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Khat (*Catha edulis*) and ethanol co-dependence modulate seizure expression in a pentylenetetrazol seizure model

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ABSTRACT

Ethnopharmacological relevance: Khat is a plant with psychostimulant properties whose parts, mainly leaves and twigs, are chewed for its euphoriant effects. Khat use and ethanol abuse are increasingly becoming global health concerns especially among many disadvantaged social groups. The present studies were undertaken to investigate seizure susceptibility and responses following chronic co-administration of khat and ethanol.

Materials and methods: Juvenile male Sprague-Dawley (SD) rats were administered khat, ethanol or combined khat and ethanol twice daily for 28 days by gavage. Khat-treated animals received 2 g/kg or 4 g/kg body weight continuously for the study period. Ethanol-treated animals received 20% ethanol at a dose of 4 g/kg body weight. The convulsant, pentylenetetrazol (PTZ), was administered intraperitoneally at a dose of 40 mg/kg body weight. Seizure responses were recorded.

Results: Twice-daily dosing of khat did not produce inhibition of weight gain. Khat alone, combined khat and 20% ethanol, but not 20% ethanol alone, significantly reduced the latency to seize. Khat treatment at 2 g/kg but not 4 g/kg body weight also significantly increased the duration of seizures. Conversely, combined khat and ethanol reduced the duration of seizures compared to controls. Ethanol alone reduced the seizure severity while khat alone, and combined khat and ethanol, produced close to the maximum seizure severity.

Conclusions: These data suggest that combined moderate to high dose khat and ethanol co-dependence produce a greater deleterious CNS effect than either drug alone. Future studies will address neurochemical effects, in addition to neuroadaptations, resulting from coabuse of these drugs.

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1. Introduction

Recreational drug use is a growing problem in Uganda and in much of the East African region, constituting a major public health concern. Khat is one of the most commonly abused drugs and its use rates vary considerably, from approximately 25% in Ethiopian youth aged 16–25 years (Ayana and Mekonen, 2004; also see Belew et al., 2000), to approximately 32% in one Ugandan survey of a similar age group (Ihunwo et al., 2004). Recent epidemiological data suggests that, in general, the use of psychoactive substances including khat is increasing in Africa and that, of the 22 countries with the highest increase in per capita alcohol consumption, 10 are in sub-Saharan Africa (Acuda et al., 2011).

A new consumption pattern of chewing khat and drinking alcohol, especially spirits, simultaneously, has recently emerged in Uganda (Anderson et al., 2007; Beckerleg, 2009, 2010) which is distinct from the traditional chewing of khat alone in countries like Ethiopia and Yemen (Gebissa, 2008). Beckerleg, a social anthropologist, has originated the term "mixers" to describe consumers who combine khat chewing with alcohol (Beckerleg, 2009, 2010). Anecdotal media reports and recent health surveys in Uganda have indicated an increasing trend of mixed khat and alcohol consumption by especially young people in Uganda (Uganda Bureau of Statistics, 2007; Beckerleg, 2009, 2010), and because khat itself is now considered to produce psychological and physical dependence on long-term use (for review see Manghi et al., 2009), this trend in drug consumption has important implications.

The khat plant, *Catha edulis*, contains several stimulant compounds including cathinone (the major stimulant), cathinine, cathidine, eduline, ephedrine, and others. Cathinone is found in greater quantities in fresh khat leaves than in the dried plant, and is considered to be the most powerful stimulant in khat (Brenneisen et al., 1990). Cathinone, like amphetamine, interacts with dopaminergic pathways to produce its psychostimulant effects (Pehek et al., 1990). Acutely, the psychostimulant khat has amphetamine-like actions when taken alone, manifested by central stimulation characterized by increased alertness, high blood pressure, excitement, euphoria, mania, aggressiveness, anxiety, garrulousness, and

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hypertension (Halbach, 1972; Brenneisen et al., 1990; Hassan et al., 2007). Chewing the bulk amount of khat leaves required to obtain the desired effects probably limits the intake of active principles, and so habitual khat consumers present with a mild dependence but do not have a typical abstinence syndrome on withdrawal (Nencini et al., 1986), although recent preclinical and clinical data have confirmed that khat has a dependence-potential (Manghi et al., 2009).

