

Effects of Catha Edulis on Anxiety-like Behavior and Prefrontal Cortex-Malondialdehyde Level in Mice

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Abstract

Background

Catha edulis, commonly called khat, is a psychostimulant plant chewed by East African people. Chewing khat has adverse effects. Anxiety is becoming one of the most common psychiatric disorders. Some psychostimulants worsen while others improve the disorder. However, the effects of khat on anxiety and brain lipid peroxidation have not been investigated. The aim of this study is, therefore, to investigate the anxiety-like and prefrontal cortex (PFC) lipid peroxidation effects of khat in animal model.

Methods

A total of 40 white albino mice aged between 7 and 8 weeks were used. They were administered with khat extract (Ke) 100 mg/kg, 200 mg/kg, 300 mg/kg b.w, and 2% tween 80 in distilled water (T80W-v/v) for thirteen weeks. The anxiety-like behaviors and PFC malondialdehyde (MDA) level were measured using elevated plus maze and spectrophotometry, respectively. One-way ANOVA, person's correlation, and independent t-tests were used. P-value <0.05 was considered statistically significant.

Results

Ke 100 mg/kg ($p < 0.05$), Ke 200 mg/kg ($p < 0.01$), and Ke 300 mg/kg ($p < 0.01$) reduced open arm entry. Ke 100 mg/kg ($p < 0.01$), Ke 200 mg/kg ($p < 0.01$), and 300 mg/kg ($p < 0.01$) also reduced open arm duration. Ke 200 mg/kg ($p < 0.01$) and Ke 300 mg/kg ($p < 0.001$) increased right PFC MDA level.

Conclusions

Ke showed anxiety-like behaviors and increased PFC MDA level. Further studies are needed on the PFC neurochemicals effects of this extract.

1. Introduction

Catha edulis is commonly called khat (1). It is psychostimulant plant grown in East African countries including Ethiopia and chewed by people in these countries (1). Khat chewing causes different adverse effects (2) and its health and socio-economic burdens are increasing (3, 4). Previous studies (5, 6) reported that mood disorders are common among khat chewers. Although some studies (7, 8, and 9) showed that khat reduced serum antioxidant levels, other studies indicated that khat possesses phenolic and flavonoids with oxygen and nitrogen free radicals scavenging activities (10, 11). Although khat reduces antioxidant levels, most chewers believed that khat reduces stress and anxiety-like behaviors (12, 13).

Anxiolytic and anxiogenic agents modulate elevated plus maze (EPM) task performances in rodents (14, 15, and 16). Rodents have an innate fear of elevated open places and stay for a longer time in closed arms of EPM (17). The innate arm preference can be worsened or improved by psychostimulant plants such as khat. Psychostimulants having anxiolytic or anxiogenic effects increase prefrontal cortex (PFC) malondialdehyde (MAD) levels (15, 16). PFV is one of the brain areas affected by anxiogenic or anxiolytic substances.

Although investigations into the cathinone in khat effects on serum lipid peroxidation have been conducted (18), none has yet investigated khat effects on the brain tissue lipid peroxidation and its association with anxiogenic response in animal models. The aim of the present study is, therefore, to evaluate the anxiety-like effects of khat in connection with the PFC-MAD level in wild-type white albino mice of both sexes.

2. Material and methods

2.1 Chemicals

Sodium dodecyl sulfate (SDS), 2-thiobarbituric acid (TBA), acetic acid, butanol, pyridine, sodium chloride, and potassium were used in this study. Besides, magnesium sulfate, calcium chloride, potassium hydrogen phosphate, sodium bicarbonate, glucose, diethyl ether,

and chloroform (Siga-Aldrich, Germany), Tween 80, and 70% ethanol were also used in this study.

2.2 Plant materials collection

As the plant is cultivated by individual formers, no permissions or licenses from particular organizations are required to collect species, only pay for the plant specimen to the farmers. Bundles of fresh khat leaves (7kg) were purchased and collected from farmers in Aweday, Eastern Ethiopia. The plant specimens were identified by Botanists in the Department of Biology, College of Natural Sciences, Addis Ababa University. The specimen was authenticated and voucher number (October 16, 2018, AA002) was given to be deposited at the National Herbarium of Ethiopia, Addis Ababa University.

2.3 Plant material extraction

After the edible parts of the leaves were separated and washed with tap water, the leaves were freeze-dried at -20°C (19) for 2 days and crushed using mortar and pestle. Two hundred grams of freeze-dried crushed leaves were placed into a conical flask wrapped with aluminum foil (19). A total of 400 ml organic solvents, i.e., 300 ml diethyl ether and 100 ml chloroform (3:1v/v ratio) were added into the flask. The mixture was shaken under the dark condition for 48hrs at 20°C using a rotary shaker (New Brunswick Scientific Co, USA) with a speed of 120 rpm. It was then filtered initially using cotton gauze followed by grade I Whatman filter paper (Cat No 1001 150). The organic solvents were then removed through evaporation using Rota- vapor under a controlled temperature of 36°C , with a speed of 120 rpm and 240 Pascal negative pressure. The water in the extract was removed through lyophilization and the dry residue was weighed using an analytical balance and stored in a desiccator till used. The amount of T80W used to extract the given weight of the leaves was determined based on the total weight of each mouse and vehicle volume (10 ml/kg b.w).

