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The effect of acute exposure to crude khat (*Catha edulis* F.) on spatial learning and memory in mice using multiple T-maze test

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Abstract

Background: The ability to learn something new and then to store the information in long-term memory is part of normal development. Khat (*Catha edulis* Forsk) is a shrub or small to a medium-sized evergreen tree that belongs to Celastraceae family. It is claimed to have a cognitive effect. The aim of this study was therefore to investigate the effect khat on spatial learning and memory in mice.

Methods : To this effect, 32 healthy Swiss albino mice of both sexes, each weighing 23-34g, were divided into one control group (Tween 80) and three experimental groups of eight animals each. Khat was orally administered in different doses (100 mg/kg, 200 mg/kg and 300 mg/kg) to experimental mice. Learning and memory performance after khat administration was measured by determining latency and wrong decision making in multiple T-maze test.

Results: Highest dose of khat showed an increment of latency period in the sixth day (100.44 sec). However, it became low in the thirteenth experimental day (76.77sec). The lower dose of Khat showed a significantly ($P<0.05$) low latency period (117.7 sec) compared to middle dose (206.46 sec) in the first experimental day. The middle dose of khat showed a better lowering of wrong decision making (errors) compared to both the control group and the other experimental groups (higher and low doses of khat). In this study, all the three doses of khat didn't show any significant improvement of learning and memory compared to the controlled drug (placebo).

Conclusion: The result collectively indicates that all the three doses of khat didn't show any improvement latency in learning and memory compared to placebo.

Keywords: mice, khat, learning, memory, latency, wrong decision

Introduction

Background

Learning is the process of acquiring new information while memory is the retention of the acquired information. Different natural products are claimed to enhance learning and memory by acting on the brain, altering the neurotransmitter system and structurally affecting the limbic Papez system. These substances can affect learning and memory by interfering with the medial temporal lobe structures and thus affecting spatial learning and memory [1].

Khat (*Catha edulis* Forsk) is a shrub or small to a medium-sized evergreen tree that belongs to Celastraceae family and cultivated in Yemen and East African countries [2]. The shrub grows to a height of 6 meters. The young shoots and leaves are the parts chewed for their psychoactive properties [3]. Since khat is freely available in Ethiopia; the number of khat chewers has increased. Khat consumption has become popular in all segments of the country [4]. Chewing the leaves of khat is a social habit in Yemen and East African countries. People regularly chew fresh khat leaves mainly in the afternoon although some people chew khat in the morning [5].

There are several names for the plant, depending on its origin [3, 6]. In Ethiopia, it is commonly known as 'chat' and has other local names such as 'Aweday', 'Beleche', 'Abo mismar', 'Gelemso', 'Wondo' and others based on place of cultivation [7, 8]. Aweday' khat, most costly local brand in Ethiopia, is collected from its natural habitat (Harar) in this study.

Because of the claimed psychostimulant effect, khat is used as a recreational drug by many people. Hence, used in formal meetings (khat sessions) where the participants are engaged in discussions and maintain social contact. During such sessions the leaves and the bark of the plant are chewed slowly over several hours and the juice of the masticated leaves is swallowed, but not the residues [9]. Similar to other psychostimulants, khat ingestion produces several CNS effects, including increased motor stimulation, euphoria, and a sense of excitement and energy [10, 11]. These effects have been observed in several clinical trials and animal studies with khat or cathinone [12].

Khat chewers believe that they think more clearly and quickly and are more alert, although their concentration and judgment are objectively impaired [13]. In view of its potency, higher lipid solubility and facilitating access in to CNS, it can be assumed that khat-induced psychostimulation is predominantly, or even exclusively due to the cathinone content of the leaves [14].

Cathinone, like amphetamine, acts by releasing catecholamines from presynaptic storage sites and inhibit their uptake, hereby increasing temporal and spatial presence of these neurotransmitters (dopamine, serotonin, and noradrenaline) at the presynaptic receptors [15]. Amphetamine and methamphetamine are two important substances with close similarity to the active constituent of khat, cathinone. Studies on these substances showed that acute amphetamine administration increase memory consolidation and reinforcement in some learning paradigms [16]. Similarly, post-trial amphetamine treatment enhanced Morris water maze (MWM) task [17]. Studies about the effect of acute khat administration on learning and memory are scanty. This study, therefore, attempted to investigate whether exposure of mice to khat had a potential to enhance learning and memory or not.

Methods

Plant material

Bundles of fresh Khat leaves and small branches were purchased (1000g) fresh at a local market from Aweday, its natural habitat, 525 km South East of Addis Ababa, Ethiopia. The fresh bundles were packed in plastic bags and transported in an icebox to the School of Pharmacy, Addis Ababa University. The plant was identified by a taxonomist and a voucher specimen (AG 001) was deposited at the National Herbarium, College of Natural Sciences, Addis Ababa University. The fresh leaves were immediately kept at -20 °C till the time of extraction.

