessary in order to separate leaf veins that could block the gavage as well as for the purpose of standardizing the particle size in different batches of khat suspension. The potency of khat bought on different days was confirmed retrospectively by the significant decrease in body weight of animals of the test groups.

Dosing: In all experiments, 1.5 ml of the suspension was administered by gavage to each mouse of the test group while control animals received an equal volume of tap water. Freshly prepared khat suspension was administered to the animals as much as possible; however, if the time between preparation and administration was more than one hour, the suspension was kept in a refrigerator to make sure that there would not be a change in potency. Every batch of suspension was utilized

within 24 hours. Animals were dosed at the same time each day and tests conducted two hours later. During preliminary screening work this dose was found to enhance exploratory behaviour of mice when introduced to a novel environment. From the third day of repeated dosing the animals' body mass decreased and their coat had an unhealthy look, similar to that of mice treated with carbontetrachloride.

Active avoidance test: Mice were conditioned to climb onto a pole suspended from the roof of a box (Cook's Pole Climbing Apparatus, Technoelectronics Lalbagh, Lucknow, India) by repeatedly pairing a conditioning stimulus (buzz) with a stimulus (electric shock). After daily training for 10 days, all animals climbed the pole upon hearing the buzz alone. The animals were then randomly divided into two groups (n=8) and a baseline test was carried out. Animals that took more than two seconds to climb onto the pole after the ringing of the bell were excluded from the study.

Khat suspension and tap water were administered daily for eight days to the test and control groups, respectively. At the end of the eight days, tests for time taken by each animal before it climbed onto the poll after hearing the buzz (latency of escape), performed alternatively from each group, were conducted in quadruplicate and the mean values recorded in each case. Animals that did not jump onto the pole within five seconds were considered response failures. The failure rate of animals is expressed as percentage of trials failed. Significance of difference between groups was analyzed using Student's two-tailed t-test; values of $P<0.05$ were considered significant.

Passive avoidance test: Passive avoidance tests have been used to investigate the effect of psychotropic drugs on memory processing (16). A shuttle box made of wood and glass that has two chambers (30 x 32 x 30 cm), with a sliding door in the middle connecting the two chambers, was used for this experiment. The walls and ceiling of one chamber were painted white and had a lamp screwed on the ceiling 25 cm from the floor, while the walls and the ceiling of the other chamber were painted black. Both chambers had sliding windows through which animals could be introduced into, or removed from, the chambers. Both chambers had grid floors through which electric shock could be delivered to the animal in either chamber.

Mice (n=6) were dosed with khat suspension or tap water for seven consecutive days and conditioning started 24 hours after the last dose. The animals were conditioned according to the method described by Norton (16).

The cumulative length of time spent on the safe side (lit chamber) as well as the number of entries into the unsafe or dark chamber (number of trials failed) during a session lasting four minutes were recorded for both groups 24 hours after conditioning. Tests were made four times for each animal and the means were recorded. Student's two-tailed t-test was employed for analysis of time spent on the safe side, while the number of trials failed was compared using the Mann-Whitney test.

Locomotor activity test: Repeated measurements of locomotor activity were made before dosing started to determine baseline values and mice with either very high or very low activity were excluded from the study. Mice selected with this screening were then randomized into the control and test groups. Locomotor activity was measured by placing each animal in a box with photocells on its sides that electronically registered the number of beam interceptions (Photo Acto Meter, Technoelectronics Lalbagh, Lucknow, India). A single measurement of locomotor activity lasting four minutes was made two hours after khat administration on each day of repeated dosing. Significance of differences between the test and control groups was measured using Student's twotailed t-test.

Test for development of tolerance: The ability of mice to remain on a rotating rod (rotarod performance test) was used as a measure for the development of tolerance. Time course changes in motor coordination on the rotarod have been used to assess the development of tolerance to drugs acting on the CNS (17). Prior to the test, the mice were screened for ability to stay on the rotarod

($\varnothing$ 28 mm, 10 rounds/min. for five minutes). Mice that could not stay on the rotarod for five minutes were excluded from the test. The animals were then randomly assigned to the two groups (n=6) and tests conducted two hours after khat administration each day for the duration of the experiment. The percentage of animals from each group staying on the rotarod for five minutes for the duration of

the experiment was analyzed using Student's two-tailed t-test; values of $P<0.05$ were considered significant.