2.4 Animal preparation

A total of 40 wild-type Swiss albino mice aged between 7 and 8 weeks of both sex weighing between 21 and 37g were used in this study. Mice with the same breeding series were purchased from the laboratory animal breeding section of the Ethiopian Public Health Institution (EPHI). Three animals per plastic cage (45 cm long, 45 cm wide, and 25 cm high) under natural light and dark (12:12hrs) cycles at room temperature were housed. Water and a standard pellet diet were available *ad libitum* throughout the experimental period. Mice were weighed every day to ensure appropriate dosing based on body weight changes. The mice were handled for 5 min every day for two weeks before the experiment.

2.5 Grouping and dosing

The mice were randomly assigned into 4 groups ($n = 10$ / group, 5 males and 5 females/group) and received tween 80 in distilled water (T80W-V/V) as "vehicle group", and grade doses of Ke 100 mg/kg, 200 mg/kg, and 300 mg/kg. The test substances were administered for thirteen weeks. T80W was used as a vehicle and the doses for Ke were selected based on previous reports (21). The selected doses in our study were nontoxic and safe in the previous study, which was the basis for dose selection in the current study.

2.6 Preparation of test substances and volume determination

Fresh Ke and vehicle were prepared every day. Ke was dissolved in T80W. The dose of the extract administered to each animal was calculated based on the total body weight (b.w) of each animal. The appropriate volume of the vehicle (10 ml/kg) was used to determine how much volume was used to dissolve the calculated dose of Ke. Each animal in the experiments group received a single daily oral administration graded dose of Ke and the vehicle received T80W. The final volume was made 1ml and all substances were administered orally using a metal gavage needle.

2.7 Apparatus and experimental procedure

EPM was used to evaluate the anxiolytic/anxiogenic, locomotor, exploratory, and anxiety-like effects of Ke. The maze was built according to the description made and used previously (22, 23). The white wood plus maze was positioned 65 cm above the floor. The maze had two closed (30 cm x 10 cm x 25 cm) and two open (30 cm x 10 cm for mice) arms separated by a center square platform (10 x 10 cm) (Fig. 1).

Figure 1: Elevated Plus Maze used to evaluate anxiety-like responses in mice

Each mouse was exposed to the EPM for 10 minutes of acclimatization on a day before actual tests have been conducted. 24hrs after the last acclimatization and 30 min after administration of the test substances, each mouse was placed at one of the ends of open arms facing away from the center square of the maze. Each mouse was allowed to explore the maze for 10 min and their behavior was

video-recorded. Transfer Latency (TL), number of closed-arm entries (CAE-#), percentage of closed-arm duration (CAD %), number of open-arm entries (OAE-#), percentage of open-arm duration (OAD %), number of total arm entries (TAE-#) and percentage of center square duration (CSD) were measured. Arm entrance was counted only when the four paws entered each arm.

2.8 Brain tissue collection and malondialdehyde assay

The lipid peroxidation effects of Ke in mice were determined through the determination of MAD level in the PFC obtained from mice of both sexes. Lipid peroxidation was estimated according to the procedure described before (24). After overnight fasting, the mice were sacrificed by decapitation and their brains were taken out quickly to dissect PFC. 0.2 ml of 10% (w/v) tissue homogenate was mixed with 0.2 ml of 8% aqueous SDS, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of TBA. 0.6 ml of distilled water was added to a final volume of 4.0 ml. The reaction mixture was incubated in a boiling water bath of 41⁰C for one hour. After cooling, 1.0 ml of distilled water and 5.0 ml of butanol/pyridine mixture (15:1 v/v) were added, mixed, and centrifuged at 10,000 x g for 15 minutes to obtain surfactant from which the absorbance was measured at 532 nm using a spectrophotometer. Level of lipid peroxide (MDA nmol/g wet tissue weight) = absorbance * (Dilution Factor (3.33)/ extinction coefficient of MAD (163.8) * wet tissue weight (g).

2.9 Statistical analysis

The statistical analysis was done using SPSS version 21.0 and graphs were plotted using Microsoft Excel. The values were expressed as mean ± SEM. One-way ANOVA followed by Tukey Post Hoc analysis, Pearson’s correlation, and independent t-test statistics were used in this study. P-value <0.05 was considered statistically significant.

3. Results

3.1 Effects of khat on elevated plus maze task performance

The TL (p < 0.001) and the TAE (p < 0.001) in mice that received Ke were significantly reduced at all doses as compared with the control. The percentage of OAD was also significantly reduced in mice administered with the extract at all doses (p < 0.01). The frequency of OAE was reduced in mice administered with Ke 100 mg/kg (p < 0.05), Ke 200 mg/kg ((p < 0.01), and Ke 300 mg/kg (p < 0.01). Percent of PFD was significantly reduced at Ke 300 mg/kg (p < 0.01). The percentage of CAD was significantly increased in mice received Ke 100 mg/kg (p < 0.05), Ke 200 mg/kg (p < 0.05), and Ke 300 mg/kg (p < 0.001) (Table 1).

Table 1
Effects of Khat Extract on the EPM Task Performance in Wild-type Mice of both Sexes.

