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 would like to express our deepest gratitude for your response and reviewer’s valuable comments on our manuscript entitled “Acute and sub-acute toxicity study of a Pakistani polyherbal formulation” (Manuscript No. BCAM-D-16-01381). We appreciate the inputs given by the worthy reviewers. These inputs really helped us to improve the manuscript. We have modified the paper in response to the extensive and insightful reviewer comments and we hope that this will comply with the reviewer’s comments.

Editor’s Comments:

1) What is the dose and duration of treatment Soshiho-tang in people? It’s impossible to judge the applicability of the toxicity study without having this information.
Answer: The polyherbal product is Hab e Kabad Noshadri not Soshiho-tang as it was an example. The dose of the Hab e Kabad Noshadri as claimed by the label is 6 tablets a day for adults and 3 tablets a day for children. The exact duration of therapy of the product is not mentioned on the label and in this study we have identified the accurate dose based on the body weight using rat model. (http://qarshi.com/product/hab-e-kabad-noshadri/)

2) Why was a limit test selected for mice? Also why were males and females dosed? In standard practice, it’s usual to only dose the females unless one expects a different toxicity profile in males?

Answer: The limit test was scheduled for the mice because we had the information that 3g/ day dose of the polyherbal product is employed for adults clinically by the herbal practitioners, so limit test at one dose was carried out to find out the mortality in the animals.

The prevalence of liver dysfunctions is more in male population. Men are 2-fold more likely to die from chronic liver disease and cirrhosis than are women. Liver transplant occurs less commonly in women than in men. On average, there were twice as many liver cirrhosis male deaths as female deaths [1, 2]. Besides, the Animal ethical Committee of University and OECD guidelines for toxicity testing suggests the use of females, as they are more sensitive towards the toxic actions of the drug. Therefore, both genders were included to evaluate the safety profile of the polyherbal product.

References:


3) How were the doses for the rats determined?

Answers: The different doses selected for the toxicity study were chosen on the basis of the claim of the polyherbal formulation label (3g/day for adults). The 50mg/kg/day was selected according to the dose employed in adult human beings on daily basis. Higher doses (100 and 200 mg/kg/day) were selected to assess the toxicity profile for sub-acute toxicity study. The dose of the individual rats in all different groups was calculated based on the body weights before the start of the study.

4) Why were the clinical pathology parameters aimed at cholesterol and lipids?

Answer: Liver disorders generally results into impairment in the lipid metabolism [1]. Therefore, the lipid profile was assessed during the sub-acute study.
5) Stats: The change in weight over time needs to evaluate by a more appropriate stats model, since the study has repeated measures.

Answer: The change in the statistics weight of animals have been done as per your requirement.

6) Stats: For the clinical pathology parameters mentioned. The study relies on a parametric statistic for significance. Was normality of the parameters shown?

Answer: During the study a control group has been designed, the parameters of which are mentioned in tables (Table 2, 3, 4, 5 & 6).

7) Data presentation: Why it is important to present the results by sex for the subacute study, the combined result independent of sex needs to be provided.

Answer: Either sex can be used for the toxicity study. However both genders are treated as separate subgroups due to significant size differences [1]. That is why we presented the results by sex difference in the results.

Reference:


8) In table 4: What is the female groups control value for bilirubin, is It meant to be 1.0, or 0.1

Answer: The value of bilirubin control for females is 1.0, it is corrected in Table 4.

9) Mention is made to normal parameters: Where did this range come from. Was genetic drift taken into consideration when choosing the range?

Answer: Yes the normal parameters range was taken from Toxicological handbook and the genetic difference was taken into consideration [1].

