Author's response to reviews

Title: Subacute administration of crude khat (Catha edulis F.) extract induces mild to moderate nephrotoxicity in rats

Authors:

Zewdneh Shewamene (zeedshow@gmail.com)
Ephrem Engidawork (ephrem.engidawork@gmail.com)

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Author's response to reviews: see over
Dear editor,

Thank you very much for the criticisms given by you and the reviewers. We have now taken into account all the suggestions forwarded and prepared the MS accordingly. We hope the MS is now in the form acceptable for publication in your esteemed journal and we hereby request your editorial hand. Please find below our response to the reviewers’ comments.

Reviewer 1 (Reem El-Naga)

1. Major comments

• What is new in this study?

Previous studies investigated toxic effects of khat administration based on measurements made on plasma or serum biomarkers (Ref 6, 7, 8, 9). The limitation of measuring circulating markers is that they could be derived from different sources, such as liver, kidney etc. In our study, antioxidant enzymes and lipid peroxidation marker were measured directly from renal tissue homogenates. This approach enables us to investigate khat-induced nephrotoxicity more specifically. Our study adds to the existing body of evidence in providing data that shed light on the potential of high dose of khat to produce nephrotoxicity.

• Why gentamicin and khat were given concomitantly?

Khat is widely chewed in Ethiopia and there are instances where khat is taken while an individual is on drugs prescribed for medical conditions. These drugs include drugs that are potentiall nephrotoxic. We wanted to see whether khat could have an antagonistic, additive/synergestic or permissive effect when given with nephrotoxic drugs. Gentamicin was chosen for tworeasons. Firstly, it is a nephrotoxic drug that is used for treatment of infections. Secondly, gentamicin-induced nephrotoxicity is a widely used animal model to study the ameliorating or accentuating effect of an agent on renal function.

• Mechanisms underlying the synergism between gentamycin and khat were not investigated in this study.
In this study, it was found that there is synergy between khat and gentamicin in inducing nephrotoxicity by measuring renal biomarkers. The biochemical mechanism(s) underlying this synergy should be addressed in future researches.

- **Sex-differences in the response**

There was no observed difference in the response between male and female rats. Now we have added a statement describing this result (first paragraph of the discussion).

- **Difference in duration of administration of the two agents**

Based on the literature on gentamicin-induced nephrotoxicity model, an extract in question has to be started 2 days before administration of gentamicin. This is important to investigate if the substance in question is able to produce a protective effect against gentamicin-induced nephrotoxicity.

- **Does 400 mg/kg have important pharmacological effects that prompted the authors to conduct the study?**

If you consult the literature, doses of khat used range from 100 mg/kg to 400 mg/kg. These doses are determined based on the amount of khat chewed daily by humans and pilot/acute toxicity studies performed in rodents. In this dose range, khat produced beneficial as well as harmful effects depending on dose and context of the study. For example, in a work done in our laboratory, subchronic administration of khat at doses of 100-400 mg/kg did not have effect on morphology and geometry of the dentate gyrus neurons, but shown to produce schizophrenic-like symptoms (MS in preparation). In addition, acute and subacute administration reversed haloperidol but not morphine-induced motor deficit (Geresu and Engidawork, 2010). One can see that the maximum dose used in rodents was 400 mg/kg. Thus, it is natural to include doses that are used in different studies to evaluate the effect of khat in the kidney. When we set out to do the study, we had the hypothesis that khat could have either an ameliorating or accentuating effect on gentamicin-induced nephrotoxicity. This was the reason why we did not think of using protective agents.

**Reviewer 2 (José Pedraza-Chaverri)**
1. Major compulsory revisions
   • More markers are needed

   Rest assured that no author can be able to exhaustively measure all parameters when s/he conducts a given study. In the present study, sufficient parameters were measured that would enable us to draw a conclusion/

   • More discussion about the specific khat compounds that may be involved in the renal damage is required

   Provided (highlighted)

2. Minor corrections:
   • Attend typo

   Eiosin is replaced with eosin; thio-barbituric is corrected as thiobarbituric and other spelling mistakes were thoroughly corrected.

Reviewer 3 (Mahmoud Rafieian-kopaei)

1. Enrich the discussion

   The discussion section is well revised based on recently published articles as recommended (highlighted)