Effects of Khat on the Brain in Male and Female mice

Abstract

Background: Substance use and related disorders are becoming public health concerns globally. *Catha edulis*, commonly called khat, is a psychostimulant plant chewed by East African people and its anxiety-like effect has not been investigated experimentally in animal models. Although the results are inconsistent, the peripheral lipid peroxidation effect of khat is investigated. However, its brain lipid peroxidation effectrepresented by an increase in the malondialdehyde level has not been investigated. The increase in the MDA level could be associated with a khat-induced increase in the generation of free radicals and oxidation of lipids. These are the main problems driving this study. The aim of this study is, therefore, to investigate the anxiety-like and prefrontal cortex (PFC) lipid peroxidation effects of khat in animal models of both sexes.

Methods: A total of 40 white albino mice in 4 groups (n= 10 / group, 5 males, and 5 females/group) aged between 7 and 8 weekswere 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, Pearson'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 the right PFC MDA level while the MDA in the left PFC was increased by the higher dose of the extract (300 mg/kg).

Conclusions: *Catha edulis* showed anxiety-likebehaviors in the elevated plus maze paradigm and increased prefrontal cortex malondialdehyde level. Further studies are needed on the prefrontal cortexneurochemicals effects of this extract.

Keywords: khat**,** elevated plus maze malondialdehyde, anxiety-like activities, prefrontal cortex

1. Introduction

The health and economicburdens of substance usedisorders are becoming more prevalent and public health concerns(1).***Catha edulis***is, commonly called khat (1, 2), a psychostimulant plant grown in East African countries including Ethiopia, and chewed by people in these and other countries (1). Khat chewing causes different adverse effects (2) and its health and socio-economic burdens are increasing (3, 4). Previous studies reported that mood disorders are common among khat chewers (5, 6). Although some studies showed that khat reduced serum antioxidant levels(7, 8, and 9), other studies indicated that khatpossesses phenolic and flavonoids with oxygen and nitrogen free radicals scavenging activities (10, 11). On the other hand, most chewers believed that khat reduces stress and anxiety-like symptoms (12, 13), indicating the presence of inconsistentfindings in studies conducted before. Anxiolytic and anxiogenic agents modulate elevated plus maze (EPM)induced anxiety-like symptoms (14, 15, 16, 17). Besides, psychostimulants having anxiogenic effects increase prefrontal cortex (PFC) malondialdehyde (MAD) levels (15, 16).

The neurobehavioral and neurochemical effects of khat are mainly attributed to the active biopharmacological*phytochemicals* found in khat leaves. Alkaloids, terpenoids, flavonoids,

sterols, glycosides, tannins, amino acids, vitamins, and minerals are some of the known biopharmacology-active *phytochemicals* found in khat (18). Alkaloids in khat are structurally related to amphetamine with sympathomimetic effects (19, 20). Cathinone and cathine are the major alkaloids in khat and are responsible for the stimulatory, excitement, alertness, motor activity, euphoria, addictive, and other neurobehavioral and neurochemical effects of khat (19, 21, 22, 23,24). Cathinone in khat increases the release of monoamines (catecholamine, dopamine, and serotonin) from nerve terminals (19). Like stressors that increase the secretion of catecholamine and induce different physiological responses (25), cathinone in khat also increases the release of noradrenaline and results in neurobehavioral changes (18, 19, 26). The anxiety-like effect of khat could be attributed to the khat effects on catecholamine and hematological indices. As determined in our previous study (27), khat extract reduced hemoglobin and other red cell indices which could worsen stress and anxiety-like effects of khat. Patients with anxiety showed low levels of hemoglobin (28, 29).

On the other hand, the oxidative stress biomarker, 8-hydroxy-2'–deoxyguanosine, plasma level was significantly increased in association with a reduction in the activity of superoxide dismutase among khat chewers (30). Previous studies revealed that although the effect was seen at the periphery, Khat increased oxidative stress and MDA levels (31, 32), khat could increase lipid peroxidation and MDA levels in the brain. Another study also revealed that cathine in khat increased the level of reactive oxygen species (33) that cause oxidative stress and lipid peroxidation and increasedlevels of MDA.