Reference:


10) The study makes the conclusion that the produce decrease liver enzymes: I have major concerns with this statement. The study made use of healthy animals. Why would the animals have raised enzymes levels? If the assertion is that the animals were not healthy, then the entire study is flawed and the results cannot be used.
Answer: Healthy animals were incorporated in this study. When the study was carried out in healthy subjects it was observed during the study that 50 mg/kg/day and 100 mg/kg/day resulted into decrease in the liver enzymes but not below the lower range. So the enzyme were within the normal range but lesser as compared to the enzymatic levels of the control group at the end of the study.

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.

11) An explanation needs to be provided for the clinical signs seen of nasal bleeding. Also an explanation needs to be provide why the clotting cascade was not evaluated after this clinical finding. Also a link needs to be drawn between the clinical pathology parameters and the clinical signs.

Answer: Liver damage results into the decreased synthesis of plasma proteins that are responsible for the clotting [1,2]. Liver damage results into portal hypertension which consequently shows the complication of bleeding due to raised pressure [3]. Compromising renal and ESRD, also results in hemostatic disorders mainly in the form of bleeding diatheses [4]. The clotting cascade was not noticed due to limited funds. The right upper paw paralysis may be due to the neurodegenerative action of the formulation in the specific part of brain controlling the right upper paw of the test subjects.

The decrease in the liver enzymes is due to the non-functional behavior 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.

The link among the clinical pathology parameters is simple. A drug after oral administration enters into liver through portal blood before it reaches the systemic circulation. The drug is first exposed to liver therefore the clinical biochemical parameters of liver were selected to be assessed for toxicity study. Also, the liver is the main site for the synthesis and metabolism of lipid, that’s why they were also assessed. Similarly, the kidney is the main organ for the excretion of drugs. Therefore, renal parameters were also included.

References:


12) An explanation needs to be provided as to why this product is not a hepato-toxin and nephrotoxin. The decrease in enzyme activity and lipid profiles is an indication of toxicity. Consideration must be given that this is a toxicity test and not a test of efficacy.

Answer: The reason for not suggesting the product as hepatotoxic and nephrotoxic because the decrease in the liver enzymes and lipid profile parameters was observed at higher dose i.e. 100 and 200 mg/kg/day. So, the product is said to be toxic if the daily recommended dose is above 7g for a 70 kg man.

The higher doses were selected in order to design the toxicity study and to find out the possible adverse effects that the drugs shows at higher doses.

13) The safety of the compound has not been properly discussed. The rodent NOEL needs to be converted to the human safe dose. The rodent toxicity study are just a basic indication of toxicity, but does not consider interspecies differences.

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.

14) The study mentions that nephrotoxicity is possible with chronic use. What is the extrapolated safe period that this product can be used, based upon the 28 days duration of treatment of the rodents.

Answer: The product label does not state any duration of therapy. In our toxicity study we have identified that the product is safe when used for 28 days at dose of 50mg/kg/day.

Reviewer Reports:

Reviewer 1

We are thankful to Meenakshi Bajpai for her appreciable comments regarding the MS.

Reviewer 2

We appreciate the comments of Ian Musgrave regarding our MS.

There is no explanation given for why mice are used for acute toxicity then rats for sub chronic, why not the same species throughout? Also, in the discussion it reads as if only rats were used.

Answer: The animal Ethical committee of our University emphasize on the use of smallest weight animals for the acute toxicity study. Therefore we used Swiss Albino mice for acute toxicity and Wistar rats for chronic toxicity study.
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.

Page 4, line 53: "After the arrival of 'medical science' the phytotherapy was given the grade of alternative therapy." This is not actually true. Some phytherapies (fox glove, cinchona) were superseded because the pure compounds (digitalis, quinine) could be given reliable and accurately, others (salicylin in willow bark, yohimbine) were speeded by more effective compounds that were produced (acetylsalicylic acid, beta blockers). Some phytherapies are alternative medicines, but many became feedstocks for purified chemicals. Why is medical science in quotes?

Answer: We agree with your comments. The part of introduction is reframed.

Page 4, line 54: "Herbal therapy encompasses … Homeopathic." Homeopathic medicine is not herbal medicine, as there is no trace of the original compounds in the homeopathic medicines.