Alcohol (ethanol) is known to act at several brain targets to produce its characteristic depressant effects. Acutely, ethanol facilitates γ-aminobutyric acid-A ($GABA_A$) receptor function to enhance inhibitory neurotransmission. Simultaneously, ethanol acutely blocks the N-methyl-D-aspartate (NMDA) glutamate receptors, inhibiting excitatory neurotransmission (Krystal et al., 2003). Chronic ethanol use leads to dependence as a result of adaptation of the $GABA_A$ and NMDA receptors. This adaptation leads to a reduced responsiveness of $GABA_A$ receptors and a sensitization of NMDA receptors (Fritschy and Brunig, 2003). On abrupt termination of ethanol intake, disruption of the receptor adaptations leads to physical and psychological symptoms seen in ethanol withdrawal, including tachycardia, agitation, anxiety, and more severe symptoms such as ethanol-induced psychotic syndrome and generalized tonic-clonic seizures.

There have been no systematic studies of combined long-term khat and ethanol use, although there have been reports of poisoning from using methcathinone (a methyl derivative of cathinone) plus bromazepam and alcohol; in overdose, methcathinone dissolved in alcohol and taken with bromazepam caused coma associated with respiratory depression and hypotension in a young woman (Belhadj-Tahar et al., 2004). In previous studies we have found that ethanol dependence increased seizure susceptibility during ethanol withdrawal, and that coadministration of pregnane neuroactive steroids with GABAergic effects, for example pregnanolone, reduced the seizure risk (Alele and Devaud, 2007).

Because many individuals abuse khat and alcohol simultaneously (Ihunwo et al., 2004; Anderson et al., 2007; Beckerleg, 2009, 2010), the aim of this study was to identify and characterize the excitotoxic central nervous system effects of concomitant khat and ethanol using a reliable chemical convulsant. We hypothesized that chronic combined khat and ethanol dependence produces greater excitotoxic effects than either khat or ethanol alone. To determine the effect of concurrent chronic khat and ethanol administration on central nervous system excitotoxicity, we assessed seizure risk and responses by comparing these seizure parameters following khat administration alone with concurrent chronic khat and ethanol administration. We used an animal model that gives reliable and reproducible behavioural manifestations seen during ethanol dependence and withdrawal and that can therefore be generalized to codependence on both khat and ethanol (Devaud et al., 2003; Alele and Devaud, 2005, 2007).

2. Materials and methods

2.1. Animals

Juvenile male Sprague-Dawley rats approximately 60 days old were divided into 5 groups of 10 animals each and the following treatments administered per group: khat at 2 g/kg; khat at 4 kg/kg; 20% ethanol at 4 g/kg; combined khat (4 g/kg) and 20% ethanol; control animals received normal saline instead of khat or ethanol. All treatments were given by gavage twice daily for 28 days between 8 and 10 AM and repeated between 3 and 5 PM daily. This dosing paradigm for ethanol was shown to reliably induce ethanol dependence in a preliminary study. Similarly, khat produced self-administration, or positive reinforcement, indicating the development of dependence, in animal studies where cathinone was used acutely (for example, see Yanigata, 1986; Nencini and Ahmed, 1989). Drinking water and standard rat chow were provided *ad libitum*. Rats were weighed twice weekly to ensure appropriate dosing based on body weight changes. The research was approved by the Faculty of Medicine Research and Ethics Committee and the Institutional Review Board of Mbarara University. All procedures were conducted in accordance with approved Mbarara University Animal Welfare Protocols and National Institutes of Health (United States) guidelines for the humane care and use of animals.

2.2. Materials

Freshly harvested khat leaves and twigs were purchased daily on the day of harvest from dealers who sell khat to consumers. Identification of these khat parts was done by a botanist, and specimens kept for reference at the Botany department. Known weights of the freshly harvested khat leaves and twigs were macerated with distilled water using an electric blender. The juice obtained was then filtered repeatedly until all soluble components had been extracted. This juice contained all undegraded active components. Maceration was preferred in order to mimic the chewing of khat leaves and twigs by persons who habitually use khat. The khat extract was used immediately to avoid degradation of the cathinone component which is known to degrade quickly after harvest, but is psychoactive within 36 h (Schorno and Steinegger, 1979). Phytochemical screening was done by testing for tannins as follows: To 5 ml of the filtrate obtained, 3 drops of 5% ferric chloride were added. A strong dark-green or black precipitate confirmed the presence of tannins