Although investigations into the cathinone in khat effects on serum lipid peroxidation have been conducted (34), the effectsof khat on the brain tissue lipid peroxidation in the PFC and its association with anxiogenicresponse have not been investigated. 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.PFC is one of the brain areas controlling stress responses and is affected by anxiogenic or anxiolytic substances. PFC coordinates cortex-wide activity patterns and controls anxiety-like behaviors and this is one of the reasons why this research focused on this area of the brain.

This research manuscript carries some scientific importance in terms of findings, especially on the regular use of khat and its effects on brain areas influencing behavior. The findings also provide some insights into the possible mechanism of action leading to observed effects in the regular khat, more specifically production of oxidative stress at the level of the brain that, in part, influences anxiety-like behaviors among other psychostimulatory behaviors. The study also highlights sex as a co-variable in terms of the effects of khat on anxiety-like responses. This research is also trying to address wrong thoughts, beliefs, and gaps among the khat chewers.

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.All chemicals used in this study were purchased from Afro-German Chemicals Est. PLC, Addis Ababa, Ethiopia.

2.2 PlantMaterials Collection

As individual farmers cultivate the plant, 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 inAweday, Eastern Ethiopia. Botanists identified the plant specimens 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^{O}C (35) 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 (35). 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 48 hours at 20^{O}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^{o}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.

2.4 Animal preparation

A total of 40 wild-type Swiss albino mice aged between 7 and 8 weeks of both sexes(20 males and 20 females)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. To prevent the effects of room temperature and humidity on animal performance and affect results, the room temperature and humidity were maintained at 25- 30^{O}_{C}and30% respectively throughout the study time.Generally, the guidelines for laboratory animal Care and Use stated in the NAP (36) were applied in this study. 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. For habituation, 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 three grade doses of khat extract (Ke) 100 mg/kg, 200 mg/kg, and 300 mg/kg. Tween 80 was used as a control since it has low toxicity, causes insignificant physiological changes, and can dissolve khat extract. The test substances were administered for thirteen weeks orally using gavage. The doses forKe were selected based on the safety of these doses as reported previously (37).

2.6 Preparation of test substances and volume determination

Fresh Ke and vehicle were prepared every day. Ke was dissolved in 2% 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 of 2% T80Wwas used to dissolve the calculated dose of Ke. Each muse in the

experimental group received a single daily oral of the extractand the vehicle received T80W. The same vehicle was used to reconstitute the khat and the final volume was made 1ml. All substances were administered orally using a metal gavage needle daily.

2.7 Apparatus and Experimental Procedure

An elevated plus maze (EPM)was used to evaluate the locomotor, exploratory, and anxiety-like effects of the extract. The maze was built according to the description made and used previously (38, 39). The white wooden 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) (**Figure 1**).

Each mouse was exposed to the maze for 10 minutes of acclimatization on a day before actual tests were conducted. 24 hours after the last acclimatization and 30 min after administration of the khat extract, each mouse was placed at one of the ends of open arms facing away from the center square platform of the maze. Each mouse was allowed to explore the maze for 10 min and their behavior was video-taped. 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 determined.

2.7 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 (40). 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 41^0_C 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).

2.8 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.The non-parametric continuous variables obtained from the groups of mice were compared using the Kruskal–Wallis test.P-value <0.05 was considered statistically significant.

2.9 Operational Definition

Transfer Latency: Time in second each mouse enters into the safe or closed arm after being placed on one of the ends of the open arms

Arm entry: entrance of each mouse into the arms of the maze and was counted only when the four paws of each mouse entered each arm.

3 Results

3.8 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 of the extract 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 receivingKe 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.

	EPM task activities, M±SEM						
Group	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

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

Each point represents the mean ± 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 army entry in number (#) and CSD(%): percent central square duration.

3.9 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 300 mg/kg ($p<0.001$) doses of Ke in the right PFC (a) and it was only at Ke 300 mg/kg ($p<0.001$) in the left PFC (b) when compared to mice received T80W (**Figure 2**).

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 that 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 (**Figure 3**).

