Review of: "Phytochemical Contents, GC-MS Analysis and Hepatoprotective Effect of the Methanol Leaf Extract of Camelliasinensis (L.) Kuntze on Paracetamol-Induced Liver Injury in Wistar Rats"

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Potential competing interests: No potential competing interests to declare.

The manuscript entitled, **The Use of Phytochemical, GC-MS Analysis and Hepatoprotective Effect of the Methanol Leaf Extract of Camellia Sinensis (L.) Kuntze on Paracetamol-Induced Liver Injury in Wistar Rats**, is interesting and creative in the implementation of methodology. It was carried out despite the limitations that one would assume the authors face in terms of infrastructure for research.

Nevertheless, its grave deficiencies in methodology and its precarious theoretical base (expressed in the introduction and discussion sections) raise serious questions about the validity of the findings. Thus, it is indispensable that the authors respond to the following points in order to clear up important questions as well as review and correct various sections of the manuscript.

Title:

I would suggest that the title not exceed 20 words. It is necessary to review the use of abbreviations. Additionally, the major variables mentioned in the title should be in agreement with those stated in the aims of the study in the abstract and introduction.

Introduction

The theoretical background information for this study, taken from previous reports, needs to be strengthened, including research related to phytochemical studies and hepatoprotection provided by the plant.

I don’t think the picture of the plant is necessary.

More bibliographic references should be consulted, including the following reports:

Pradhan, S., & Dubey, R. C. (2021). GC-MS analysis and molecular docking of bioactive compounds of Camellia sinensis and Camellia assamica. Archives of microbiology, 203(5), 2501–2510. https://doi.org/10.1007/s00203-021-02209-6

Hasan, M. R., Haque, M. M., Hoque, M. A., Sultana, S., Rahman, M. M., Ali Shaikh, M. A., & Sarker, M. K. U. (2023). Antioxidant activity study and GC-MS profiling of Camellia sinensis Linn. Heliyon, 10(1), e23514.

https://doi.org/10.1016/j.heliyon.2023.e23514

Salminen WF, Yang X, Shi Q, Greenhaw J, Davis K, Ali AA. Green tea extract can potentiate acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol. 2012 May;50(5):1439-46. doi: 10.1016/j.fct.2012.01.027. Epub 2012 Jan 28. PMID:

22306919.

Lu Y, Sun J, Petrova K, Yang X, Greenhaw J, Salminen WF, Beger RD, Schnackenberg LK. Metabolomics evaluation of the effects of green tea extract on acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol. 2013 Dec;62:707-21. doi: 10.1016/j.fct.2013.09.025. Epub 2013 Sep 27. PMID: 24080264.

Oz HS, Chen TS. Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity. Dig Dis Sci. 2008 Nov;53(11):2980-8. doi: 10.1007/s10620-008-0239-5. Epub 2008 Mar 29. PMID: 18373199.

After laying out an exhaustive analysis of the theoretical basis of the study, it is paramount that the authors explain the importance of this study to fill a scientific need for greater information in a certain area of knowledge. Please pay attention to the following questions when explaining the reason for the present study.

1. Why was this study designed and carried out despite the existence of various studies in the literature related to the qualities of hepatoprotection of this plant?
2. On what basis was the decision made to consider only the methanolic extract in the study?
3. Why was gas chromatography performed despite the existence of previous studies that report this procedure in relation to the plant?

Methodology

Why was the analysis of the plant conducted with stems and leaves together? How was that decided? Why didn't the authors carry out an extraction of each plant part separately?

It is essential to mention in the methodology of the extraction the degree of purity of alcohol and the percentage of water comprising the final mixture.

The *n* is missing for each experimental group.

Please explain in section 2.7 why the animals were selected at a weight of 150 g. The majority of previous studies were carried out with adult rats weighing 250 g.

It is crucial to expand upon section 2.8. How was this phase of the experiment performed? Please describe in a timely manner each of the parameters observed and how they were measured.

It is indispensable to substantiate why heterogeneous groups of male and female rats were used, and to state that if the hormonal condition influenced the results, it would be necessary to repeat the experiments, preferably with male rats.

It is essential that the authors substantiate in section 2.9 the reason for deciding on the particular dose of paracetamol used in the study and the reason for administering the compound for 8 weeks. The dose employed presently differs from that of previous reports, which administered paracetamol at a dose greater than 2 g daily in order to provoke hepatotoxic effects.

Results:

The results of section 2.8 are missing.

The images of the photographs included in Figure 3 should be improved to bring them up to a professional level (less pixelated). The main changes should be indicated with arrows or some other symbol. The images in 3d and 3e appear to have an amplification distinct from that of the other images.

To accurately measure histopathological changes (necrosis, steatosis, and fibrosis), with a suitable score, and graph the results properly, it is vital that the authors read about how to prepare tissues and evaluate them correctly, following a methodology similar to that described in the following study:

Feki, F., Mahmoudi, A., Denev, P., Feki, I., Ognyanov, M., Georgiev, Y., Choura, S., Chamkha, M., Trendafilova, A., & Sayadi, S. (2022). A jojoba (Simmondsia chinensis) seed cake extracts express hepatoprotective activity against paracetamol-induced toxicity in rats. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 153, 113371. https://doi.org/10.1016/j.biopha.2022.113371

Discussion

Based on the aforementioned points, the first paragraph of the discussion should be omitted, and the discussion of the principal results should be broadened. The results should be compared to those previously published, offering a possible explanation for the similarities and differences.

It is necessary to have a section on the limitations of the study.