

Review of: "Hepatoprotective Effect of the Ursolic Acid-Oleanolic Acid Mixture Administered Intragastrically in Mice with Liver Damage Induced by Anti-TB Drugs"

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Potential competing interests: No potential competing interests to declare.

I suggest that the manuscript will be rejected

It is undoubtedly an article that shows interesting data on the potential use of compounds of natural origin with a hepatoprotective effect on damage induced by antituberculosis drugs; however, it is almost equal to other studies conducted by the same authors such as Sordia-Reyes et al., 2021 and Gutierrez-Rebolledo et al., 2016. So, that this study can contribute something new in relation to Previous studies should include additional data about the pharmacokinetic, principally the difference in the absorption, of compounds when they are administered orally in comparison to when they are administered subcutaneously.

In addition, the authors should demonstrate the hepatoprotective effect of the ursolic acid and oleanolic acid by separated; as well as their combination but with known doses of each one since in the mixture of UA/OA derived from the methanolic extract of *Rosmarinus officinalis*, they do not know how much of each one drugs are present; and this is very important because it is necessary to know if the hepatoprotective effect is not due to the effect of only one of the drugs or if in another proportion of both drugs they could obtain a better effect. I think that the design of experiments that address these points could contribute much more to the potential hepatoprotective effect of UA/OA.

GENERAL Comments

1. The manuscript needs an exhaustive review of English, since there are several words in Spanish throughout the text and some phrases do not have the grammatical order in English
2. I suggest attaching a list of abbreviations at the beginning or at the end of the manuscript, because there are many abbreviations.

ABSTRACT

1. In the abstract there are missing methodological details and, in the results and conclusions in relation to the BWG they do not show differences between the animals of groups II, III, IV and V.

INTRODUCTION

1. The references that are indicate in the introduction do not correspond to the information they refer; For example, authors mention that the mixture of UA/OA induces antinociceptive and anti-inflammatory effects but the information

corresponds to references 23 to 25, not until 20 as it was mentioned in the fifth paragraph of the introduction.

2. In this same paragraph, authors refer to the results of a research of Gutierrez-Rebolledo et al., 2016 (15), but first they mention a dose of the mixture of 0.1 mg and, later, they put the dose of 0.1 µg, they must correct those details.
3. It seems to me that the justification of the work is not correct, because in previous studies, the same authors already demonstrated the usefulness of this mixture (UA/OA) for its hepatoprotective effect on the damage induced by antituberculosis drugs, as they refer to in the last paragraph of the introduction. They must reflect on the contribution they could be doing with this work unlike the other works that they already published (Gutierrez-Rebolledo et al., 2016 and Lordia-Reyes et al., 2021).

Materials and Methods

1. A subsection of “drugs” that mentions all the compounds used in this study, as well as its origin, its preparation and its therapeutic utility must be included before “in vivo trials”.
2. In the “In Vivo Assay”, it is necessary to mention the total number of animals that they used in this study and the specific conditions in which the animals were in the bioterio the 60 days that they were in treatment.
3. In the description of the experimental groups, I suggest to clear that the Group I is a group without liver damage (veh), the group II is the group with liver damage (negative control); Group III is the group with liver damage and a hepatoprotective drug (positive control) and the other two groups are those that have liver damage and the treatments that are being evaluated.
4. It is important to know, of the doses of 10 and 20 mg/kg of the mixture of UA/OA, what amount corresponds to each drug, since that is not clarified. Once this information is known, the corresponding dose of each drug must be administered separately to demonstrate if some of the drugs alone induce a hepatoprotective effect.
5. Lack to mention the amount of blood that was extracted of each mouse for the biochemical tests carried out.
6. In the “Histological Analysis” section, authors said: “This Trial was performed in accordance with that previously described by ...” I suggest that authors describe briefly the histological technique that they used so that it is not necessary to check the article they refer.
7. Authors mention a section of “Quantification of Paramers of Oxidative Stress”, but there are no results of this part in the manuscript.
8. In the statistical analysis section, lack to put the statistical justification of the number of animals used for each treatment, the number of animals in total used in this study and why for the body weight registration the authors initially used 10 animals per group and in the following tests the number of animals were decreased. In addition, authors mention that SEM is the standard error of the average, but at the feet of the figures it appears as E.S. or S.E.; correct this. Also, in this section an abbreviation of GPC appears, but authors did not put the meaning.