4 Discussion

In this study, the transfer latency to the closed arm was significantly reduced in mice that received khat extract. This indicates that mice administered with the extract returned to the closedarms more quickly than the control group and showed the presence of anxiety-like

behavior. It means that the natural aversion of mice to openareas and elevationwas worsened by the extract and showed the stress its stress effect. The previous report also indicated that amphetamine worsened stress responses in the elevated plus maze test (41). Simone *et al*. (17) indicated that quick entry to closed arms indicated the presence of stress and anxiety.

On the other hand, these were the lower and the higher doses of the extract that decreased the transfer latency in females. However, the lower dose of the extract didn't affect the elevated plus maze performance of males, revealing that females showed stress and anxiety-like behaviorsat the lower doseof khat extract than males.Variations in sex hormones, higher activation of brain areas (limbic system), and staying for a longer time involved in anxiety response in females than males, and variation in the neurotransmitter levels could be some of the reasons for females' stress and anxiety-like response to khat was higher than in males.

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 this extract and it has shown stress and anxiogenic effects. Another study also indicated that methamphetamine, cathinone in khat–like chemicals, and synthetic cathinone reduced open arm entries and duration (39, 42, and 43). 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 extract (16). Sestakova *et al*. (44) reported that rodents with lower locomotor activities revealed a greater level of anxiety and reduced exploratory behavior.

Effects of khat on dopamine (43), glutamate, and GABA transmissions (45, 46) could be attributed to the lower psychomotor and anxiety-like effects of extract 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 (47) and showed anxiety-like symptoms (48). A magnetic resonance spectroscopy study conducted on healthy humans showed that stress response reduced the level of GABA in the PFC (49). On the other hand, the administration of GABA (Gamma-aminobutyric acid)-transaminase inhibitor, vigabatrin, in rats showed anxiolytic-like behaviors in EPM (50). 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 returned into the closed arms. This means the approach-avoid conflict was high in mice administered with extract and avoidance is one of the symptoms of anxiety disorder and fear responses (51, 52). A previous study revealed that khat use was associated with enhanced conflict responses in humans (53). Cohen *et al*. (54) 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 extract, 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 (46), 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 (55, 56). On the other hand, Shawqi *et al*. (46) reported that khat reduced GABAergic transmission. At the same time, a previous study (49) reported that stress reduced GABA levels in the PFC. Alfaifi *et al*. (35) also

reported that ethanolic extraction of khat reduced the number of heads 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 (57). Previous reports showed that estrogen treatment increased stress-inducible C-fos mRNA (58) and women are markedly more vulnerable than men to the negative consequences of drugs and stressors (59, 60.).

The prefrontal cortex is one of the brain regions involved in anxiety-like behavior (61, 62). In this study, the prefrontal cortex lipid peroxidation, designated by malondialdehyde level, was significantly increased dose-dependently by the extract in mice. However, the differential effects of the extract on lipid peroxidation between the left and right prefrontal cortex were not observed. A study reported by Al-Zubairi *et al*. (34) showed that fasting plasma malondialdehyde level was non-significantly increased in human subjects chewing khat. The increase in prefrontal cortex malondialdehyde level in our study could be attributed to the reduction of antioxidant levels induced by the extract. A study conducted previously indicated that serum glutathione was reduced among khat users (63). 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*. (64) also reported

that the prefrontal cortexmalondialdehyde increased in rats administered with amphetamine at a dose dependable.

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5 Conclusions

Our study has shown that *Catha edulis* administered subchronicallytomice modelexhibited anxiety-like symptoms. It significantly increased prefrontal cortex malondialdehyde. The elevated plus maze performance was more affected by *Catha edulis*in females than in males, showing that the anxiety-like behavioral effect of*Catha edulis*was higher in females than in males.

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.

7. Declaration

7.1 Ethical approval and consent to participate

7.1.1 Ethical approval

The studies were approved by the 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 (36).