2.3. Seizure latency, duration and severity

Following uninterrupted twice daily administrations of ethanol and khat for 28 days, test animals were administered a bolus dose of pentylenetetrazol intraperitoneally, at a dose of 40 mg/kg body weight. This dose of PTZ was based on preliminary studies that showed reliable inductions of seizures without a maximal effect on seizure severity (death) in most of the animals. The time to first sign of seizure (seizure latency), duration of seizure and the severity of seizure were recorded for each animal following the injection of the convulsant drug pentylenetetrazol. Seizure severity was graded using the scoring system modified from Marsh et al. (1999) as follows

Grade **0**: no signs of motor seizure activity during the 30 min observation period; Grade **1**: staring, mouth or facial movements; Grade **2**: head nodding or isolated twitches; Grade **3**: unilateral/bilateral forelimb clonus; Grade **4**: rearing; Grade **5**: loss of posture, jumping; Grade **6**: clonic/tonic seizures; Grade **7**: full tonic; Grade **8**: death.

Mortality following testing with PTZ was also recorded for up to 96 h post-administration of PTZ.

2.4. Data analysis

As an initial step in the data analysis numerical summaries were obtained and presented graphically (as means ± standard errors of the mean) providing an insight into the patterns and distribution of the data. Further statistical testing entailed using one-way analysis of variance (ANOVA) for parametric data, and Kruskal–Wallis test (non-parametric ANOVA) for data that did not meet the assumptions for one-way ANOVA (i.e. where normality and constant variances of the error terms were violated). The independent variable was treatment and comprised four levels compared to a control group [2 g/kg khat; 4 g/kg khat; ethanol only; and combined khat and ethanol]. The initial sample size of

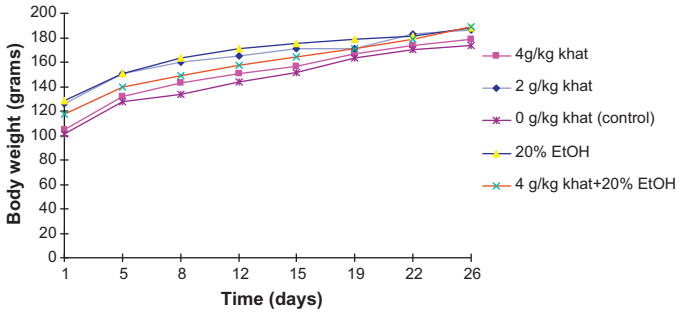


Fig. 1. Changes in body weight with time (days) of administration of khat alone, ethanol alone, or combined khat and ethanol. There was no significant difference among the treatment groups. All the treatment groups gained weight during the course of the treatments; $n = 8–10$ animals per group. Two animals in the 4 g/kg of khat group, and one animal in the control group died during the feeding period and were excluded from these data.

10 animals/group was chosen based on previous studies with PTZ that showed a high variability in some of the seizure responses measured (Alele and Devaud, 2007), and on our expectation that the psychostimulant khat would be proconvulsant (Oyungu et al., 2009). Outliers in the seizure responses studied were determined using Grubbs' method (extreme studentized deviate) and excluded to avoid minimizing the mean responses and the *F*-statistic; thus, smaller group sizes are reported than the initial sample size. A small number of animals died prior to completion of the khat and ethanol dosing. Statistical significance of means was taken for $p < 0.05$. Significant main effects were further analyzed using Bonferroni multiple comparison test to compare treatment groups and least significant difference (LSD) was used to assess statistical significance among treatment groups following the Kruskal–Wallis test. Statistical analysis was done using SAS version 9.13 software.

3. Results

The twice-daily dosing regimen of khat used did not produce inhibition of weight gain in the male SD rats (Fig. 1). Both doses of khat used in this study, 20% ethanol, and combined khat and 20% ethanol had no significant effect on weight gain in juvenile male rats, despite significant differences in mean consumption by the respective treatment groups studied (Fig. 2). Overall, the 2 g/kg khat-treated male rats consumed nearly 50% less khat than did the rats that received 4 g/kg body weight.

