Author’s response to reviews

Title: The effect of Liv-52 on liver ischemia reperfusion damage in rats

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Dear editor,

the revision made. The comments and the responses added. And the PMID added to the non-validated references.

Sincerely…

From editor;

1- justify dosage of test compound and discuss the chosen dosage (does it match dosages for clinical use?); discuss the pharmacological aspects in more depth;
Response: Dosage of test compound (Liv-52) in previous studies added to material method section. Clinical use added to the discussion section. The pharmacological aspect of Liv-52 needs to be enlightened. The pharmacology of medicinal plants extracts that Liv-52 contains studied as in PMID:
2- follow ARRIVE GUIDELINES very carefully in reporting the study; justify the low number of animals; revise methods as suggested by the referees;
Response: Arrive Guidelines followed, and methods revised as the reviewer’s commended. And according to the 3R(Reduction,Refinement,Replacement) rule, the minimum number of animals that would yield statistically significant results was used.
3- report all data in supplementary material, if needed;
Response: A table that contains all data added as the reviewers commended.
4- make sure high quality images are provided;
Response: It’s provided.
5- revise English style
Response: It’s revised.
Reviewer reports:
Giovanni Dothel (Reviewer 1):
• Phenolic compounds contained in herbal extracts are currently under study for their possible clinical application in anti-inflammatory therapy and, lately, in liver damage. Recently, Liv-52, an ayurvedic herbal formula, showed scavenging activities in hepatic oxidative stress. These properties are noteworthy in the therapy of surgery-related complications such as hepatic ischemia/reperfusion injury. In the present study the authors used a rat model of liver ischemia/reperfusion to analyze the activity of Liv-52 on hepatic enzymes and tissue morphology, through spectrophotometry and histology.
Although the adequate experimental design, some concerns arise from the methods and formal dissertation presented by the author:
Given the low experimental number (N=6) used, a table presenting raw data of the biochemical analysis could be added in the supplementary material, in order to ease a comparison with previous analogous studies (Oguz A PMID: 31436305; Kim SK PMID: 31493918)
Response: According to the 3R(Reduction,Refinement,Replacement) rule, the minimum number of animals that would yield statistically significant results was used. Also table including datas added.
• the number of experimental replicates are not clearly mentioned.
Response: The number of experimental replicates are mentioned in material method section.
• In addition, a formal revision, especially of the material and method, as well as the result paragraphs, is mandatory.
Response: The material and method and the result paragraphs revised as you commended.
Beibei Huang, Ph.D. (Reviewer 2): Researchers have studied the effect of Liv-52 on liver damage induced by ischaemia-reperfusion in rats. This topic fits the scope of Pharmacology and Toxicology and the results attractive, although its hepatoprotective effects has been reported for years that may reduce its attractiveness.
1. Some literature reported two major types of liver damage that are attributable to ischaemia-reperfusion (PMC3577927), have you consider it respectively and discuss the effect of Liv-52 on them separately.
Response: As it specified in the same literature (PMC3577927), “The activation of liver Kupffer cells and neutrophils, the production of cytokines and chemokines, the generation of ROS, increased expression of adhesion molecules and infiltration by circulating lymphocytes and/or monocytes are immunological cascades present in both types (warm and cold) of IRI.” And also the same literature “limited the discussion of other essential IRI pathogenic mechanisms, such as parenchymal cell death programmes, the complement system and the role of mitochondria in generating reactive oxygen species (ROS) and nitrogen species”.
As same in this study, we have limited our study area by evaluating the common mechanisms in both types (warm and cold) of IRI because the study area is very large and includes various mechanisms.
So we could not discuss the effect of Liv-52 on them separately.

2. In statistical analyses, what is Shapiro-Wilk test? Kindly explain why do this?
Response: Two well-known tests of normality, namely the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test. The Shapiro-Wilk Test is more appropriate for small sample sizes (< 50 samples), but can also handle sample sizes as large as 2000. For this reason, we will use the Shapiro-Wilk test as our numerical means of assessing normality. Also some studied reported that Shapiro-Wilk test has good power properties for a wide range of alternative distributions (Investigation of Four Different Normality Tests in Terms of Type 1 Error Rate and Power under Different Distributions by Oztuna et al.).

3. In discussion, any difference of the effect of Liv-52 between different species? Please explain how to extrapolate influence on rat to human.
Response: There studies in the literature explaining the hepatoprotective effect of Liv-52 in cirrhotic patients. (PMID: 16194047). Of course Further studies with appropriate dose ranging and in larger cohorts of patients are required to establish whether or not Liv-52 effective treatment modalities for organ IRI. Also we still need to establish how relevant currently used animal experimental models are for patients who receive liver transplants.
Please make the figures for the plots more demonstrative as well as attractive by using color.
Response: It’s done.