Author’s response to reviews

**Title:** Biochemical and histological alterations induced by the smoke of allethrin based mosquito coil on mice model

**Authors:**

Abdulla Al Mamun (mamungeb26@gmail.com)
Ataur Rahman (ataurm1@gmail.com)
Habibur Rahman (habib.geb.ru@gmail.com)
Faisal Hoque (kmfhoque03_gen@yahoo.com)
Zennat Ferdousi (ferdousi04@yahoo.com)
Abu Reza (rezaru@gmail.com)
Nurul Matin (nmatin2@yahoo.com)

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**Author’s response to reviews:**

Reviewer #1: In this paper, Al-Mamun and co-workers investigated the effects of exposure to smoke of allethrin based mosquito coil on a mice model, focusing on biochemical alterations in blood and histopathological alterations in lung and liver tissue.

The authors have used increased levels of exposure and found alterations in the mice subjected to higher exposure periods.

This paper deals with a relevant health issue in several countries as this type of insecticide is widely used and its side effects might be deleterious.

The overall experimental design seems appropriate for the aims of the study. Nevertheless, there are several issues that require clarification/correction:
Global issues:

1. There are many typos and mistakes (e.g. "vains" instead of "veins", etc). Thus, language editing is mandatory.

2. The quality of the photos, especially those of the lung are suboptimal and this needs correction.

Material and Methods

1. How do the conditions of exposure of the several groups of mice relate with conditions of human exposure, e.g. in terms of concentration of allethrin? It could be that the higher duration of exposure is not replicated in real life conditions.

2. Why alkaline phosphatase and/or bilirrubin were not included as biochemical parameters? Is there any indication in the literature? Could it provide additional information?

3. Concerning the histopathological evaluation, how many blocks were made from each organ? If only one how many sections were visualized and evaluated? Was all tissue from lungs and liver included for histopathological examination?

4. The authors performed ANOVA and student’s t test. This means that they assume a gaussian distribution of the values. How was this tested? Admitting that the distribution is, indeed, normal, then student’s T test is not appropriate for pairwise comparisons and a more stringent test must be used (e.g., Scheffe).

Results

1. The histopathological alterations reported are mostly qualitative. Were there quantitative differences?
Discussion

1. The discussion is too long and should be made shorter

2. In page 11, paragraph 3, a study with discordant findings is mentioned, but no reason for the discrepancies is provided. Please elaborate more.

3. The paragraphs about ROS and P53, are mostly speculative as the authors did not evaluate any of those parameters. This should be corrected.

4. How can the authors discard the possibility that the lesions and biochemical alterations found are due to allethrin and not to other compounds present in the coil (although they are considered inert and adjuvant)?

5. No pulmonary functional studies were performed and this is a limitation of the study that must be acknowledged.

Conclusion

1. No effect on cardiac function can be inferred from the data presented, thus this conclusion is not valid and must be removed.

Our responses

Global issue

1. The grammatical mistakes and poor sentences throughout the manuscript have been emended.
2. We used X40 objective lens during capturing the images and select the area of interest in the photo. However, according to reviewer suggestions, we have already increased the resolution of both the figures and embodied in the text.

Materials and method

1. We have used the commercially available mosquito coil from local market. The concentration of allethrine was same in all mosquito coil (MC) used in the experiment, but the duration of the treatment was different based on the groups. In south Asian region, people generally use mosquito coil as an easy and cheap option. The duration of burning of a typical MC is generally 8-10 hour. People in this region are exposed for prolong period with this insecticide regularly. Some people use MC for whole night, but some people use in the evening and early night inside the room to get rid of mosquito and use mosquito curtain only when sleeping. In our experiment, we created such mimicking condition for those people who use MC in evening and early night in different short duration based on their need. Therefore, we organized the current dose of treatment in this manner.

2. We did not do the alkaline phosphatase and/or bilirubin observation due to insufficiency of reagent. Previously, someone in our laboratory tested the status of bilirubin in mice exposed to the smoke of MC for 2 months, but did not find significant variation comparing with control. Therefore, we did not proceed to test these parameters.

3. I have made single blocks from each organ of each animal from each group, and two sections from each block. Therefore, there were in total thirty mice, sixty blocks and one hundred twenty sections for both the organs.