Group	EPM task activities, M±SEM						
	TL(s)	CAE(#)	CAD (%)	OAE(#)	OAD (%)	TAE(#)	CSD (%)
T80W(10 ml/kg)	20.00±1.18	11.30±.89	57.20±1.34	12.20±.86	32.30±1.44	23.60±.81	10.50±0.91
Ke 100 (mg/kg)	12.80±1.29***	7.80±.74*	62.10±1.30*	9.20±.74*	27.10±1.30**	16.70±.56***	10.80±1.32
Ke 200 (mg/kg)	10.60±.96***	9.70±.80	61.80±1.11*	8.30±.63**	27.00±0.82**	17.90±1.18***	11.20±1.03
Ke 300 (mg/kg)	10.60±.97***	9.30±.63	66.00±0.77***	7.60±.60**	27.10±0.38**	17.10±.67***	6.90±0.74**

Each point represents the mean ± SEM of TL (s), CAE (#), CAD (%), OAE (#), OAD (%), TAE (#), and CSD (%) of mice (n = 10/group) which received T80W and khat extract (Ke) (100 mg/kg, 200 mg/kg and 300 mg/kg). ***P < 0.001, **P < 0.01, and *P < 0.05 when each group of mice was compared with those which received T80W. EPM: elevated plus maze, TL: transfer latency in second (s), CAE (#): frequency of closed arm entry, CAD (%): percent closed arm duration, OAE (#): frequency of open arm entry, OAD (%): percent open arm duration (%), TAE (#): total army entry in number (#) and CSD (%): percent central square duration.

The TL was significantly reduced in male mice administered with the middle (p < 0.001) and higher (p < 0.001) doses of extract, while it was significantly reduced at the lower (p < 0.05) and higher (p < 0.01) doses in female mice. The frequency of CEA was also

significantly reduced in female mice administered with the middle ($p < 0.01$) and higher ($p < 0.01$) doses, whereas the extract didn't affect this parameter in males. Although significant differences were not observed in males, the percentage of CAD was also significantly increased in females at the middle ($p < 0.05$) and higher ($p < 0.001$) doses. The percentage of OAD in females was significantly reduced at Ke 200 mg/kg ($p < 0.01$), and Ke 300 mg/kg ($p < 0.01$) in males, while no change in males. The total arm entry was significantly reduced at all doses of extract ($p < 0.01$) in females but not in males at the middle dose of the extract ($p > 0.05$) (Table 2).

Table 2
Effects of Khat Extract on the EPM Task Performance in Wild-type Mice Stratified by Sex

Group	EPM task activities, M $\pm$ SEM							
	sex	TL(s)	CAE(#)	CAD (%)	OAE(#)	OAD (%)	TAE(#)	CSD (%)
T80W (10ml/kg)	M	21.00 $\pm$ 2.26	9.40 $\pm$.81	60.00 $\pm$ 1.48	13.40 $\pm$ 1.36	30.00 $\pm$ 2.09	23.00 $\pm$ 1.18	10.00 $\pm$.71
	F	19.00 $\pm$.84	13.20 $\pm$ 1.07	54.40 $\pm$ 1.40	11.00 $\pm$.84	34.60 $\pm$ 1.50	24.20 $\pm$ 1.16	11.00 $\pm$ 1.76
Ke(100 mg/kg)	M	14.40 $\pm$ 1.86	6.40 $\pm$.87	63.40 $\pm$ 2.38	11.00 $\pm$.45	26.00 $\pm$ 2.21	17.40* $\pm$.81	10.60 $\pm$ 1.69
	F	11.20* $\pm$ 1.66	9.20 $\pm$.86	60.80 $\pm$ 1.07	7.40 $\pm$.81	28.20 $\pm$ 1.46	16.00** $\pm$.71	11.00 $\pm$ 2.24
Ke (200 mg/kg)	M	8.00*** $\pm$.45	11.20 $\pm$.58	60.80 $\pm$ 1.80	8.40** $\pm$.40	28.20 $\pm$ 0.97	19.40 $\pm$.51	11.00 $\pm$ 1.67
	F	13.20 $\pm$.80	8.20*** $\pm$ 1.20	62.80* $\pm$ 1.36	8.20 $\pm$ 1.28	25.80** $\pm$ 1.16	16.40*** $\pm$ 2.20	11.40 $\pm$ 1.40
Ke(300 mg/kg)	M	10.40*** $\pm$ 1.36	10.40 $\pm$.81	66.00 $\pm$ 1.14	6.40*** $\pm$.68	28.00 $\pm$ 0.32	17.20* $\pm$ 1.02	6.00 $\pm$ 1.14
	F	10.80** $\pm$ 1.53	8.20** $\pm$.73	66.00*** $\pm$ 1.18	8.80 $\pm$.66	26.20** $\pm$ 0.37	17.00** $\pm$ 1.00	7.80 $\pm$.86

Each point represents the mean $\pm$ SEM of EPM task performance measuring parameters in mice ($n = 10$ /group) that received T80W and khat extract (Ke) (100 mg/kg, 200 mg/kg, and 300 mg/kg). *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$ when male mice in each group were compared with male mice which received T80W, and females in each group with female in T80W received mice. EPM: elevated plus maze, TL: transfer latency in second(s), CAE(#): frequency of closed arm entry, CAD(%): percent closed arm duration, OAE(#): frequency of open arm entry, OAD(%): percent open arm duration (%), TAE(#): total arm entry in number (#) and CSD(%): percent central square duration.

3.2 Effects of Khat on Brain tissue Malondialdehyde

A Kruskal–Wallis test showed that there was a statistically significant difference in right PFC MDA level ($X^2 = 27.81$, $p < 0.01$) and left ($X^2 = 27.03$, $p < 0.01$, $n = 40$) between groups. The Post Hoc Pairwise Comparisons showed that the level of MDA in nmol/g wet brain tissue weight increased at Ke 200 mg/kg ($p < 0.01$) and Ke300 mg/kg ($p < 0.001$) doses of Ke in the right PFC and it was only at Ke 300 mg/kg ($p < 0.001$) in the left PFC when compared to mice received T80W (Fig. 2).