Crude extract preparation

Extraction was performed as described elsewhere with slight modification [18]. The freeze-dried plant was finely minced, weighed and placed in Erlenmeyer flasks (400 g per flask) wrapped with aluminum foil to avoid light-induced decomposition. Chloroform (150 mL) and diethyl ether (450 mL) (1: 3 v/v) were added to cover the minced leaves. The resulting mixture was shaken under a dark condition for 24 h using a rotary shaker (New Brunswick Scientific Co, USA) at 120 rpm and 20 °C. The mixture was later filtered through a folded filter paper. The filtrate was again passed through a round filter paper with the help of a mini filter pump. The organic filtrate collected in this way was pooled to gether in a wide mouth amber bottle and placed in a hood for 24 h to remove the organic solvents. The residue was left overnight in a deep freezer and then lyophilized using a freeze dryer (Christ 100400, Bioblock Scientific, France). The yield was calculated and found to be 1.02%, which was similar to previous works [3, 18].

Experimental animals

Thirty-two (16 males and 16 females) healthy Swiss albino mice (age 6–8 weeks and weight of 23–34g) were used in this study. Swiss albino mice bred in the animal house of Ethiopian public health institute (EPHI) were used for the experiment. The mice were housed in cages and maintained at room temperature (22–25 °C) and 12 h light and dark cycle. Standard pelletized feed and tap water were provided *ad*

libtum. All animals were handled according to internationally accepted guidelines [19].

Grouping and dosing of animals

Animals were divided into 3 experimental groups and 1 negative control group of 8 (four male and four female) each. The first group served as control (CON) and given vehicle (Tween 80, 2% v/v in water) orally. The second, third and fourth groups received crude khat extract at three different doses of 100 mg/kg (K100), 200 mg/kg (K200) and 300 mg/kg (K300) orally for four days. The various doses for the khat extract were selected based on previous reports [20]. Khat extract was weighed and mixed with the vehicle in predetermined concentration and mixed with agitator. The prepared solution will be administered through oral route of administration by using oral gavage.

Multiple T-maze test

The multiple T-maze (MTM) is a land maze used to assess spatial cognitive ability. Animals learn to find the goal box based on their memory from extra-maze cues. The MTM was constructed of wood and consisted of a wooden platform with seven choice points and the dimensions was 150 cm x 130 cm x 15 cm and a path width of 8 cm.

Khat was administered for 4 consecutive days. In the fifth day, the mice was placed in the multiple maze box to search for the goal box. Prior to testing, mice were deprived of food for 16 h to motivate food searching. Mice were placed in a start box and the first trial was started. Mice were searching for the reward and the trial was completed when mice had reached the goal box or, if failed, after 5 min. After getting the goal box, mice were allowed to consume a small piece of food pellet as provided reward and transferred to their home cage. Each mouse performs three trials per day.

Immediately after each trial, the entire maze was cleaned with a dilute alcohol solution. After testing, animals were given food into the home cage, representing the amount to preserve their body weight but keeping them hungry for the following day for MTM tests. Mice were trained with 3 trials per day for 4 days. Trials were carried out using 20 min intervals. Trials were recorded using a video camera and the following parameters were measured; correct or wrong decisions and latency to reach the goal box. Latency is the time taken to reach the gal box in second within the given 5 minutes. While wrong decision refers to the mice wrong path taken in attempt to reach goal box, it is number of paths they take.

On the sixth and thirteenth experimental day, subjects were undergoing a probe trails for 5 min to determine the long-term effect of khat. Mice were allowed to explore the maze and time to reach the goal and correct and wrong decisions was recorded.

Statistical analysis

All data are presented as a mean \pm standard error of the mean and SPSS data analysis software version 19 was used for data processing. The analysis was performed by one way ANOVA followed by Tukey multiple comparison tests. Level of significance was set at $p < 0.05$.

Results

Effect of khat on latency

The negative control group has exhibited a day dependent decrement of latency from the first day up the fourth day (D1=152.24, D2=108.96, D3=55.61, D4=37.67) but it started to be elevated on sixth experimental day (67.37sec) and

thirteenth day (89.08 sec). On the contrary, the higher dose of khat showed increment in the sixth day (100.44 sec) and it became low in the thirteenth experimental day (76.77 sec). In the first four days, the higher dose of khat showed a lowering of latency in the fourth (83.54 sec) day. In each of the experimental days, the negative control resulted a better lowering of latency compared to the higher doses of khat, but this was not found to be statistically significant. The lower dose of khat showed a significantly ($P < 0.05$) low latency period (117.7 sec) compared to middle dose (206.46 sec) in the first experimental day.

