Author's response to reviews

Title: Effect of oral administration of ethanolic extract of Vitex negundo on thioacetamide-induced nephrotoxicity in rats

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Author's response to reviews: see over
Dear Editorial Board,

First, we would like to admire reviewers for the comments. We highly appreciated their time and attention to evaluate our work. Below, are the comments and our respective responses.

Best Regards,

Prof. Dr. Normadiah M. Kassim

**Report 1:**

1. **Authors want to indicate the effect of *Vitex negundo* on kidney disease? Then, authors need to discuss more about the effect of this plant extracts in the biological points of view.**

   Done as suggested. Please refer to the revised manuscript page 5.

2. **Materials and methods:**
   
   *Page 5: Please include the number of Voucher specimen.*

   Done as suggested. Please refer to the revised manuscript page 6.

   *Page 6:*

   1. **How much the % yield of plant extract**

      Percentage yield of VN crude extract was 18%. Please refer to the revised manuscript page 6.

   2. **The author used two dosages (100 mg/kg and 300 mg/kg). What are the rationales of using these two dosages?**

      A dose-response relationship describes how the likelihood and severity of adverse health effects (the responses) are related to the amount and condition of exposure to the drug or the agent (the dose provided). As the dose increases, the measured response also increases. At low doses there may be no response. Hence, 2 different doses of this plant in TAA-induced nephrotoxicity is used to determine the differences on the biochemical and histopathological parameters that can be obtained per feeding the rats. And many previous studies have been done using two doses [1-3].
2. The authors mention: 30 mg pure TAA which is in crystal form in 100 ml distilled water (0.03% w/v) until all the crystals were dissolved. The solution was given to the rats as their daily drinking water. Can you estimate the amount of TAA taken by the rat daily, I believe this will strengthen (the amount consumed per day) yours results and gives evidence about the effect of the plant.

Normally, the amount of daily water that can be taken by a rat of (200 g) body weight is about 10-20 ml. Therefore, the daily amount of TAA that can be given to the rat is about 3-6 mg.

3. Animals prepared by the National Academy of Sciences and outlined in the Guide for the Care and Use of laboratory Animals” were complied. Please add reference

Reference was added. Please refer to the revised manuscript page 7.

Page 7
- The kidneys were excised into two halves. One half was kept in isotonic formalin for determination of histopathological assessment and the other half was kept in freezer under -80 °C until processed for detection of MDA, CAT and SOD assays.

Page 8
- Kidney samples were dissected and washed immediately with ice cold saline to clean. Kidney homogenates (10 % w/v) were prepared by homogenizing kidney tissue.
- Why both statements are different? Please explain why you used different approach.

Rewritten. Please refer to the revised manuscript page 7 and 8.

Page 9
- For immunohistochemical examination, 4µm-thick paraffin-embedded tissues were deparaffinized and blocked for endogenous peroxidases and the slides immersed in 0.3% hydrogen peroxide in methanol for 20 min.
- Deparaffinized sections were treated in a microwave oven in the citrate buffer at 95 °C for 15 minutes, and immersed in 3% hydrogen peroxide in methanol for 10 min to abolish endogeneous peroxidase activities.
- Why both statements are different? Please make them clearer why you used different % and time

The paragraph was corrected. Please refer to the revised manuscript page 9 and 10.
3. Results
Page 11
Table 1 should be reconstructed as follow
• Body weight and kidney weight should be arranged in horizontal form, while
  Normal control, TAA, VN 100 + TAA, and VN300 + TTA should be arranged in
  vertical column form
• Replace the superscript letters with Asterisk symbol (*)

Done as suggested. Please refer to the revised manuscript page 22.

Page 11 & 12
• Table 2 & 3 do it similar to Table 1

Done as suggested. Please refer to the revised manuscript page 22 and 23.

4. Discussion
Page 15
• role in the development of many kidney diseases [4, 31]. The font is not the same
• Authors need to state the limitations of the work in few lines.

Done as suggested. Please refer to the revised manuscript page 14 and 15

Page 20 and 21
• Legends of the figures not clear which one fig.1
• Figure with 5 Photomicrographs of renal sections :
  • plz clarify which Ai: Normal control- cortical area, showing normal glomeruli
    (GL) with intact Bowman’s capsule (BM) and proximal convoluted tubules. Aii:
    Normal control - Medullary area of the, showing intact collecting ducts.

Done as suggested. Please refer to the revised manuscript page 21 and (Figure 1).
**Report 2:**

This is a well-planned study. Its findings would be important to those with closely related research interests. However English is poor. There are serious grammar mistakes. It needs corrections before being published.

Editing was done.

Information concerning SOD and CAT present in Results section should be placed in discussion section. It makes the Results section unnecessarily long. It can be accepted for publication after minor corrections

Done as suggested. Please refer to revised manuscript page 11 and 14.

**Editorial comments:**

- Please provide details in your manuscript on who formally identified the Vitex negundo used in your study.

  Done as suggested. Please refer to revised manuscript page 6.

- Please confirm whether a voucher specimen of the plant material used in your study has been deposited in a publicly available herbarium, and include a statement to this effect in your manuscript. You should also include a deposition number, if available.

  Done as suggested. Please refer to revised manuscript page 6. [3]

**References**


Notice:

All the authors are kindly request to take note from the following few changes:

1. The discussion part was rephrased.
2. Additional grant No is added (UM/MoHE/HIR Grant E000045-20001) replacing (HIR F000009-21001) as shown in the acknowledgments.
3. The whole manuscript was reedited and rewritten according to the journal format.

Thank you in advance your consideration,

Corresponding author,

Prof. Dr Normadiah M. Kassim