Answer: The word Homeopathic is deleted.

Problems with references

Page 4, line 60: "a number of botanical drugs, have proved to be very efficient in curing sicknesses [4]." Reference 4, "Subacute toxicity and stability of Soshiho-tang, a traditional herbal formula, in Sprague-Dawley rats" does not support this statement, but is a toxicological study of one herbal therapy.

Page 4, line 64: "These combinations are employed for the betterment of various chronic disorders [6]" Reference 6, "Acute and subacute toxicity studies on the polyherbal antidiabetic formulation Diakyur in experimental animal models" does not support this (and should be reference 7, as it supports the following sentence).

Page 4, line 71: "In addition to this, conventional people and even still certain physicians invoke the usage of medicinal 72 and curative herbs to aid the medication therapy for better clinical outcomes [8]" is not supported by reference 8, "Evaluation of acute and sub-chronic oral toxicity study of baker cleansers bitters - a polyherbal drug on experimental rats".

Reference 23 is in the wrong format and has no journal, volume of page number.

Answer: All the problems with the references are addressed properly.

Statistics

The data are expressed as mean and standard error of the mean, not "Values are expressed as standard error of mean" as is used throughout.
Answer: Corrected

"*** represents highly significant p<0.01" highly significant is not an appropriate statistical term
"*** represents significance of p<0.01" is more appropriate.

Answer: Corrected

Interpretation: Page 11, line 192 "This effect indicates that the formulation considerably decrease
the raised liver enzymes" the control values for ALT and AST are within the reference ranges of
normal rats, these values are not raised.

Answer: It was a typing error. The statement is reframed.

What is the normal dose of Hab e Kabad Noshadri for an adult human? How does this compare
to the doses in this study? As the preparation is to be given to people with liver disease, the
adverse liver effects must warrant more caution than suggested in this paper.

Answer: The study was designed based on the label claim of the product available in the
Pakistani market and is recommended by the herbal practitioners to the population majorly
suffering from hepatic disorders. The study was emphasized on the toxicity of the herbal product
at different doses on different organs rather its hepatoprotective action.

Minor issues

Page 2, line 1: "Acute and sub-acute toxicity study of Pakistani polyherbal formulation" would
be better as "Acute and sub-acute toxicity study of a Pakistani polyherbal formulation"

Answer: Corrected

Page 3 line 30: "methodology, has a wide spread to people at risk of contracting." Risk of
contracting what?

Answer: “has a wide spread to people at risk of contracting the side effects of the herbal
medicines.”

Page 3, line 31: "The aim of study was to assess the acute and sub-acute toxicity of polyherbal
formulation" is better as "The aim of this study was to assess the acute and sub-acute toxicity of
the polyherbal formulation"

Answer: Corrected

Page 3, line 32: "In acute study, single dose of 2000mg/kg was administered to the mice and
were observed for physical symptoms and behavioral changes for 72 hrs" would be better as "In
the acute arm of the study, a single dose of 2000mg/kg was administered to Swiss Albino mice
which were observed for physical symptoms and behavioral changes for 72 hrs".
Page 3, line 33: "In sub-acute toxicity studies repeated doses of polyherbal preparation was administered in rats of both genders, separately." Is better as "In the sub-acute toxicity studies repeated doses of the polyherbal preparation was administered to rats of both genders, separately."

Page 3, line 36: "On 28th day of experiment, blood sampling of animals were done" is better as "On the 28th day of experiment, blood sampling of animals was done"

Page 4, line 50: "Herbal medicines are focused as the popular therapies to treat diseases by the largest group of world population" do the authors mean something like "For most of the world's population, herbal medicines are the most popular form of therapy"? For many, it is the only available or affordable therapy.

Page 4, line 51: They have accomplished widespread appropriateness as medicinal agents." I am uncertain as to what this sentence means.

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.