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** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
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.		
Is the title of the article suitable? (If not please suggest an alternative title)		
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.		
Is the manuscript scientifically, correct? Please write here.		
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.		

Review Form 3

Is the language/English quality of the article suitable for scholarly communications?		
Optional/General comments	This research article by Author presents comprehensive investigation into ***Catha edulis*** plant (commonly known as Khat) extract which contains psychoactive compounds to show stimulant effects in mice. People of East Africa often chew the leaves of ***Catha edulis plant*** for their stimulating effects, and it's known to affect the central nervous system. Author uses this plant extract (denoted by Ke) to investigate the anxiety-like effects of khat in connection with the PFC-MAD level in wild-type white albino mice of both sexes. MDA is a marker of oxidative stress and lipid peroxidation. The prefrontal cortex is involved in decision-making, emotion regulation, and higher cognitive functions. By assessing MDA levels, the study would explore if khat extract causes oxidative damage or stress in this area of the brain. The study design is well-structured, and the findings, demonstrating showed anxiety-like behaviors of plant extract in the elevated plus maze paradigm and increased prefrontal cortex malondialdehyde level in mice provide valuable insights into understanding how khat affects the brain at a molecular level could have implications for human health, especially in regions where khat consumption is prevalent. While the author effectively discusses the significance of their findings, further exploration of prefrontal cortex neurochemicals effects with lipid peroxidation would strengthen the overall contribution of the paper. Author uses two hundred grams of freeze-dried crushed leaves to prepare extract by using organic solvents, i.e., 300 ml diethyl ether and 100 ml chloroform (3:1v/v ratio) through stages like using a rotary shaker at 120 rpm, then filtered using I Whatman filter paper followed by evaporation and extract removable by through lyophilization. Although specification on instrument of lyophilization needed for better understanding of process. Further author use tween 80 in distilled water (T80W-V/V) as "vehicle group", and prepare three grade doses of khat extract (Ke) 100 mg/kg, 200 mg/kg, and 300 mg/kg. Mice as animal model used and divide 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 freshly prepared khat extract (Ke) 100 mg/kg, 200 mg/kg, and 300 mg/kg administered repeatedly through elevated plus maze (EPM) to evaluate the locomotor, exploratory, and anxiety-like effects of the extract. Author further demonstrated experiment on following parameter like 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 using statistical analysis(One-way ANOVA followed by Tukey Post Hoc analysis, Pearson's correlation, and independent t-test statistics). The main strengths of this paper is that it addresses an interesting and timely question, finds a novel solution based on a carefully selected set of rules, and provides a clear answer. As such this article represents an excellent statistical analysis to demonstrate PFC-MAD level associated with anxiety like behavior in animal model (mice), which will almost certainly influence our thinking about neurochemicals effects of PFC-MAD level as marker of oxidative stress and lipid	

	peroxidation for anxiolytic and anxiogenic effects in future for better understanding of neurodegenerative disorder. Another possible criticism could be that, to determine the maximum dose of any drug molecule toxicological studies is compulsory to be carried out which illustrate the minimum and maximum therapeutic range for drug, which is an important parameter, without toxicological studies, it remain only overall conclusion which can only allow statistical considerations. It could of course be argued that this is good enough to reach meaningful conclusions in specific case.	

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)***	

Reviewer Details:

Name:	**Aditi Jyotishi**
Department, University & Country	**Millenium College of Pharmacy, India**