7.2 Availability of data: the data will be available when requested from the corresponding author

8. Abbreviation

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

9. Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

10. References

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Figure 1

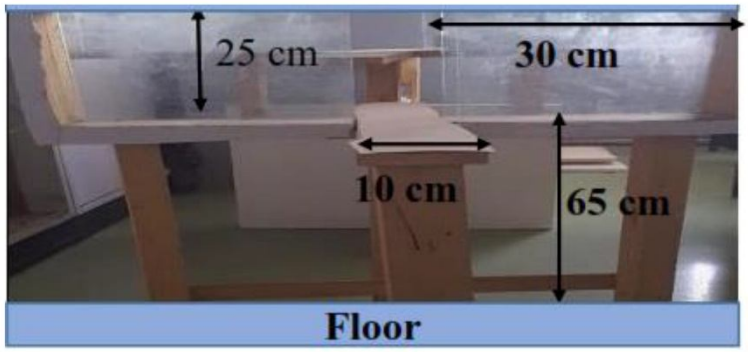

Fig 1- Floor plan

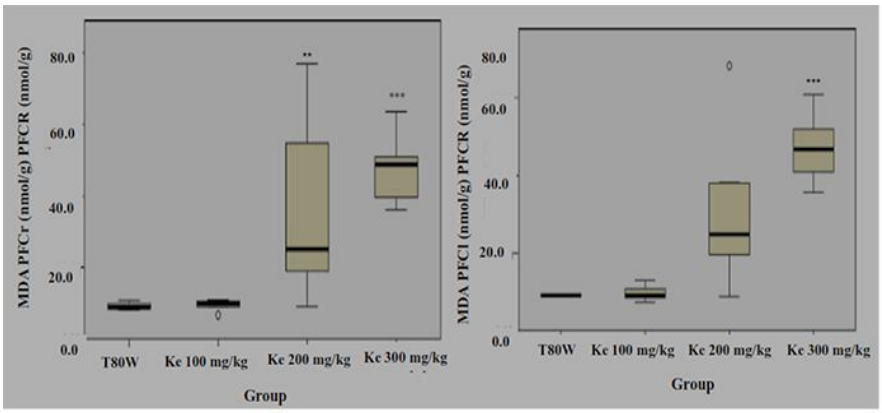

Fig 2- Box plot

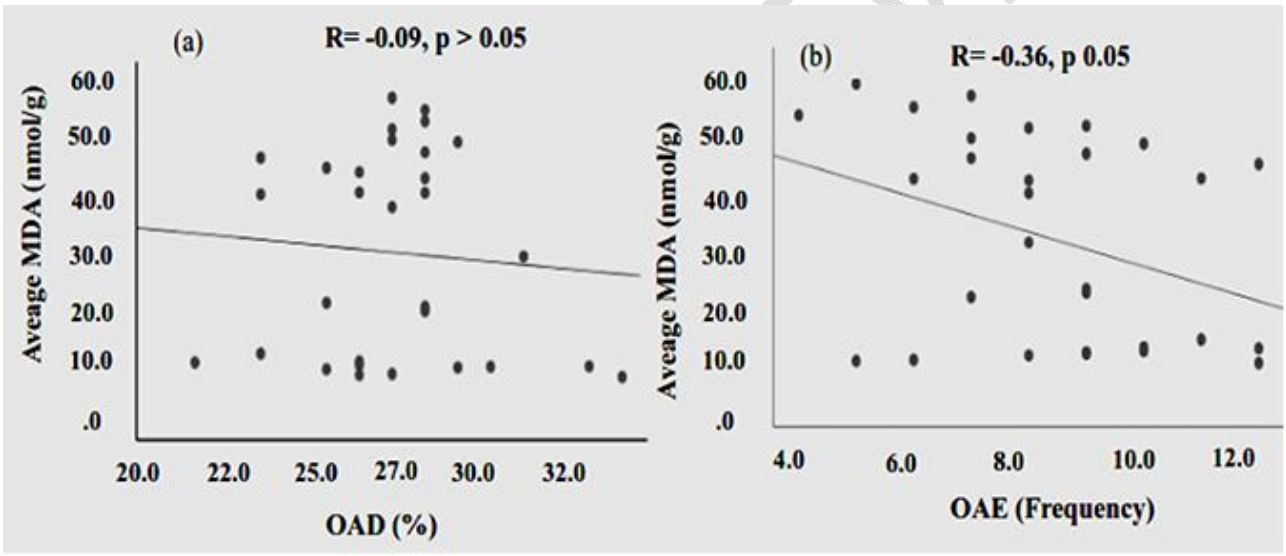

Fig 3- Result of regression analysis