Reviewer’s report

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

Version: 1 Date: 11 Jun 2017

Reviewer: Ian Musgrave

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

The authors have done substantial work to accommodate the suggestions by the reviewers. The manuscript is substantially improved by their work.

There are still some issues that remain:

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 behaviour 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.

Page 10, Line 209: "The liver parameters of male and female rats of all treatment doses groups showed that the levels of ALT and AST declined highly significantly as compared to control at dose of 200 mg/kg/day". ALTH and AST were significantly lower at the 50 mg/kg and 100 mg/kg doses, even if they were still within the normal range. This is problematic.

The authors state in the response to the first reviewer "The decrease in the liver enzymes is due to the non-functional behaviour of hepatocytes this happened due to the coalescence of the hepatocytes. The (atrophy) shrinkage and fusion of the hepatocytes can be observed in the histopathological slides of the liver as well." While this applies to the highest dose, that fact that 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.

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 Pakistan community at the dose of 3g as claimed by the label. The study was designed to scientifically prove the safety of the claim dose and higher doses were chosen to assess the toxicity profile of the product.

The question is the "3 g dose clinically in use safe" 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.

"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 web site of a provider, there is no citation to clinical studies showing efficacy.

"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 and reframe, it will certainly be done." The sentence should be reframed, especially if it was taken directly from the book.

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").

Page 8 line 70. "Echinacea for the treatment of common cold71 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".

Page 21 line 323: "During the sub-acute toxicity studies 200 mg/kg/ day showed significant decrease in the324 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.

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)

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 5 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.

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"

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"

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

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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