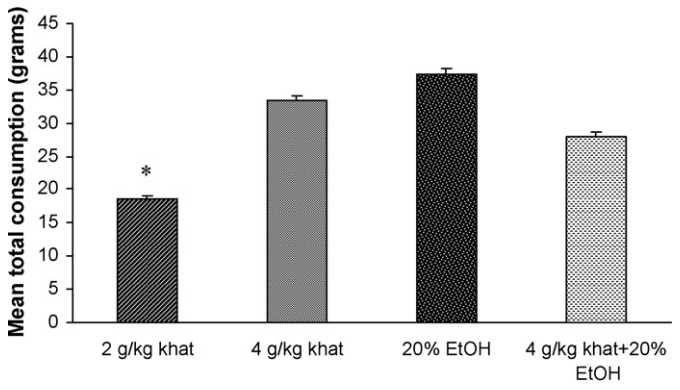


Fig. 2. Total consumption of khat alone, ethanol alone, or combined khat and ethanol, by male rats during 4 weeks of drug administration. Control animals received normal saline and are not represented. Consumption is represented in grams (g) as the mean ± SEM; $p < 0.05$, compared with the 4 g/kg khat and 20% ethanol treatment; $n = 8–10$ animals per treatment group. Two animals in the 4 g/kg of khat group, and one animal in the control group died during the feeding period and were excluded from these data.

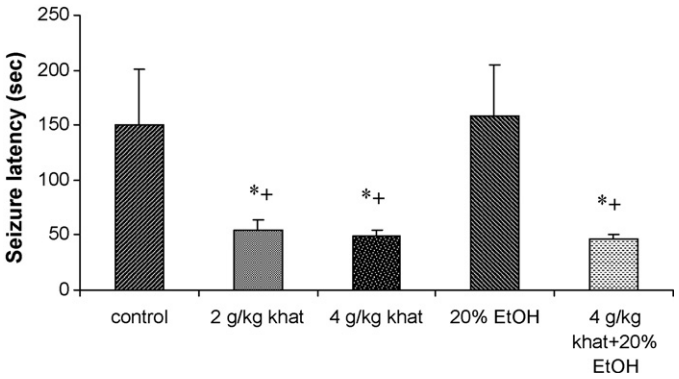


Fig. 3. Effects of khat alone, ethanol alone, or combined khat and ethanol, on seizure latency following chronic drug administration. Khat-treated rats at both 2 g/kg and 4 g/kg showed notably greater tendency to have seizures compared to controls. In rats administered combined khat (at 4 g/kg) plus ethanol, there was also a notably shortened time to start seizures compared to controls, and compared to ethanol-only treated rats. $^{*}p < 0.05$ compared with controls; $+p < 0.05$ compared with ethanol-only treated rats. Seizure latency is represented in seconds (s) as mean ± SEM; $n = 6–9$ rats per treatment condition. Two animals in the 4 g/kg khat group and 1 animal in the control group died during the feeding period and were excluded from these data. One control animal, 1 animal in the 4 g/kg khat group, and 2 animals in the 20% ethanol group did not show seizure activity. There were 2 statistical outliers in the control group, 1 in the 2 g/kg khat group, 2 in the 20% ethanol group, and 2 in the khat/ethanol group.

There was a significant difference in the effect of treatment (Kruskal–Wallis $H = 11.51$; Critical χ^2, 4 df, for α of 0.05 = 9.49, $p = 0.0214$) on seizure latency. As shown in Fig. 3, khat at both 2 g/kg and 4 g/kg, and combined khat and 20% ethanol, but not 20% ethanol alone, significantly reduced the latency to seize compared to controls ($p < 0.05$). There was also a significant difference between khat treatment only and combined khat and ethanol treatment versus ethanol treatment alone ($p < 0.05$).

We also found a significant difference in the effect of treatment (Kruskal–Wallis *H* statistic = 17.410; Critical χ^2, 4 df, for α of 0.05 = 9.49, $p = 0.0016$) on seizure duration. At 2 g/kg, but not 4 g/kg body weight, khat treatment significantly increased the seizure duration compared to controls ($p < 0.01$), Fig. 4. Combined khat and ethanol treatment reduced the seizure duration significantly compared to khat at 2 g/kg, and compared to 20% ethanol alone