Results

The ability of mice to remember a learned experience deteriorated between days two, five of repeated khat administration as evidenced by the significant increase in latency of escape (Fig. 1). Maximum disruption of the memory

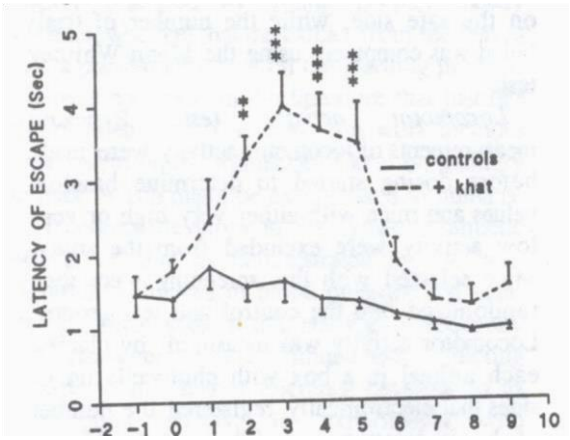

Figure 1: **increase in latency of escape of mice (n=8) in a one-way active avoidance test after repeated administration of 1.5 ml of an aqueous suspension of khat leaves. Day - 1 = baseline; Day 0 = 2 hrs after the first dose. Values are means ± SEM. *** p<0.001; ** p<0.05; student's t-test**

process occurred on days three to five of repeated dosing with khat ($p< 0.001$). Interference with memory was relatively short-lived; values returned to control levels on the 6th day of repeated dosing. In a similar paradigm, but where percentage of trials failed (inability to jump onto the pole within five seconds of the ringing of the bell) was measured, comparable results were obtained except for the slightly longer period of time when disruption persisted (Fig. 2). The experiment could not proceed beyond 10 days due to the deterioration of the animals' condition as indicated in "Methods".

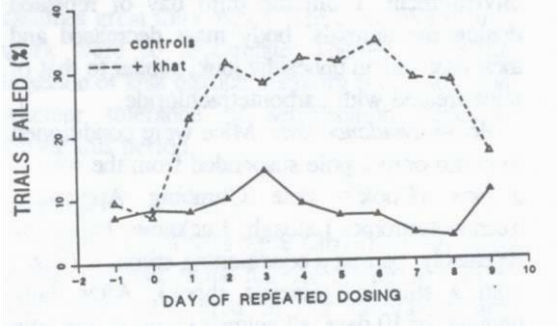

Figure 2: **Effect of repeated administration of an aqueous suspension of khat leaves on the ability of mice (n=8) to antcipate shock in a pole-climbing test. Each point represents the % value of trials that failed out of 24 to 32 trials. Day -1=baseline; Day 0=2 hrs after the first dosing. Failure rate of khat-treated animals was increased significanly compared to base;ome ($p<0.01$; student's t-test)**

Results from the passive avoidance test showed that treatment of mice with khat for one week had a disinhibiting effect on carrying out a task that had earlier been accompanied by punishment (Fig. 3). Although Fig. 3 does not show the dynamics of memory processing, it clearly demonstrates that sub-chronic ingestion of khat has a deleterious effect on memory.

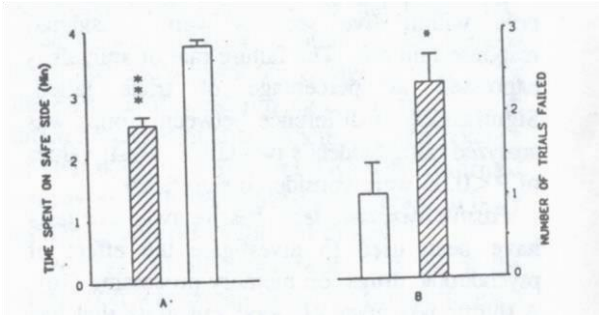

Figure 3: **Failure of khat-treated mice to withhold a preferred response (entering a dark chamber) despite punishment for doing so before the administration of khat. A - test animals demonstrated a significant decrease (*** $p<0.0001$; Student's t-test) in the length of time spent on the safe (lit) side of the box B - significant increase in the number of trials failed (entrance into the dark chamber) by khat-treated animals (*$p<0.05$; Mann-Whitney test). Animals (n-8) were dosed for 7 dayes.**
☐ khat-treated ☐ controls