4. The data present here follow normal/ Gaussian distribution. We have checked the data using histogram and Shapiro-Wilk W test. Therefore, we performed student t-test for pair comparison. Additionally, we conducted “trend for p” test using regression method to evaluate the relationship between the effect and treatment doses as a continuous variable. Some statistical errors have been corrected.
Results:

1. I have used only one stain, hematoxylin and eosin and do not have advanced quantitative histological analytical techniques such as automated quantitative histological facility in our Lab. Therefore, quantitative histological analysis was not possible. There are numerous examples of published papers in different international journal, only used qualitative histological analisis. For example-Ricciardi BF, Nocon AA, Jerabek SA at al. BMC Clin Pathol. 2016;16:3.

Discussion:

1. The discussion part has been shorten as per reviewer suggestion. Some point with less related have been removed from the text.

2. The sentence pattern of page 11, paragraph 3 has been changed, showing information more accurately. The detail information is in reference no 28.

3. We did not test the over-expression of p53 and the generation of ROS in this study. But it is establish by some worker that allethrine based MC induce up-regulation of p53 and generation of ROS. We tried to establish the present outcome of tissue injury would be correlated with high level of p53 and ROS, using the relevant reference. However, we have reorganized the sentence describing about p53 and ROS by shortening.

4. MC composes of some biological and chemically inert material other than allethrine. These materials include wood powder, coconut shell powder and starch, are very natural and these materials might not causes of toxicity to living organism. Actually, there are many reports, published in different international journal, did not take the inert substances into consideration as a harmful substance. For example-


Therefore, we thought that we could discard the possibility that the lesions and biochemical alterations found are due to allethrin and not to other compounds present in the coil.

5. We have acknowledge regarding the point of debar from conducting pulmonary function analysis such x-ray (due to limitation of Lab facility) at the end of discussion section.

Conclusion:

1. Higher activity of some parameters of lipid profile including cholesterol indirectly indicates poor cardiac condition. However I have emended the sentence in conclusion by truncating this point.

Reviewer#2

General comments:

This manuscript describes the adverse health effects of allethrin from mosquito coil using mouse as an experimental animal. As previous study, it has been shown that allethrin may cause an inflammation or injury of lung and liver as well as biochemical alterations from long term exposed to mosquito coil in human and rats. Anyhow, this study provides some new data such as poor cardiac condition and apoptosis of hepatocytes. Finally, there are some points that deserve to be changed in order to improve the quality of the paper.

Text

1. Abstract

Although not absolutely necessary, it would be nice to show new finding such as apoptotic mediated cell death of hepatocyte or lipid alteration after inhalation in the abstract.

2. Materials and Methods
a. Why did you choose to expose mice with smoke of mosquito coil ½, 1, 2 and 3 hours respectively for 120 days? Idowu and his coworkers (2013) exposed rats with the duration 16 weeks or 112 days. The duration and results of this study and Idowu and his coworkers (2013) quite the same.

b. Why did you use mice as an experimental animal? Ventilation rate of human is around 12-20 breaths per minute, rat is around 85 breaths per minute, and mouse is around 160 breaths per minute. It means that mice inspire toxic agent more often than human and rat. Effects of more frequent contact may show more severity in the result of mouse injury than human and rat.

3. Results/Discussion
   a. Fig 1 and 2 have to improve the contrast, brightness and add the magnification.

4. Typing error:
   Please correct "vain" to "vein" in page 6 line 9, page 10 line 5 and page 25 line 39.

Our responses

Text:

1. According to reviewer suggestion to make better in the abstract, the new finding of this study such as apoptosis of hepatocyte and elevated activity of lipid profile have been described in the abstract with more emphasis.
Materials and methods

1. The duration of burning of a typical MC is generally 8-10 hour. People in this region are exposed for prolong period with this insecticide regularly. Some people use MC for whole night, but some people use in the evening and early night inside the room to get rid of mosquito and use mosquito curtain only when sleeping. In our experiment, we created such mimicking condition for those people who use MC in evening and early night in different short duration based on their need. Therefore, we organized the current dose of treatment in this manner.

2. Laboratory mouse shares 99 % of their genes with human being along with very similar biochemical organization and serve as a safe and reliable platform to manipulate the toxic substances in a manner which would be impossible to perform on live human. In different famous inhalation studies, mouse was used as model organism and these studies were conducted on the perspective of human physiology. The probability of mouse to be affected more severely with inhaling of toxic smoke rather than human can be addressed by a point that, in this study, mouse was treated for only four months but in human cases, a typical MC user uses MC for long time regularly even until death.

Result and discussion:

We have improve the contrast, and brightness of the fig. and used in the revised text. Moreover, some magnified portion has been added.

Typing mistakes:

All typing mistakes have been corrected and checked by English expart person.