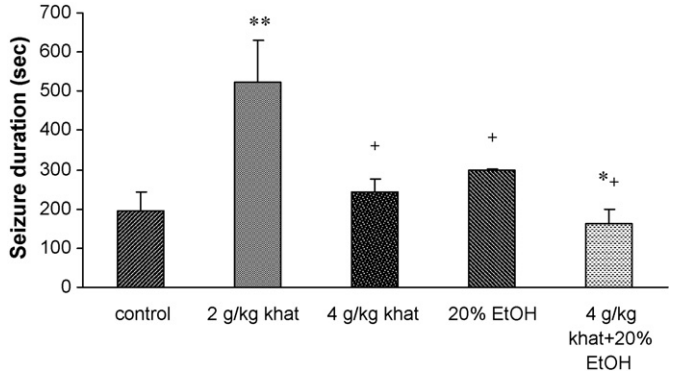


Fig. 4. Effects of khat alone, ethanol alone, and combined khat and ethanol, on seizure duration following chronic administration. Khat-treated rats showed a notably increased duration of seizure activity at 2 g/kg of khat compared to controls and other treatment groups. $^{*}p < 0.05$, compared with ethanol treatment; $^{**}p < 0.05$ compared with control; $+p < 0.05$, compared with the 2 g/kg khat treatment. Seizure duration is represented in seconds (s) as mean ± SEM; $n = 6–9$ rats per treatment condition. Two animals in the 4 g/kg khat group and 1 animal in the control group died during the feeding period and were excluded from these data. One control animal, 1 animal in the 4 g/kg khat group, and 2 animals in the 20% ethanol group did not show seizure activity. There were 2 statistical outliers in the control group, 1 in the 2 g/kg khat group, 2 in the 20% ethanol group, and 2 in the khat/ethanol group.

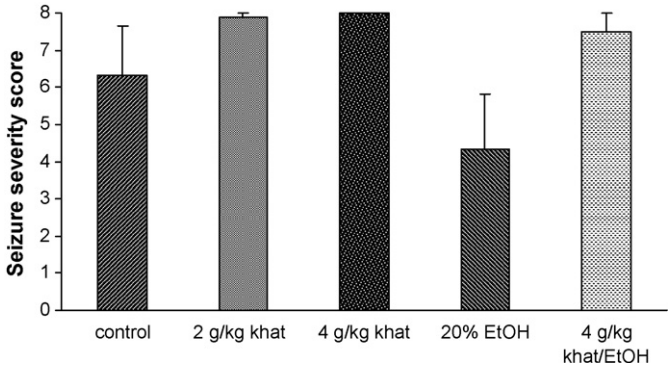


Fig. 5. Effect of khat alone, ethanol alone, and combined khat and ethanol on the severity of seizures following chronic administration. Ethanol-pretreatment alone showed a modest reduction in seizure severity compared to khat-treated rats. Seizure severity is represented as mean ± SEM; $n = 6–9$ rats per treatment condition. Two animals in the 4 g/kg khat group and 1 animal in the control group died during the feeding period and were excluded from these data. One control animal, 1 animal in the 4 g/kg khat group, and 2 animals in the 20% ethanol group did not show seizure activity. There were 2 statistical outliers in the control group, 1 in the 2 g/kg khat group, 2 in the 20% ethanol group, and 2 in the khat/ethanol group.

($p < 0.01$). Ethanol treatment alone reduced the seizure duration significantly compared to khat at 2 g/kg, but not 4 g/kg (Fig. 4). Of the two khat doses studied, there was a significant reduction of seizure duration by the 4 g/kg dose compared to the 2 g/kg dose ($p < 0.01$).

There was a mild reduction in seizure severity of the ethanol-only treated animals that was not statistically significant (Kruskal–Wallis $H = 2.0278$; Critical χ^2, 4 df, for α of $0.05 = 9.49$, $p = 0.7306$). Both Khat treatments, and combined khat and ethanol treatment had no statistically significant effect on seizure severity in relation to control treatment (Fig. 5).

Ethanol had a protective effect on seizure-induced mortality (33.3% mortality for ethanol treatment alone versus 66.7% for controls), while 77.8% of the group given khat at 2 g/kg died, and 85.7% of the group given khat at 4 g/kg died. Additionally, combined khat and ethanol treatment produced mortality of 100% by 96 h, an effect not seen in all the other experimental groups (Table 1).

4. Discussion

Several studies have shown that many individuals who chronically abuse khat combine it with alcohol (Ihunwo et al., 2004; Anderson et al., 2007; Beckerleg, 2009). Because seizures are one of the most serious consequences of chronic drug abuse or withdrawal, we wanted to study the effect of khat and ethanol on seizure behaviour following chronic administration. We mimicked the chewing of freshly harvested khat leaves and twigs in this study by obtaining an extract of all soluble components as a juice. Using the juice containing the undegraded active components allowed determination of the modulatory effect of khat on seizure expression.

