Author’s response to reviews

Title: Acute oral toxicity and antioxidant studies of an amine-based diselenide

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BCAM-D-18-01189

Acute Liver toxicity tests and antioxidant profile of an amine-based diselenide

Mohammad Ibrahim, PhD; Niaz Muhammad; Musadiq Ibrahim; Muhammad Ishaq Ali Shah; Muhammad Said; Jean Paul Kamdem; Joao Batista Teixeira Rocha; Muhammad Idrees Khan

BMC Complementary and Alternative Medicine

Dear Dr. Ibrahim,

Your manuscript "Acute Liver toxicity tests and antioxidant profile of an amine-based diselenide" (BCAM-D-18-01189) has been assessed by our reviewers. They have raised a
number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Complementary and Alternative Medicine.

Their reports, together with any other comments, are below. Please also take a moment to check our website at https://bcam.editorialmanager.com/ for any additional comments that were saved as attachments. Please note that as BMC Complementary and Alternative Medicine has a policy of open peer review, you will be able to see the names of the reviewers.

If you are able to fully address these points, we would encourage you to submit a revised manuscript to BMC Complementary and Alternative Medicine.

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I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

Patrick Amoateng, PhD

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We would like to thank the reviewer for the suggestions and corrections made in the manuscript. We realize that your comments contribute to improve considerably the quality of the manuscript. The responses to the questions raised are given in sequence.

Editor Comments:

1. Research Justification.

The authors need to provide further information on the research justification. There are enough evidence suggesting that such selenium based compounds possess antioxidant properties,
however, the rationale for this compound should be justified further beyond just the general knowledge.

Response # Organoselenium compounds have gained global attention due to their potent pharmacological activities. APDP is a simple synthetic organoselenium compound widely reported to exhibit antioxidant, anti-inflammatory and neuroprotective effects in several chemically-induced toxicity and disease models [Ibrahim et al., 2012, lopes et al., 2012, 2015]. The antioxidant activity of APDP has been associated with its ability to mimic glutathione peroxidase [Ibrahim et al., 2015]. In another study APDP provide neuroprotection against 6-hydroxydopamine (6-OHDA) toxicity in differentiated human SH-SY5Y cells. However, there is paucity of scientific information of APDP on the acute toxicity model of mice. Therefore, the aim of the present study was to assess the acute toxicity of APDP in mice. it can be seen in the text under section. Background, line 98-109, page 4

2. Experimental Animals.

This section seems to provide information only for the animals used for the in vitro assay. Authors must revise this section to include the animals used for the in vivo assay. The numbers per experiment should be removed. However, such details can be provided when describing the exact procedure where they are used.

Response# Thank you for your valuable suggestion, Animal detail are revised and the numbers per experiment are removed, and mention is appropriate place.

3. Language.

There are several typographical and grammatical errors in the manuscript. Authors are advised to seek professional proof-reading.

Response# Thank Sir, The Paper has been checked by someone who is fluent in English and I hope now it will Ok for Publication.

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Reviewer reports:

SYED WADOOD ALI SHAH, M.Phil (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as
attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Karuppusamy Arunachalam (Reviewer 2):

This manuscript deals the effect of Acute Liver toxicity tests and antioxidant profile of an amine-based diselenide. It is very important study about toxicity and antioxidant activities of amine-based diselenide. However, the present study lacking of detailed pre-clinical experimental data as well as cytotoxicity analysis of the diselenide. Therefore, this study needs to be revised completely as per the following comments:

Inadequate title.
Response# The title has now been changed to "Acute oral toxicity and antioxidant studies of an amine-based diselenide" title page section, line 1, page 1

Abstract
In materials and methods, check the typographical mistakes.
Response# In materials and methods Section all the typographical mistakes are removed.
Indeed this study used rat or mice? Experimental section mentioned mice.
Response# Yes. As mention in the abstract the mice were used as experimental Model.

How the authors decided the diselenide toxicity effect based on the 72 h lethality test and conclude this study like…… in vitro and in vivo clearly demonstrated that this potential compound has no acute toxicity towards mice among all the tested parameter.

OECD guidelines recommend the acute toxicity study in animal 14 days with suitable vehicle control groups. After the treatment period, biochemical analysis, and liver may removed for macroscopic and histopathological analysis.
Response # Yes both Protocol/Model are used, but we have followed the experimental model which is already used by Avila et al., 2011. i.e.


Introduction

In this part authors should start with importance of the amine-based diselenide and corroborate with present study.

Response# Thanks for the suggestion, the same pattern is followed in the Introduction Part

Higher dose of selenium has some toxic effects but in this manuscript hide the information’s including nausea, vomiting, diarrhea, fatigue, and skin lesions.

Response# We have included the toxic effects of the organoselenium compounds with references.

Materials and methods

Every pre-clinical procedure should be planned. It should be included the sample concentrations, reagent preparation with absolute concentrations.

Response# It is now discussed in the procedure. Experimental animals section, line 131-136, page 5

Primarily, on what basis the acute liver toxicity study doses were selected?

