

Review Form 3

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	Ms_IJTDH_130803
Title of the Manuscript:	Effects of Repeated Oral Administration of Catha edulis Extract on Anxiety-like Behavior and Prefrontal Cortex-Malondialdehyde Level and Sex Differences to the Responses in Mice
Type of the Article	Original Research Article

PART 1: Comments

	Reviewer's comment	Author's Feedback <i>(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.	The manuscript carries some scientific importance in terms of findings especially on regular use of khat and effects on brain areas influencing behaviour. 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 than, in part, influences anxiety-like behaviours among other psychostimulatory behaviours. The study also highlights sex as a co-variable in terms of effects of khat.	Incorporated in the introduction section of the manuscript (page 5)
Is the title of the article suitable? (If not please suggest an alternative title)	Title needs to be focused; it talks about 3 things: anxiety-like behavior, Malondialdehyde levels in prefrontal cortex and sex of mice. The title will appear more focused if it just talks about effects of khat on the brain in male and female mice	Corrected accordingly

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<p>Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.</p>	<p>What exactly is the problem that drove the study? Specification on sex of animals and grouping is lacking. What does increased levels of malondialdehyde in the brain mean owing to the understanding that khat is a psychostimulant?</p>	<p>Now it is incorporated in the background of the abstract</p> <p>Sex specification and grouping now included: 4 groups (n= 10 / group, 5 males, and 5 females/group</p> <p>What dose increased MDA?</p> <p>Now is mentioned in the manuscript as below:-</p> <p>The middle (200 mg/kg) and the higher (300 mg/kg)doses of extracts increased the right PFC MAD and only the higher dose (300 mg/kg) of the extract increased the lipid peroxidation in the left PFC</p>
<p>Is the manuscript scientifically, correct? Please write here.</p>	<p>The manuscript is generally good and results finding elaborate. However, a few areas of concern:</p> <ol style="list-style-type: none"> 1. There is need to specify sources and other details of the chemicals. Under section 2.3, how were housing conditions of temperature and humidity managed? This information is missed yet it is important especially on performance of animals hence can influence results. Under section 2.4, Why was tween 80 used for the control? Was the same vehicle used to reconstitute the khat doses? If so, please make it clear in the description. Was dosing done daily or how often? Under section 2.6, there is contradiction of information under 2.3 where you mention fresh leaves were prepared through freeze-drying for 2 days. How did you determine that you were strictly working with the prefrontal cortex? You have not mentioned about use of stereotactic atlas, for example. The process of brain tissue preparation for spectrophotometry is not clearly outlined; details are missed out. 2. There is need to add some literature on what khat does to the user in terms of metabolites produced hence observed effects. Some information of phytochemical composition of khat will provide the flow of information towards where the research is going. 3. Under section 2.8: Specify each statistical test for the measures analyzed. Kruskal-Wallis is mentioned under results but it is not illustrated here 4. Under results section, figure 2 is missed. 5. Under discussion, paragraph 1, authors state that female mice at lower dose showed stress and anxiety-like behaviors more than males but explanation is not given 6. Authors need to state how they determined anxiety and anxiogenic behaviours. What indicators were used to determine presence of such behaviours? These could have been just explorative behaviours, may be. 	<ol style="list-style-type: none"> 1. In 2.3, the fresh leaves free-drying for two days was initially to prepare the dry extract. In 2.6, it is not about the preparation of dry extract but was about the preparation of fresh extract solution by dissolving the dry extract with the 2% T80W. How did you determine that you were strictly working with the prefrontal cortex? The brain tissue was taken from the préfrontal cortex (PFC). Anatomically PFC is located in the anteriormost portion of the frontal lobe. After brain dissection, a sagittal cut down at the midline was made to separate the two hemispheres (the right and the left). Then, the olfactory bulb was removed. After that coronal cut directly in front of the tip of the corpus callosum was made to isolate the frontal cortex and then the front part of this cortex (PFC) was dissected. Generally, it was teamwork, anatomist was there. I, a physiologist, was part of the team. Thus, it was specialists engaged in dissection to isolate the PFC and homogenize the tissue from this section of the brain 2. Literature review included (page 3-4) 3. Kruskal-Wallis now is mentioned: as The non-parametric continuous variables obtained from the groups of mice were compared using Kruskal–Wallis test. 4. Figure 2 is mentioned: Page 10 5. The possible explanation is provide now 6. Anxiety and anxiogenic behaviours in this study was assessed using elevated plus maze (EPM). In this model, time spent in the closed arm, open arm and number of entries into the open arm was determined. In this case, a decrease in the proportion of entries into, and time spent in, the open arms indicates the presence of anxiety and showed the anxiogenic effect of khat. Generally, Transfer Latency, number of closed-arm entries , percentage of closed-arm duration, number of open-arm entries, percentage of open-arm duration, number of total arm entries and percentage of center square duration were assessed and used as indicators
<p>Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.</p>	<p>References are sufficient</p>	<p>Okay</p>

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Is the language/English quality of the article suitable for scholarly communications?	The language is suitable except a few grammatical errors pointed out in the manuscript	We tried to improve the language
Optional/General comments	The manuscript requires minor changes	Minor changes have been made

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details)	All procedures were performed following institutionally approved protocols by the Institutional Review Board at Addis Ababa University, No. 021/19/Physio). And mentioned in the manuscript . thanks