Figure 2: Effect of Ke on the Content of Lipid Peroxidation in the PFC of Swiss Albino Mice ($n = 10$ / group). Mice received T80W and khat extract (Ke) (100, 200, and 300 mg/kg). MDAPFCr: right the prefrontal cortex malondialdehyde and MDAPFCl: left the prefrontal cortex malondialdehyde level.

The average PFC MDA level was also significantly higher in mice that received Ke 200 mg/kg ($p < 0.01$) and Ke 300 mg/kg ($p < 0.001$). When MDA level was compared in mice stratified by sex, though a significant difference was not observed in female mice, male mice which received Ke 300 mg/kg had significantly greater right ($p < 0.01$) and left ($p < 0.01$) PFC MDA level when compared with the same sex of mice which received T80W. On the other hand, a significant correlation between average MDA level and OAE or OAD was not observed in mice that received Ke (Fig. 3).

Figure 3: Correlation between average PFC MDA level and OAD (a) or OAE (b) in mice received Ke ($n = 40$). R: correlation coefficient. MDA: malondialdehyde in nmol/g of wet brain tissue weight, OAD (%): percent open arm duration, OAE (#): open arm entry in number.

4. Discussion

In this study, the transfer latency to the closed arm was significantly reduced in mice that received Ke. This indicates that mice administered with the Ke returned to the closed arms more quickly than the control group and shows the presence of anxiety. The

natural aversion of mice to open arms and elevation-induced anxiety was worsened by the extract. The previous report also indicated that amphetamine worsened stress responses in the EPM test (25). Simone *et al.* (17) indicated that quick entry to closed arms indicated the presence of anxiety. While the open-arm duration and the number of total arm entries were significantly reduced, the closed-arm duration was significantly increased at all doses of the extract. Reduction in the open arm duration indicates that the exploration ability of mice was affected negatively by Ke and the extract has shown stress and anxiogenic effect. Another study also indicated that methamphetamine, cathinone in khat-like chemicals, and synthetic cathinone reduced open arm entries and duration (23, 26, and 27). Similar to our study, a study conducted before also indicated that the total arm entries, revealing the locomotor activities, were reduced in mice administered with Ke (16). Sestakova *et al.* (28) reported that rodents with lower locomotor activities revealed a greater level of anxiety and reduced exploratory behavior.

Effects of khat on dopamine (27), glutamate, and GABA transmissions (29, 30) could be contributed to the lower psychomotor and anxiety-like effects of Ke in this current study. According to studies conducted before, the administration of monosodium glutamate, which increases glutamate levels in the body, reduced the number of open-arm entries and duration in the EPM test (31) and showed anxiety-like symptoms (32). A magnetic resonance spectroscopy study conducted on healthy humans showed that stress response reduced the level of GABA in the PFC (33). On the other hand, the administration of GABA (Gamma-aminobutyric acid)-transaminase inhibitor, vigabatrin, in rats showed anxiolytic-like behaviors in EPM (34). In addition, the increase in the closed-arm duration in our study might be due to the effects of khat on the approach-avoid conflict. Mice administered with the extract tried to come into the open arms but immediately return into the closed arms. This means the approach-avoid conflict was high in mice administered with Ke and avoidance is one of the symptoms of anxiety disorder and fear responses (35, 36). A previous study revealed that khat use was associated with enhanced conflict responses in humans (37). Cohen *et al.* (38) showed that an anxiolytic dose of nicotine reduced the number of avoidance in rats, indicating that anxiolytic agents reduced approach-avoid conflict. Amphetamine administered to rats also significantly increased the number of returns to closed arms and open-arm avoidance (16).

The time spent in the center square, particularly at the higher dose of Ke, was also significantly reduced in this study. This less time spent in the center square of the maze indicates the presence of anxiety. The reduction in the time taken by mice at the center square of the maze might be attributed to the effects of the extract on GABAergic cells in the brain. Studies before indicated that anxiolytic drugs, mediating GABAergic transmission (30), increased exploration of open arms and the number of head-dipping, while anxiogenic drugs reduced the frequency of head-dipping and exploration at the central platform of the maze (39, 40). On the other hand, Shawqi *et al.* (30) reported that khat reduced GABAergic transmission. At the same time, a previous study (33) reported that stress reduced GABA levels in the PFC. Alfaifi *et al.* (19) also reported that ethanolic extraction of khat reduced the number of head dipping into holes in mice. These findings revealed the presence of relationships between Ke administration, GABAergic transmission, stress, and anxiety-like responses.

Furthermore, anxiety-like behaviors of female mice administered with the extract were compared to the same sex of mice administered with T80W. This comparison was also made in male mice administered with the extract and T80W. The cumulative anxiogenic effects of khat were more pronounced in female than male mice. This could be attributed to the neuroendocrine effects of khat and variations in hormonal response to stressors between sexes. Sex hormones and autonomic response to exogenous stressors contribute to gender differences in different conditions such as drug abuse, anxiety, and depression (41). Previous reports showed that estrogen treatment increased stress-inducible C-fos mRNA (42) and women are markedly more vulnerable than men to the negative consequences of drugs and stressors (43, 44).