RESULTADOS

1. In the “Obtaining and identification of the UA/OA mixture” section, authors mention that the dust obtained from the R. Officinalis methanol extract had a fusion point of 265-269 ° C and that this melting point was compared to commercial standards of reference, but they do not mention what is the melting point of the UA or what is the OA; Or, will Sigma

sell the mixture of both compounds? In fact, the melting point is a physical property of pure substances, not mixtures; so I do not understand how this test can ensure that the mixture used in this study was composed of UA/OA.

2. In Figure 1, for a better identification of the result in the BWG of each treatment I suggest putting the treatments in the brands of each group instead of group I, Group II, etc. In addition, I do not understand very well why the authors used BWG instead of the body weights of the animals of the different experimental groups, since the authors did not explain how the BWG was calculated. In the same figure, authors did not mention what the "a" that appears in some points of the temporal course means.
3. On the other hand, in relation to the results presented in Figure 1, it is very strange that the animals with vehicle (Group I) did not show a body weight gain, on the contrary, they showed a weight loss in the course of time, so BWG values of this group were below zero; While, animals with anti-TB (Group II) show an important weight gain as shown in Figure 1. Exactly the opposite result was reported in the Gutierrez-Rebolledo et al., 2016 research, in which, the same procedure to produce hepatotoxicity was carried out.
4. The authors must explain how they obtain the relative weights of the organs that they showed in Table 1, since they do not explain it and, in the foot of the table they should put SEM instead of S.E. and vehicle instead of CMC 0.5% in Group I. In the same table, I suggest that the line that says Group I, Group II, etc. was deleted because in the next line of the table the treatments appear and is better understood with the treatments. In addition, in the description of Table 1, it should be clear that there are no differences in the "relative weights" of any of the organs with any treatment, since although there is a "slight increase" if it is not significant, it has no biological relevance.
5. In relation to the description of the results represented in Table 2, it is not necessary to put values in the text since the values are in the table.
6. On the other hand, the values of Creatinine and Urea on the vehicle, anti-TB and Anti-TB + SIL groups are exactly the same to those reported by Sordia-Reyes et al 2021; Although in the 2021 report the treatments were for 91 days and in this case animals were treated by 60 days; Could it be that authors used historical controls?
7. I do not understand why the values reported in Table 2 represents a n=3 and in other cases with a n = 5. Why did authors not used the blood samples of the 10 animals with the different treatments?
8. In the results of the histological analysis of livers, how do authors determine the degree of steatosis, liver hepatic hematopoiesis, the presence of microabese and the lymphoid infiltrate that they refer to in Table 3? By the way, I believe that the description of these damages can be described in the results, in a paragraph; and Table 3 must be eliminated.
9. In Figure 2, I suggest that instead of putting group I, Group II, etc., authors should put the treatments in each photograph; also, the authors must put the magnification in each image and arrows within the photographs that indicate the changes shown in animals with liver damage in comparison with animals in the vehicle group (without liver damage) to be able to identify them easily. Because according to the photographs, it seems that any treatment produced hepatoprotection.

DISCUSSION

1. In the first paragraph of the discussion, the authors said: "In this investigation, it was observed that the UA/OA mixture

(at 10 and 20 mg/kg) and SIL administered by i.g. route during 60 days favored BWG with regard to the anti-TB group and controls”; although this was not showed in the graph, rather it seems that only the group with the combination with 20 mg/kg has differences in some points in comparison to animals with liver damage. I think that authors must review the data showed in this figure; because seems that it have some mistakes that do not allow an adequacy interpretation of the results; For example: there are two symbols that represent Group IV and there is not Group V, so I suggest better putting the treatments in the graph instead of group I, Group II, etc.

2. Then, in the next part of the paragraph, where the authors said: "This behavior was similar to that reported previously by... “ is wrong, such as in the first place, behavior is not being evaluated, so the idea is not expressed properly. In second place, the results are the same to the previous reports that authors refer because they made the studies under the same experimental conditions only changed the route of administration of the UA/OA mixture, and just why their discussion should have directed it to the differences in the absorption of these compounds with respect to the Different routes of administration of these natural compounds, but casually authors did not discuss anything about this. On the other hand, the discussion of this manuscript does not contribute anything new to that of the articles of the same research group (Gutierrez-Rebolledo et al., 2016; and lordia-real et al 2021)
3. In the “Author contributions” appears two authors more than in the first page did not.
4. I don’t understand why appears “other references” that did not included in the references section.
5. Finally, I don’t know what means the letters in some references.