Author’s response to reviews

Title: Acute and sub-acute toxicity study of a Pakistani polyherbal formulation

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Author’s response to reviews:

Respected Editor;

We are thankful for the reviewer’s cherished comments on our manuscript entitled “Acute and sub-acute toxicity study of a Pakistani polyherbal formulation” (Manuscript No. BCAM-D-16-01381R1). We appreciate the feedback given by the praiseworthy reviewer. These inputs really helped us to improve the manuscript. We have reframed the paper in response to the extensive and insightful reviewer comments and we hope that this will comply with the reviewer’s comments.

Answers to Reviewer’s comments

Reviewer 2 – Ian Musgrave

Significant interpretation issues:
In regard to the herbal preparation reducing liver enzymes

"So, if it is decreasing the liver enzymes in healthy animals, then this could be interpreted that the product might also be given to population suffering from liver disorders”.

This is not an appropriate interpretation at all. There is no link to suggest that the reduction in healthy enzyme levels will work in a pathological situation as you have no mechanistic link. Especially as later on the authors say "The decrease in the liver enzymes is due to the non-functional behavior of hepatocytes this happened due to the coalescence of the hepatocytes." If anything this suggests that this preparation could exacerbate liver damage. Please be more careful in your interpretations.

Answer: The interpretation was made on the basis of the product formulation. The label claims the presence of Embelia and Senna as an ingredients in the formulation that have proved to be effective in liver disorders [24, 25]. The formulation also contain chebulicmyrobalan yellow (Terminalia chebula), that has phenolic content which inhibits phenotype change in HSC in liver and reduces the infiltration of neutrophils in the liver and protecting liver from inflammational damage [26].

However your comment is appreciable as our sentence is not properly structured and presented, the changes in the normal liver enzymes cannot interpret the effects of the medicine in diseased state.

References:


AST and ALT are falling at the lowest dose of herbal is concerning. While no frank histopathology is seen, changes in enzymes may precede gross histopathological changes.

Answer: There is continuous oxidative stress naturally present inside the liver, as a result of which AST and ALT have a constant level inside the blood. The reason behind falling of enzymes at lower levels with lower doses (50 mg/kg and 100 mg/kg) is because the formulation ingredients contain flavonoids and phenolic contents. These species are reported to encounter the oxidative stress inside the body. As a result they are lowering the normally present oxidative stress due to which the enzymes levels fell but remained within the normal range. Therefore, no abnormal histopathology was observed at these doses.

From the first reviewer:

"The rodent NOEL needs to be converted to the human safe dose. …..[deletion]

Answer: The product is already been used clinically in Pakistani community at the dose of 3g as claimed by the label. The study was designed to scientifically prove the safety of the claimed dose and higher doses were chosen to assess the toxicity profile of the product."

The question is "whether 3 g dose clinically used is safe or not" and converting the NOEL to human values is important to answer this question.

Given that there is very little margin between the 50 mg/kg dose, and the 100 mg/kg dose where problematic changes are seen, the margin of safety would appear to be very low in this preparation. This should be more clearly indicated.

Answer: The doses are converted according to the following chart [1].

Dose 1 = 50 mg/kg/day

Dose for 0.15 kg rat = 50 x 0.15 = 7.5 mg/ 0.15 kg

Conversion into Human equivalent dose (HED) = 7.5 x 0.162 = 1.215 mg/kg

Dose for 60 kg (Avg.) human = 1.215 x 60 = 72.9 mg/ 60 kg man ≈ 75 mg / 60 kg

Dose 2 = 100 mg/kg/day

Dose for 0.15 kg rat = 100 x 0.15 = 15 mg/ 0.15 kg

Conversion into Human equivalent dose (HED) = 15 x 0.162 = 2.43 mg/kg

Dose for 60 kg (Avg.) human = 2.43 x 60 = 145.8 mg/ 60 kg man ≈ 150 mg / 60 kg

Dose 3 = 200 mg/kg/day
Dose for 0.15 kg rat = 200 x 0.15 = 30 mg/ 0.15 kg

Conversion into Human equivalent dose (HED) = 30 x 0.162 = 4.86 mg/kg

Dose for 60 kg (Avg.) human = 4.86 x 60 = 291.6 mg/ 60 kg man ≈ 300 mg / 60 kg

According to product’s regimen = 2 Tab. TID (Wt. of Tab. is 500 mg), it means 6 tablets a day

So, 50 mg/kg = 75 x 6 = 450 mg ≈ 500 mg / 60 kg / day

100 mg/kg = 150 x 6 = 900 mg ≈ 1000 mg / 60 kg / day

200 mg/kg = 300 x 6 = 1800 mg ≈ 2000 mg / 60 kg / day

The clinically used dose i.e. 3 g doesn’t appear to be safe. As from our study it was seen that the margin of safety is narrow that’s why the conclusion was made to prescribe 100 mg/kg cautiously to the patients unless otherwise the therapy should be continued with 50 mg/kg.

Reference


"There is no citation to show that the preparation being studied is effective in liver disorders.