The PFC is one of the brain regions involved in anxiety-like behavior (45, 46). In this study, the PFC lipid peroxidation, designated by MDA level, was significantly increased dose-dependently by Ke in mice. However, the differential effects of Ke on lipid peroxidation between the left and right PFC were not observed. A study reported by Al-Zubairi *et al.* (18) showed that fasting plasma MDA level was non-significantly increased in human subjects chewing khat. The increase in PFC MDA level in our study could be attributed to the reduction of antioxidant levels induced by Ke. A study conducted previously indicated that serum glutathione was reduced among khat users (47). Another study also indicated that synthetic cathinone reduced and increased antioxidant enzyme activity and lipid peroxidation dose-dependently in the limbic areas of mice respectively (4). Hatami *et al.* (48) also reported that the PFC MDA increased in rats administered with amphetamine at a dose dependable.

5. Conclusions

Our study has shown that Ke administered subchronically exhibited anxiety-like symptoms in mice. In addition, Ke significantly increased PFC MDA.

6. Recommendations

The substrates in the brain modulated by khat for altered behaviors we observed in this study are required to be investigated. The in-vitro neurotoxicity, neurotransmitters, and brain tissue antioxidant level effects of khat should be also investigated.

Abbreviations

CAD: closed arm duration; CAE: closed arm entry; CSD: central Square duration; EPM: Elevated plus maze; GABA: Gamma-Aminobutyric acid, Ke: khat extract, l= left; MDA: Malondialdehyde; mRNA: messenger ribonucleic acid, OAD: open arm duration; OAE: open arm entry; PFC: Prefrontal cortex, r- right; rpm: revolution per minute; TAE: total arm entry; TL: transfer latency

Declarations

Ethical approval and consent to participate

Ethical approval

The studies were approved by Institutional Review Board committee, Addis Ababa University (021/19/Physio). All the studies were conducted under the guidelines for animal research as detailed in the NAP guidelines for the Care and Use of Laboratory Animals (20).

Consent to participate: Not applicable

Consent for publication: not applicable

Availability of data: the data will be available when requested

Competing interests: The authors declare that no competing of interest.

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Author Contributions

Conceptualization, data curation, formal analysis, investigation, methodology, original draft: and article writing were done by Abebaye Aragaw. Editing both the original draft and article were done by Eyasu Mekonnen, while supervision of the research work and editing was conducted by Tesfaye Tolessa.

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References

1. Abebe M, Kindie S and Adane K. Adverse Health Effects of Khat: A Review. Family Medicine & Medical Science Research. 2015; 4(1): 2-5.<http://dx.doi.org/10.4172/2327-4972.1000154>.
2. Al-Habori M. The potential adverse effects of habitual use of Catha edulis (khat). Expert Opin. Drug Saf. 2005; 4(6):1129-1138.