Previous studies have shown the amphetamine-like effects of acute khat, as well as its chronic systemic effects (Nencini and Ahmed, 1989). In this study, the doses of khat used, as well as ethanol, and combined khat and ethanol did not have anorexiant effects seen with amphetamine-like compounds, signifying that the seizure responses to pentylenetetrazol were not a result of malnutrition. Amphetamines act mainly by stimulating the presynaptic release of dopamine to increase synaptic dopamine levels, rather than by preferential blockade of the dopamine transporter as happens with other oral stimulants (Volkow et al., 1999; Schiffer et al., 2006). The absence of inhibition of weight gain by khat and the khat-ethanol combination seen in this study can be attributed to the short-term efficacy of khat as an anorexiant. Indeed, several other oral stimulants such as fenfluramine that are amphetamine-like and prescribed for weight reduction are known to have short-term efficacy because of the development of tolerance (Brunton et al., 2006). Furthermore, studies that have found reduced food intake in rats, have used potent doses of d-norpseudoephedrine, a metabolite of synthetic cathinone (Brenneisen et al., 1986), and d-amphetamine and other khat components (Eisenberg et al., 1987), rather than the total khat extract. Regardless, humans who chew khat for its stimulant action such as increased alertness have to habitually chew bulk quantities continuously in order to obtain its CNS actions, and it takes several hours before the full sequence of effects including euphoria and anorexia are achieved (Nencini et al., 1986; Beckerleg, 2009).

We also found a dose-dependent effect of khat in modulating both seizure latency and duration, but not seizure severity. Whereas the low and high doses of khat used produced a comparable time to initiation of seizure activity (seizure latency), there was a divergence of effect for the 2 g/kg and 4 g/kg doses of khat, on seizure duration. Low dose khat prolonged the seizure activity significantly while the high dose khat shortened seizure activity to a time similar to that of control animals. This suggests that the mechanisms for seizure initiation and propagation extending to seizure generalization may be differentially influenced by the dose of khat and by combined khat and ethanol.

Recent studies have shown that fresh aqueous khat extract at an intermediate dose (3 g/kg) to the doses used in this study, lowered PTZ seizure thresholds (Oyungu et al., 2009), and stimulated locomotor activity and induced seizures. It is thought that the alkaloid cathinone is a potent amphetamine-like compound that modulates the release of catecholamines from storage sites in the synaptic terminal (for reviews, see Kalix, 1984, 1990 and Feyissa and Kelly, 2008). Of interest was the finding that the lower dose (2 g/kg) of khat used in this study increased the seizure duration, while the higher dose (4 g/kg) of khat had the opposite effect that tends to produce a more favourable seizure outcome. This finding is consistent with a previous study on the acute effects of orally administered khat extracts on the rat electroencephalogram (EEG) that reported a stimulant effect of lower doses of oral khat, with the highest amount of cortical activation resulting from administration of the lowest dose (Saleh et al., 1988). The same study reported that the largest dose used resulted in a state of EEG depression. It is also notable that, while there was a divergence in the seizure duration of the 2 g/kg and 4 g/kg doses of khat used, there was a convergence in the seizure severity for the two doses, suggesting the possibility of a ceiling effect of the two khat doses in influencing seizure mortality by different pathways following this administration paradigm.

In order to better evaluate the delayed effect of the convulsant PTZ on animals treated with khat only, ethanol only, or combined khat and ethanol, we assessed mortality for up to 96 h post administration of PTZ. We found that, although there were no statistically significant differences, further deaths occurred by 48 h for the animal group that received combined khat, a psychostimulant, and ethanol, a CNS depressant. These deaths for the combined khat and ethanol group would indirectly be attributable to seizure activity because no overt seizures were observed at this time. Subsequent experiments will study the role of withdrawal phenomena in altering seizure behaviour at different times following administration of PTZ. Conversely, the simultaneous shortening of seizure duration and maximal seizure severity (death) for the combined khat and ethanol-treated animals are directly attributable to seizure activity. It can be postulated that altered brain chemistry as a result of coadministration of khat and ethanol influenced the modulation of brain responses following PTZ administration. Overall, the findings in this study suggest that in a rat model combined khat and ethanol co-dependence, there may be an increased risk of seizure activity