Administration of khat for one week significantly reduced the locomotor activity of mice on the 3rd and 4th days of repeated dosing ($p<0.001$ and $p<0.01$, respectively). The results are depicted in Fig. 4. Likewise, repeated administration of khat to mice reduced their motor coordination by more than five times on the 2nd day of dosing (Fig. 5). Tolerance to the performance-disrupting action of khat suspension was manifested starting on the third day of repeated dosing. The steep nature of the segment of the graph for Days three to five is an indication of the development of acute tolerance.

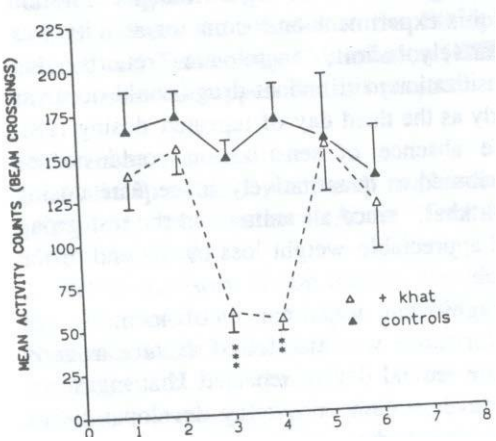

Figure 4: **Effect of repeated oral administration of an aqueous suspension of khat leaves on the locomotor activity of mice (n=6). Activity was measured during 4 min. periods two hours after dosing. Values are means ±SEM. Significant decreases in activity on days 3 (Student's t-test) and 4 (** p<0.001; test subjects, n=4). *** p<0.001;**

We have found the rotarod performance test to be the most sensitive of all tests in this work in screening the performance-disrupting effect of khat and a useful and simple technique to follow the dynamics of the development of tolerance.

Discussion

It has been shown that amphetamine is more potent in disrupting performance than cathinone (7). However, although no direct comparison was made with amphetamine, the results of this work demonstrated the significant dulling effect of repeated khat ingestion on the ability of mice to perform an acquired task. Data from both the active and passive avoidance tests demonstrate that repeated ingestion of khat dulls memory and disrupts performance in mice which have no prior exposure to khat. The work also demonstrated that interference with memory processes is transitory and baseline values are

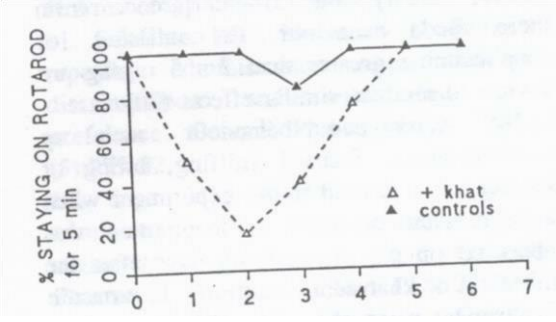

Figure 5: **Effect of repeated administration of an aqueous suspension of khat leavs on the rotarod performance of m ice (n=6). Baseline performance of both groups=100% khattolerance developed -treated animals showed a significant (p<0.05; Student's t-test) impairment in performance before**

re-established within one week. The transient nature of tolerance which develops after repeated dosing with amphetamine and cathinone has been demonstrated in experiments on the feeding and drinking behaviours of rats (26, 27). Our data from the active and passive avoidance tests (Figs. 1 and 2) show that a similar pattern of tolerance develops to the disruption of memory under the influence of khat. Whether the return to baseline performance level achieved by the end of one week will be maintained further, if khat ingestion continues for a longer period, needs further investigation.