3. Ageely HM. Health and socio-economic hazards associated with khat consumption. J Family Community Med. 2008; 15(1): 3–11.
4. Safhi MM, Alam MF, Hussain S, Hakeem Siddiqui MA, Khuwaja G, Jubran Khardali IA, Al-Sanosi RM, FIslam F. Cathinone, an active principle of *Catha edulis*, accelerates oxidative stress in the limbic area of swiss albino mice. J Ethnopharmacol. 2014; 156:102-6. doi: 10.1016/j.jep.2014.08.004.
5. Bahhawi TA, Albasheer OB, MaKeen AM, Arishi AM, Hakami OM, Maashi SM, Al-Khairat HK, Alganmy OM, Sahal YA, Sharif AA and Mahfouz MS. Depression, anxiety, and stress and their association with khat use: a cross-sectional study among Jazan University students, Saudi Arabia. Neuropsychiatric Disease and Treatment 2018; 14: 2755–61.
6. Yeshaw Y, Mossie A. Depression, anxiety, stress, and their associated factors among Jimma University staff, Jimma, Southwest Ethiopia, 2016: a cross-sectional study. Neuropsychiatric Dis Treat. 2017; 13: 2803–2812.
7. Masood AM, Al-Shehari BA, Al-Hattar LN, Altaeizzi MA, Al-khadher WA and Zindal YN. Alterations in Antioxidant Defense System in the Plasma of Female Khat Chewers of Tamar City, Yemen. Jordan Journal of Biological Sciences. 2012; 5(2): 129 – 133.
8. Al-Ashwal RH, Al.maqtari MA, Naji KM, Al-wsabai NA and Al Hazmy SM. Potential health effects of daily khat leave chewing: Study on the biochemical blood constituents change among adults in Sana'a city, Yemen. International Journal of Biochemistry and Biotechnology. 2013; 2(6): 461-463.
9. Naji KM, Al-Maqtari MA, Al-Asbahi AA, Abdullah Q, Babu RN and Devaraj VR. Effect of Daily Chewing Soft Buds and Leaves of *Catha edulis* (Khat) on the Antioxidant Defense System and Oxidative Stress Markers in Blood. Arab J Sci Eng. 2015; 40:1–6: Doi: 10.1007/s13369-014-1492-x.
10. Hailu YM, Atlabachew M, Chandravanshi BS, and Mesfin Redi-Abshiro MR. Composition of essential oil and antioxidant activity of Khat (*Catha edulis* Forsk), Ethiopia. Chemistry International. 2017; 3(1):25-31. <https://www.researchgate.net/publication/305347104>
11. Abdelwahab SI, Alsanosy R, Mohamed M, Taha E, and Mohan S. Khat Induced Toxicity: Role on Its Modulating Effects on Inflammation and Oxidative Stability. Bio Med Research International. 2018;1-7:<https://doi.org/10.1155/2018/5896041>.
12. Gebremariam TB, Mruts KB and Neway TK. Substance use and associated factors among Debre Berhan University students, Central Ethiopia. Substance Abuse Treatment, Prevention, and Policy. 2018; 13(13):1-8. <https://doi.org/10.1186/s13011-018-0150-9>.
13. Wondemagegn AT, Cheme MC and Kibre KT. Perceived Psychological, Economic, and Social Impact of Khat Chewing among Adolescents and Adults in NeKemte Town, East Welega Zone, West Ethiopia. BioMed Research International. 2017; 1-9. <https://doi.org/10.1155/2017/7427892>.
14. Mechan AO, Moran PM, Elliott JM, AMJ · Michael H. Joseph and Green AR. A study of the effect of a single neurotoxic dose of 3, 4 methylenedioxymethamphetamine (MDMA; “ecstasy”) on the subsequent long-term behavior of rats in the plus maze and open field. Psychopharmacology (2002) 159:167–175 DOI 10.1007/s002130100900.
15. Aduema W, Lelei SA, Osim EE, Osim EE, Koikoibo W and Nneli RO. Effect of chronic consumption of powdered tobacco (snuff) on anxiety, fear and social behaviors. International Journal of Basic, Applied and Innovative Research. 2012; 1(4): 161 – 169. www.antrescentpub.com
16. Pometlova M, Nohejllova-Deykun K, Slamberova R. Anxiogenic Effect of Low-dose Methamphetamine in the Test of Elevated Plus-maze. Prague Medical Report. 2012; 113 (3): 223–23.
17. Simone H, Pinheiro SH, Zangrossi-Jr H, Del-ben CM and Graeff FG. Elevated mazes as animal models of anxiety: effects of serotonergic agents. Anais da Academia Brasileira de Ciencias. 2007; 79(1): 71-85. www.scielo.br/aabc.
18. Al-Zubairi A, Al-Habori M and Al-Geiry A. Effect of *Catha edulis* (khat) chewing on plasma lipid peroxidation. Ethnopharmacology. 2003; 87:3–9.
19. Alfaifi H, Abdelwahab SI, Mohan S, Mohamed M, Elhassan T, Shaala Lamiaa A, Alsanosy R, Syame SM. *Catha edulis* frosk (Khat): Evaluation of its antidepressant-like activity. Phcog Mag. 2017; 13: 354-8. DOI: 10.4103/pm.pm_442_16.
20. National academies press. Guide for the care and use of laboratory animals, 2010; 8th edition.
21. Mohammed F., Engidawork E, Nedi T. The Effect of Acute and Subchronic Administration of Crude Ke (*Catha Edulis* F.) on Weight in Mice. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS). 2015; 14 (1): 132-141.
22. Walf AA and Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007; 2(2): 322–328. doi:10.1038/nprot.2007.44.