Effect khat in wrong decision

In order to investigate the learning and memory enhancing effect of khat three doses of crude extract were given through oral route of administration and determined using the wrong decision (error) they make in the multiple T-maze box. The result indicated that, though all of the groups exhibited a day dependent lowering of wrong decision making in short term memory (day1-day4), the middle dose of khat showed a better lowering of wrong decision making (errors) compared to both the control group and the other experimental groups (higher and low doses of khat). Low dose of khat showed an elevated error in the 13th experimental day (27.53) compared to the previous days (day1,2,3,4 and 6), and it also exhibited the higher decision making in 13th day compared to control (8.53), group three (4.83), and group four (5.1).

Since it needs 14 correct decisions to reach the reward box, in the first experimental day the middle dose took 25.58 paths to reach to the goal box but in the fourth day it only took 15.27 paths to get the reward.

As it is depicted in the table 2 both the experimental and control groups did not show a statistically significant decrement in the wrong decision making.

Though it is not statistically significant, the middle dose of khat exhibited day dependent lowering of both latency and wrong decision making in good manner. it exhibited a higher number of wrong decision making (15.78) and increased latency period (206.49 sec) in the first experimental day, but the lowest figures of both latency (27.05 sec) and wrong decision (1.27) is recorded from middle does compared to both the experimental (high and low dose of khat) and control group.

Discussion

This Study found a total yield of 1% which is almost similar to previous studies done on khat extract [14, 16, 21]. In this study, the effect of khat administration in learning and memory was studied by using multiple T-maze tests. Rats use spatial information provided by the distal cues in the room to efficiently locate the baited arms. The multiple T-maze allows the study of spatial reference and working memory processes in the rat [22]. Therefore, Multiple T-maze is a good model for the study of this effect.

Recent evidence from rodent research implicates an association between daily khat use and spatial learning and memory [23] as well as behavioral sensitization with repeated dosing [24]. Due to some pharmacological similarities between the active components of khat (cathinone and cathine) and amphetamines, future studies examining these same domains of cognitive functioning in chronic khat users and abstinent khat users appears to be warranted, if possible using some of the same or similar laboratory measures.

The finding of the present stud was in line with other study done in Ethiopia, which showed no significant effect khat on learning and memory enhancement [25]. But the study focused on the short-term effect of khat while the present study stress was given to both short term and long term memory effect of khat.

This study's result is opposite to other reports about the effect of the crude extract on learning and memory in animal models. kimani *et al* [26] reported that khat enhances learning and memory in CBA mice after acute administration of it. But this study didn't find any significant difference between khat and control group in learning and memory.

Conclusion

In the present study, multiple T-maze test was employed to determine the effect khat on learning and memory in mice. Since some experimental researches also showed a paradoxical result regarding the khat effect on learning, this study tried to investigate this effect in mice. All the three doses of khat didn't show any improvement latency in learning and memory compared controlled drugs (placebo). It did not also show a significant lowering of wrong decision making in the multiple T-maze test.

Table 1: Effect of crude khat administration in latency to reach goal box.

Group	Latency					
	Day 1	Day 2	Day 3	Day 4	Day 6	Day 13
CON	152.24±4.49	108.96±3.99	55.61±6.08	37.67±2.25	67.37±7.02	89.08±1.66
K100	117.17±2.75 ^{a*}	58.49±1.18	72.28±8.12	40.33±7.07	71.94±7.52	59.44±2.81
K200	206.49±5.99	115.52±8.71	66.76±3.77	27.05±5.69	102.15±1.16	71.89±1.12
K300	157.94±7.47	109.60±3.45	110.50±3.36	83.54±3.24	100.44±1.81	76.77±3.61

Values are mean ± SEM. n = 8; a compared to K200, CON: control; K100, khat 100 mg/kg; K200, khat 200 mg/kg; K300, khat 300 mg/kg). * = p-value < 0.05; latency = time taken to reach goal box expressed in seconds

Table 2: Effect of crude khat administration in wrong decision making to reach goal box.

Group	Wrong Decision					
	Day 1	Day 2	Day 3	Day 4	Day 6	Day 13
CON	13.25±2.09	6.52±1.88	2.75±0.79	2.31±1.52	5.32±1.56	8.53±1.86
K100	12.27±1.09	9.83 ±1.49	5.40±0.83	1.66±0.71	5.63±0.74	27.75 ±16.20
K200	15.78 ±1.49	9.83±1.49	4.30 ±1.99	1.27±0.46	5.05±0.47	4.83 ±1.27
K300	11.41±1.33	9.94±2.47	7.98±1.89	5.61±2.11	6.38±1.97	5.1±2.18

Values are mean ± SEM. n = 8; (CON: control; K100, khat 100 mg/kg; K200, khat 200 mg/kg; K300, khat 300 mg/kg). Wrong decision = number of paths wrongly taken to the goal box

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

All authors involved in the design and write up of the study, and all authors conducted the actual study and the statistical analysis.

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