Author’s response to reviews

Title: Effects of pharmaceutical formulations containing thyme on carbon tetrachloride-induced liver injury in rats

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Author’s response to reviews:

Dear Reviewers,

Firstly, on behalf of the co-authors, I would like to thank you for helping us with your kind and precise comments and suggestions to improve our initial version of the manuscript.

We accepted the suggestions and they are included in this modified and improved version of our manuscript. We have highlighted these changes in the text.

In this letter we tried to state point by point the changes we made to the manuscript in accordance with your comments.
Reviewer #1:

1. Is the plant authenticate by any Botanist, If so then what is the specimen No?

- A voucher specimen of the plant (Thymus vulgaris L. 1753 subsp. vulgaris No 2-1516) was confirmed by a Botanist, which is also now stated in the manuscript.

2. Have you done Phytochemical Screening of the plant? If so then what are the active phytoconstituent which responsible for pharmacological response?

- Thymol and carvacrol, the main chemical constituents that, based on literature, mostly contribute to pharmacological effects of thyme, have been identified and quantified using gas chromatography coupled with mass spectrometric detection (GC/MS) and flame ionization detection (GC/FID). Several studies investigating the influence of thymol and carvacrol on hepatic function have been included in Discussion section, and have been compared to our results.

3. Without performing acute toxic study how you calculate the dose of the formulations?

- In determining the pharmacological dose of thyme preparations in rats, we used the guidance of US FDA and Center for Drug Evaluation and Research. Human doses were retrieved from Thyme ESCOP monograph, and then these doses were converted to doses for rats according to the Guidance. This is now also explained in the main text, and the proper reference was added.

4. Give proper reference regarding the dose of carbon tetrachloride.

- The proper reference (No. 27) related to the dose of carbon tetrachloride has been moved right after that information in the text.

5. Functional parameters like sleeping time, wet liver weight, wet liver volume also may be used for calculating hepato protective effect of any medicine.

- We have measured liver weights of rats after being sacrificed, but there were no significant changes and no differences between groups. Body weights for all experimental groups ranged from 276.3±26.2 g to 285.8±14.8 g, livers weighted from 9.6±0.9 g to 10.9±1.2 g, but the percentages of liver weights normalized to body weights were in much narrower range: from
3.5% to 3.8%, with no statistical differences between groups. This was expected since this is a model of acute liver injury and animals were sacrificed only 24h after administration of carbon tetrachloride. According to the literature, this method is much more suitable to the models of chronic hepatic damage. Thus, we chose not to present these results in the manuscript, since they are not applicable to a large degree to this model of hepatic injury.

6. There is no such specific table containing antioxidant parameters results.

- Free radical scavenging activity (RSC) and total phenolic content has been measured only for tincture (and ascorbic acid as a reference for RSC), and therefore we chose to present those results only in text, which we compared in Discussion with similar studies.

7. The histopathological slides not interrupted i.e. portal triad, total degeneration, central vein, sinusoids etc…

- We have largely rearranged the section ‘Influence of thyme preparations on liver histology’ within ‘Results’ and changed photomicrographs of the liver sections in order to provide more detailed explanation of our obtained results. Figure 3 is now more clear and has also been more in-depth explained.

8. Check and correct grammatical/spelling errors.

- All grammatical, spelling and other language-related errors have been corrected by the consultation with native English speaker.

Reviewer #2:

1. You could give some data on the culinary use of thyme.

- We accepted your suggestion and added some data on main uses of thyme in culinary and food processing. These changes are highlighted in the main text and references.

Our team hopes that we have managed to properly consider and act upon the comments and suggestions raised.
Yours sincerely,

Nebojša Pavlović, corresponding author