23. Miladi-gorji H, Fadaei A and Bigdeli I. Anxiety Assessment in Methamphetamine - Sensitized and Withdrawn Rats: Immediate and Delayed Effects. *Iran J Psychiatry*. 2015; 10(3): 150-157. <https://www.researchgate.net/publication/284747233>.
24. Ohkawa H, Ohishi N, and Yagi K. Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Analytical biochemistry*. 1979; 95: 35-358.
25. Atchley D. The Time-Course of the Effects of Stress on Behavior in Rodents. *Eukaryon*. 2011; 7:1-9.
26. Lapin IP. Anxiogenic Effect of Phenylethylamine and Amphetamine in the Elevated Plus-Maze in Mice and Its Attenuation by Ethanol. *Pharmacology Biochemistry and Behavior*. 1993; 44:241-243.
27. Safhi MM, Alam MF, Khuwaja G, Hussain S, Siddiqui MH, Islam F, Khardali I, AL-Sanosi RM, Alhazmi HA, Khan A and Islam F. Catha edulis active principle, cathinone, suppresses motor coordination, accelerates anxiety and alters the levels of dopamine and its metabolites in the limbic areas of male Swiss albino mice. *Acta Pharm*. 68 (2018) 485–495. <https://doi.org/10.2478/acph-2018-0038>.
28. Sestakova N, Puzserova A, Kluknavsky M, Bernatova I. Determination of motor activity and anxiety-related behavior in rodents: methodological aspects and role of nitric oxide. *Interdiscip Toxicol*. 2013; 6(3): 126–135. doi: 10.2478/intox-2013-0020
29. Al-Awdi SH, Al-Kadi HO, and Shehab MM. Effect of Khat Habituation on Psychomotor Behavior in Mice. *Journal of Pharmacology & Clinical Toxicology*. 2014; 2(1):1-3.
30. Shawqi A-A, Hussein A-K and Mohanad S. Effect of khat-habituation on GABA levels in the brain. *Asian Journal of Pharmacy and Life Science*. 2013; 3(2):74-80. <https://www.researchgate.net/publication/258847846>.
31. Onaolapo OJ, Onaolapo AY, Mosaku TJ, Akanji OO and Abiodun OR. Elevated Plus Maze and Y-Maze Behavioral Effects of Subchronic, Oral Low Dose Monosodium Glutamate in Swiss Albino Mice. *Journal of Pharmacy and Biological Sciences*. 2012; 3(4): 21-27. www.iosrjournals.org.
32. Quines CB, Rosa SG, Da Rocha JT, Gai BM, Bortolatto CF, Durate MM and Nogueira CW. Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young rats. *life Sci*. 2014; 27;107(1-2):27-31. doi: 10.1016/j.lfs.2014.04.032.
33. Hasler G, Willem J, Grillon C, Drevets WC, and Shen J. Effect of Acute Psychological Stress on Prefrontal GABA Concentration Determined by Proton Magnetic Resonance Spectroscopy. *Am J Psychiatry*. 2010; 167(10): 1226–1231. doi:10.1176/appi.ajp.2010.09070994.
34. Sherif F and Orelan L. Effects of chronic treatment with the GABA-transaminase inhibitor vigabatrin on exploratory behavior in rats. *Behav Brain Res*. 1994; 63(1):11-5.
35. Martin PP. Neural systems underlying approach and avoidance in anxiety disorders. *Dialogues Clin Neurosci*. 2010; 12(4): 517–531. Neural systems underlying approach and avoidance in anxiety disorders
36. Holmes A, Kinney JA, Wrenn CC, et al. Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. *Neuropsychopharmacology*. 2003; 28 (6):1031–1044. Doi: 10.1038/sj.npp.1300164.
37. Colzatol LS, Ruiz MJ, Wery P. M, Wildenberg van den and Hommel B. Khat use is associated with increased response conflict in humans. *Human psychopharmacology Hum. Psychopharmacology Clin Exp*. 2012; 27: 315-21.
38. Cohen A, Young RW, Velazquez M, Groysman M, Noorbehesht K, Ben-Shahar O and Ettenberg A. Anxiolytic effects of nicotine in a rodent test of approach-avoidance conflict. *Psychopharmacology (Berl)*. 2009; 204(3): 541–549. Doi: 10.1007/s00213-009-1486-7.
39. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of “fear”-motivated behavior. *Naunyn-Schmiedeberg’s Archives of Pharmacology*. 1984; 327 (1):1–5
40. TaKeda H, Tsuji M and Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. *Eur J Pharmacol*. 1998; 350(1):21-9.
41. Verma R, Balhara Y and Gupta C. Gender differences in stress response: Role of developmental and biological determinants. *Indian Psychiatry J*. 2011; 20(1):1-10. DOI: 10.4103/0972-6748.98407
42. Handa RJ and McGivern RF. Stress Response: Sex Differences. *Reference Module in Neuroscience and Biobehavioral Psychology*. 2017; 1–6. Doi.org/10.1016/B978-0-12-809324-5.02865-0
43. Fox HC and Sinha R. Sex Differences in Drug-Related Stress-System Changes: Implications for Treatment in Substance-Abusing Women. *Harv Rev Psychiatry*. 2009 ; 17(2): 103–119. doi:10.1080/10673220902899680.
44. Salem GM, Allah MBA, Said RM. Prevalence and predictors of depression, anxiety, and stress among Zagazig University students. *Med J Cairo Univ*. 2016; 84(2):325–334.

45. Arnsten A. Stress signaling pathways impair the prefrontal cortex structure and function. *Nat Rev Neurosci*. 2009; 10(6): 410–422. Doi: 10.1038/nrn2648.
46. Zarrindast MR and Khakpai F. The modulatory role of dopamine on anxiety behavior. *Arch Iran Med*. 2015; 18(9): 591 – 6.
47. Farag, R., Gunaid, A., Qirbi, A., 1989. Effect of khat on the metabolism of erythrocytes. *Biochemical Pharmacology* 38, 563–566.
48. Hatami S, Hatami H, Sheikhzade F and Dehgha G. Chronic administration of crystal meth in parallel with oxidative stress impaired spatial memory in the prefrontal cortex of male rats. *Trakia Journal of Sciences*.2016; 2(14): 126-134. doi:10.15547/tjs.2016.02.002.