Answer: The citation is added (http://qarshi.com/product/hab-e-kabad-noshadri/) in the introduction." This is just the website of a provider, there is no citation to clinical studies showing efficacy.

Answer: There are no clinical studies available on internet but the formulation is still being prescribed by the herbal practitioners on the basis of the individual ingredient pre-clinical data available. The pre-clinical data is mentioned in the discussion. The references of individual ingredients activities are mentioned below;

References


"Page 4, line 52: "Before 1800, when medicinal therapy was introduced in the scientific era, the herbal therapy was the only obvious choice" is better as "Before 1800, when science-based..."
medicinal therapy began to be introduced, herbal therapy was the only available choice” (this is not actually true, mineral drugs such as mercury were in use since medieval times, and the use of sulphur as a medicine dates back to classical Greece).

Answer: We highly appreciate your comments. We comply with your statement about sulphur and mercury. The sentence was taken from the book as mentioned in the reference "In PDR for Herbal Medicines." If you want us to remove or reframe, it will certainly be done.” The sentence should be reframed, especially if it was taken directly from the book.

Answer: The sentence is reframed. “In the 18th century, when the medicinal therapy era was being introduced, herbal treatment was the most preferred and available therapy.”

Page 8 line 66: "have proved to be very efficient in curing various sicknesses. For example; Digitalis (Foxglove) as cardiotonic for heart failure,” digitalis treats, but does not *cure* heart failure. This needs to be reworded. Also the most recent reviews on ginseng do not provide good evidence for cognitive support https://www.ncbi.nlm.nih.gov/pubmed/26268331 (let alone efficient "cure").

Answer: The sentence is reworded. The “curing,” is replaced with the word “management.” The line regarding, ‘Ginseng,’ is removed from the introduction.

Page 8 line 70. "Echinacea for the treatment of common cold etc." The most recent Cochrane systematic review (2014) https://www.ncbi.nlm.nih.gov/pubmed/24554461 concludes "Echinacea products have not here been shown to provide benefits for treating colds”.

Answer: The line regarding, ‘Echinacea,’ is removed from the introduction.

Page 21 line 323: "During the sub-acute toxicity studies 200 mg/kg/day showed significant decrease in the liver enzymes in male rats.” Liver enzymes decreased significantly at ALL doses in both male and female, if you intend to say the levels only fell below the reference range at 200 mg/kg/day you need to say that as well.

Answer: The sentence is reframed as directed. “During the sub-acute toxicity studies, a significant decrease in the liver enzymes with 200 mg/kg/day was observed in both male and female rats and this decrease was below the normal reference range.”

Page 22 line 351. "The 28 days sub-acute toxicity study, revealed no significant changes with 50 mg/kg/day." AST and ALT were significantly reduced. In light of the histopathology at higher doses this is concerning. Increasing body weight is also a concern, given there is no nutritional benefit to this preparation. Did the authors check for oedema? (This would be consistent with alterations in serum proteins leading to coagulation dysfunction at higher doses)

Answer: There was a gradual increase in the body weights of the male and female rats of both untreated (control) and treated groups (50 mg/kg/day, 100 mg/kg/day, 200 mg/kg/day) as shown in the Figure 1 and 2. The comparison of weight was made with their respective body weights from day 1. The increase was there as the animals were on standard pelleted diet throughout the
experiment. There was no nutritional value associated with the formulation. Besides, there wasn’t any oedema observed in the treated animals groups.

Page 22 line 353: "So, it is concluded that the formulation is safe to use at dose of 50 mg/kg/day for a period of 28 days" the 50 mg/kg dose and the 100 mg/kg dose is quite low, and should be commented on. "Whereas the 100 mg/kg/day should be cautiously employed" given the results, this recommendation is over optimistic. An explicit margin of safety should be calculated.

Answer: The recommended regimen of the product is 2 Tab. TID. The weight of the tablet is 500 mg. So, the prescribed daily dose is 3g/ day. According to our calculation human daily dose is approx. 500 mg to 1 g maximum for avg. 60 kg BW human, in three divided doses per day but as this research was conducted in normal animals so further scientific analysis should be done in different liver disorders to calculate the dose depending upon the severity of the condition. Our data provides a safe therapeutic range to be suggested to avoid toxicity.

The duration of 28 days was suggested on the basis that if high dose i.e. approx. 1 g for avg. 60 kg BW is recommended than of the therapy should be cautiously continued for longer period of time along with the analysis of LFT’s.

Grammar and typographical:

Abstract: "has a wide spread to people at risk of contracting the side effects of the herbal medicines" may be better as "is widely used in the general population exposing them to the risk of the side effects of the herbal medicines"

Answer: The sentence is reframed.

Page 8 line 58: "Many compounds from herbal origin have accomplished widespread appropriateness as medicinal agents" is better as "Many compounds from herbal origin have achieved widespread use as medicinal agents"

Answer: The sentence is reframed.

Page 8, line 69. Garlic is Allium sativum, not Zingiber (that is ginger).

Answer: It is corrected.