Table 1
Proportions of animal deaths following bolus injection of PTZ. Ethanol-treatment alone had a protective effect against mortality, but increased mortality when combined with khat. One animal that had been pretreated with ethanol only died after 24 h, and two animals that had been pretreated with both khat and ethanol also died after 24 h. However, there were no new deaths between 48 and 96 h post-administration of the different treatments. Data are presented as number of animals that died/number of animals tested, with percentages in parentheses. Two animals in the 4 g/kg khat group and 1 animal in the control group died during the feeding period and were excluded from these data. One control animal, 1 animal in the 4 g/kg khat group, and 2 animals in the 20% ethanol group did not show seizure activity. There were 2 statistical outliers in the control group, 1 in the 2 g/kg khat group, 2 in the 20% ethanol group, and 2 in the khat/ethanol group.

	Mortality at 24 h	Mortality at 48 h	Mortality at 96 h	Total
Controls	4/6 (66.7%)	0/6 (0%)	0/6 (0%)	4/6 (66.7%)
2 g/kg khat	7/9 (77.8%)	0/9 (0%)	0/9 (0%)	7/9 (77.8)
4 g/kg khat	6/7 (85.7%)	0/7 (0%)	0/7 (0%)	6/7 (85.7%)
20% ethanol	2/6 (33.3%)	1/6 (16.7%)	0/6 (0%)	3/6 (50%)
4 g/kg khat + ethanol	6/8 (75%)	2/8 (25%)	0/8 (0%)	8/8 (100%)

and responses that could arise in this case, using a chemical convulsant, but also from other causes such as drug withdrawal in the acute phase.

Using only male rats in this study allowed the separation of possible action of circulating sex steroids (more abundant in females) and their metabolites in influencing the actions of khat or ethanol, and also modulating seizure expression. It is uncertain why a small set of animals from the control group, the ethanol-only group and the 4 g/kg khat group did not show seizure behaviour following administration of PTZ. An important caveat in this study is that the cathinone content of khat leaves, like for many other plant extracts, depends on environmental and climatic factors such as rainfall and soil quality, which can influence the amount and concentration of the active cathinone content. This study did not compare the khat cathinone content between seasons. However, the two doses studied were from the same khat extracts obtained from the same geographical area where khat has been used for several years. Additionally, administration of khat for the two doses, and for the other test substances occurred at the same times twice daily in the morning and late afternoon and was done consistently throughout the study. Administration of all the substances was completed within an hour for all the groups. Later, generation of seizures with PTZ followed the same order as the administration of test substances. Controlled pharmacokinetic studies of oral khat have shown a peak plasma concentration of between 1.5 and 2.5 h, and a terminal elimination half-life of between 2.5 and 6 h (for example, see Widler et al., 1994). Thus, even though it is technically impossible to administer the khat doses by gavage to all the animals concomitantly, the duration during which substances were given to all the animals was sufficiently short enough not to confound the behavioural parameters in this chronic study.

Given the current trends in the rise in consumption of individual drugs especially khat and alcohol in Africa (Claasen, 1999; Othieno et al., 2000; Acuda et al., 2011), it can be expected that coabuse of these drugs will also increase because of easy access to them. It is important then to understand and predict the physical effects in the brain, especially epileptogenic effects, of long-term coabuse of khat and ethanol, these drugs being among the most commonly abused substances in Africa (Nkowane and Jansen, 1999). In view of the major health problems associated with recreational drug use such as khat chewing and alcohol abuse, coupled with growing evidence for a causal link between khat use and mental illness, (Warfa et al., 2007), the pathophysiological implications of combined khat and ethanol abuse need to be further explored and addressed through well designed animal and human studies. Future studies will compare the extent to which rainy and dry seasons influence khat cathinone content and activity, and which other chemical components in khat modify the CNS excitation observed. It will also be interesting to investigate and compare seizure expression in female rats as well, following chronic khat and ethanol administration considering that males and females have markedly different levels of baseline sex hormones such as 17 β-estradiol and other neuroactive steroids like allopregnanolone (these being higher in females). The findings of this study should be confirmed in larger series and extended to encompass withdrawal phenomena, which can include severe seizures. Ultimately, data from studying long-term khat and ethanol co-abuse or co-dependence can help guide decisions for appropriate intervention strategies both for prevention of khat plus ethanol abuse, and help guide strategies designed for appropriate treatment of dependence on these drugs of abuse.

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