Figures

Figure 1

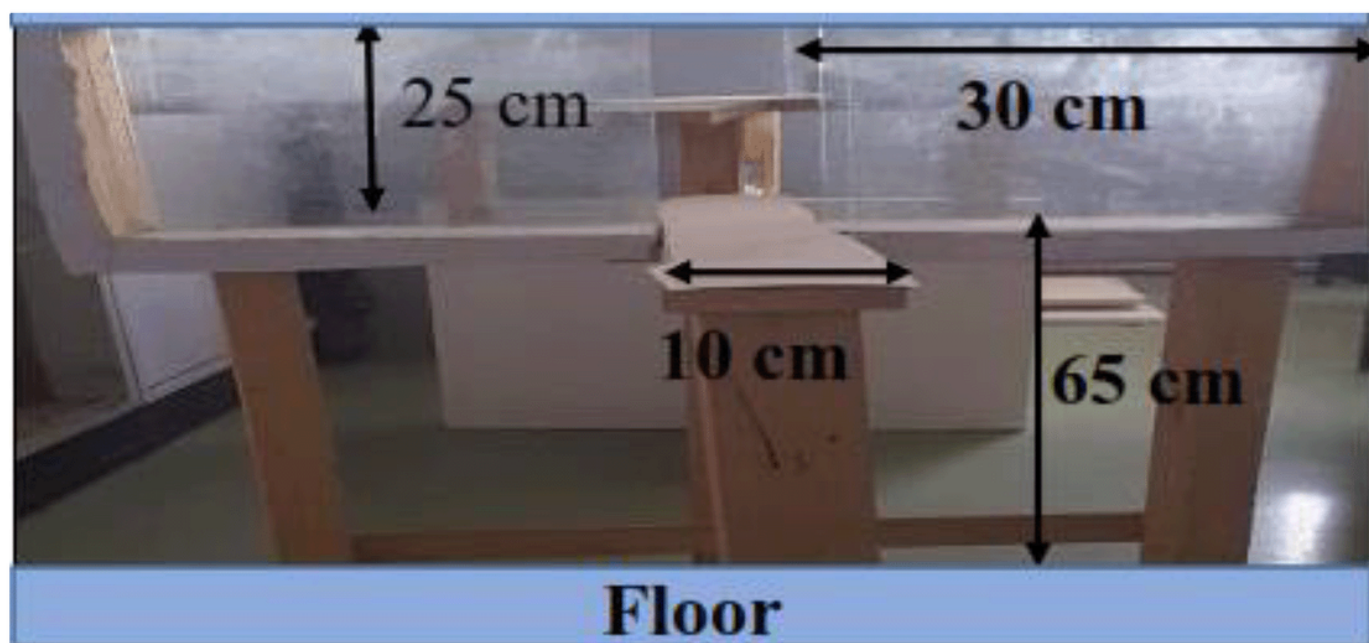


Figure 1

Elevated Plus Maze used to evaluate anxiety-like responses in mice

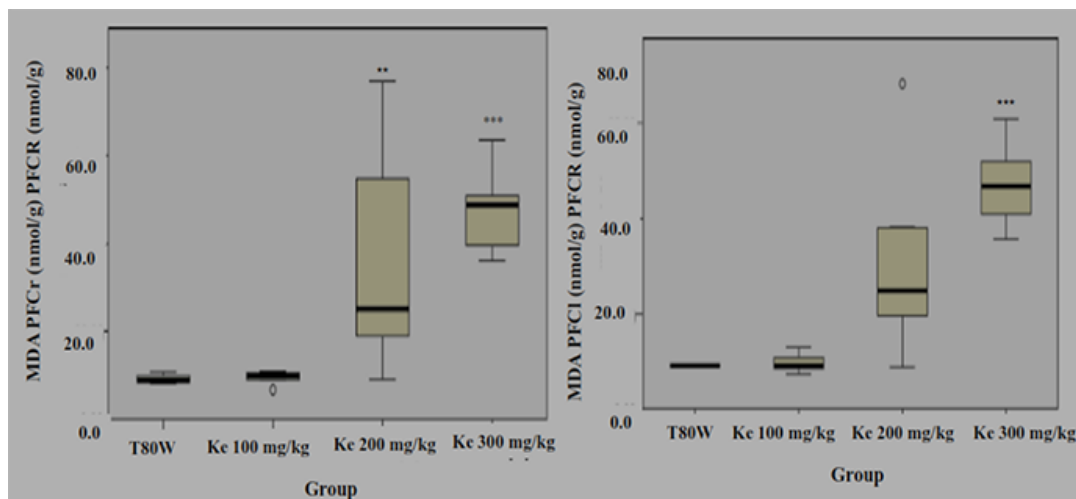


Figure 2

Effect of Ke on the Content of Lipid Peroxidation in the PFC of Swiss Albino Mice ($n = 10$ / group). Mice received T80W and khat extract (Ke) (100, 200, and 300 mg/kg). MDAPFCr: right the prefrontal cortex malondialdehyde and MDAPFCl: left the prefrontal cortex malondialdehyde level.

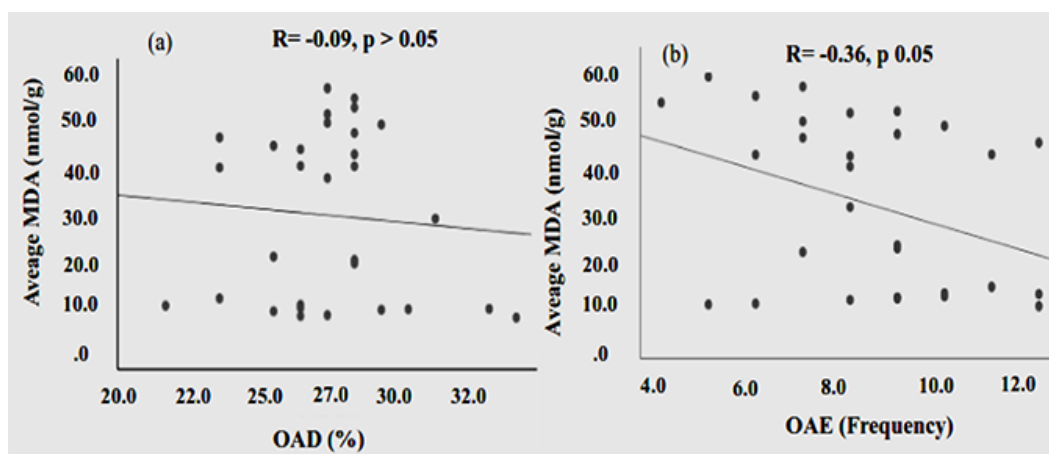


Figure 3

Correlation between average PFC MDA level and OAD (a) or OAE (b) in mice received Ke ($n=40$). R: correlation coefficient. MDA: malondialdehyde in nmol/g of wet brain tissue weight, OAD (%): percent open arm duration, OAE (#): open arm entry in number.