Response# For dose selection mainly we followed the literature of organoselenium i.e the range already tested for toxicity of organoselenium.
Have you tested the in vitro cytotoxicity test before the start the acute toxicity study?
Response# yes. Please see the below the reference i.e lopes et al., 2012,

DMSO is toxic reagent for in vivo studies. What is the percentage used?
Response# Yes. It is safe up to 0.04% see reference below

Need more details of 1-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine synthesis process and identification and characterization.
Response# It is already published please see reference [Ibrahim et al., 2012, lopes et al., 2012, 2015].

For in vivo study design not clear.
Response# It is modified now i hope it will be clear.

In vitro experiments
The in vitro experiments were carried out to evaluate antioxidant effect of APDP, an organoselenium compound ……what are the in vitro assays? Where is the procedure and results?
Response# In vitro experiment we performed the TBARS assay see material and methods section and for ex vivo experiment we treated mice orally with APDP compound and the biochemistry were analyzed. Experimental animals section, line 227-231, page 9

How the TBARS assay results were expressed?
Response# % MDA values

What is the difference between TBARS assay and lipid peroxidation procedures?
Response# Both are same assays
What is the concentration of tissue homogenates and other reagents? Authors should clearly describe in all methods.

Response#

Liver tissues were immediately homogenized in cold 10mM Tris–HCl, pH 7.5 (1/10, w/v) with 10 up-and-down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 4000×g to yield a pellet and a low-speed supernatant (S1). An aliquot of 100 µl of S1 was incubated for 1 hour at 37 °C in the presence of both organodiselenide with and without the prooxidants (iron final concentration 10µM).

Need clear procedure for Non-Protein Thiols (NPSH) and δ-ALA-D activity assays.

Response# its now modified

How the δ-ALA-D activity results were expressed?

Response #

Liver δ-ALA-D activity was assayed according to the method of Sassa (1982) (Sassa, 1982), by measuring the rate of product (porphobilinogen, PBG) formation, except that 84mM potassium phosphate buffer, pH 6.4, and 2.5 mM ALA were used. All experiments were carried out after a 15 min preincubation of S1 with the medium, starting the reaction by adding the substrate, aminolevulinic acid. Incubation was carried out for 1 h at 37 °C. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of 6.1×104 M−1 for the Ehrlich-porphobilinogen salt. The reactions rates were linear with respect to time of incubation and added protein for all the experimental conditions. Simultaneously, a set of tubes was assayed in the presence of 8 mM of dithiothreitol (DTT) to observe the possible reversion of the δ-ALA-D inhibition.

Response #

Have you used positive and negative controls for all experiments?

Response# yes

Statistical analysis.....mentioned as all data were expressed as means ± S.D..............but Fig. 2 data show mean ± SEM values, which one is correct?

Response# all the data are statistically defined now.
Results

All the results were expressed in statistical significant p-values, the suggestion is use some experiment results in another way of expressions like % and times.

Response# all the data are statistically defined now.

In Table and figures…. all abbreviations should be explained in legend….Figures should be in self explanatory.

Response# ok now it is modified

Results section should be revised, reviewer do not able to understand the results because of the confusing words and sentences.

Response# now it is modified

Discussion

In discussion section many contradictory sentences for example……. our in vitro study to in vivo we demonstrated the acute oral toxicity study of amine-based diselenide 1-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine in mice at different doses (10mg/kg, 100mg/kg and 350mg/kg). This section completely rewrite and discuss the present study results with previous reports of 1-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine.

Response# is ok now it is modified Discussion section, line 270, page 10

Many citations missing in discussions part….e.g. our earlier publications related to the acute toxicity effects of diphenyl diselenide and binapthyl diselenide have reported that these compounds were nontoxic and showed promising pharmacological properties.

Response# now they are included

Conclusion

This section should be revised. Conclude the present study results with appropriate experimental data.

Response# ok It is now modified . Discussion section, line 314, page 12
Raghdaa Hamdan AlZarzour, PhD (Reviewer 3):

It seems that the manuscript is providing good tests for evaluating Liver toxicity and antioxidant profile. It is also written with very good academic language and flow of ideas. However, the following two points should be amended:

1. Why the tests didn't include Immunohistology or even histology for the liver samples. This is essential for evaluating liver toxicity and cannot be omitted.

Response: we have already published Immunohistology in our previous paper. lopes et al., 2012

2. For the Preparation of tissue homogenate for (TBARS) assay In page 5 line 36 it is mentioned that the homogenate was centrifuged for 10 min at 4000xg. usually the homogenate for this test should be centrifuged at 10000 g in an automatic high speed cold centrifuge for 30 min at 4C°. Please justify your method

Response: Please see the following reference. We have a well established this protocol in our lab.


4. Waseem Hassan, Mohammad Ibrahim, Joao Batista Teixeira Rocha, Towards the mechanism and comparative effect of diphenyl diselenide, diphenyl ditelluride and

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Declarations

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- Consent to publish
- Availability of data and materials
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