Increased locomotor activity observed in rats following administration of amphetamine, cathinone, and cocaine is believed to be a consequence of dopamine (DA)-releasing property of these

psychostimulants in the brain, whereas agents that block release of DA attenuate the locomotor activity (18, 19). However, this relationship is not always linear and the locomotor activity observed in laboratory animals as a result of DA-releasing agents like amphetamine is progressively replaced by stereotyped behaviour due to increasing levels of serotonin (5-HT) as the dose of the monoamine releasing agent increases (20). For example, in an acute experiment, increasing the dose of cathinone from 15 mg/kg to 30 mg/kg decreased the locomotor activity of mice by more than 100%, mainly due to interference from stereotyped behaviour (21). Doses of amphetamine greater than 2 - 5 mg/kg are known to produce similar effects (20).

No stereotyped behaviour such as verticalization, focused sniffing, biting or licking was observed in our experiment when a significant decrease in locomotion was observed on the 3rd and 4th days after the initiation of khat administration. In an acute experiment using the pure alkaloids of khat leaves, Zelger et al (21) reported that low doses of cathinone (up to 1.25 mg/kg) not only failed to increase locomotor activity, but actually decreased it significantly.

As dopaminergic transmission in the mesolimbic and mesocortical pathways of the brain is thought to regulate stimulant-induced locomotor activity (22, 23), the decrease in locomotor activity observed on the 3rd and 4th days after the beginning of dosing with khat suspension (Fig 4) could have resulted from changes in neuronal functions in these dopaminergic pathways. However, these changes are short-lived and functional equilibrium is re-established by the 5th day of dosing. The absence of stereotypy in conjunction with the reduction in locomotor activity suggests that at the dose employed, khat leaves may not have a pronounced 5-HT releasing action. At the doses employed in this experiment, khat leaves interfered with memory processes in mice without increasing locomotion or inducing stereotypy.

The decrease in locomotor activity could have occurred as a result of decreased availability of monoamine neurotransmitters, particularly DA, or downregulation of receptors to which these monoamines bind. Such reasoning is in line with the findings that chronic stimulant administration may set in motion compensatory mechanisms like transient reduction in brain norepinephrine and DA concentrations (24) or downregulation of post-synaptic DA receptor sensitivity (13, 25). The development of acute tolerance to repeated administration of khat, and its transient nature, is also evident from rotarod results (Fig. 5).

There are reports that repeated administration of amphetamine or related stimulants causes behavioural sensitization (12, 13). We did not observe any manifestation of sensitization that could have been displayed by increased locomotor activity. Rather, we found a decrease in locomotor activity (Fig. 4). Although it could be argued that the duration of this experiment on locomotor activity was relatively short, there are reports that sensitization to stimulant drugs could occur as early as the third day of repeated dosing (28). The absence of sensitization could not be attributed to quantitatively inadequate dosing with khat, since all animals in the test group had appreciable weight loss by the end of the week.

Significant impairement of memory and performance was manifested in mice as early as the second day of repeated khat ingestion. However, tolerance quickly developes to the performancedisrupting and memory-dulling effects of khat. The results of this work showed that acute tolerance, rather than sensitisation, develops to repeated administration of khat to mice lasting from seven to 10 days. It is not clear whether sensitization occurs after a much more prolonged use of whole khat leaves, and this issue needs to be resolved through future work. We have also found that interference with memory and performance was brought about by a dose of khat suspension that did not increase locomotor activity or induce stereotypy.

In many respects, it is difficult to extrapolate results from laboratory animals to humans, and it is more so when the results are about animal behaviour. However, the effects of cathinone in experimental animals have been shown to be similar to the effects of khat chewing in humans (1). Thus , failure of mice to “remember” the consequence of performing an act which had earlier been accompanied by punishment (electric shock) in this experiment could be equated with unrestrained behaviour in humans. If this assumption is valid, novices who have just embarked on the use of khat may fail to appreciate the consequences of performing an act which they know will be accompanied

by punishment. Users of khat, especially students and machine operators, including drivers, could show memory impairment that adversely affects performance if they had no previous experience with khat.

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