Carmen Ruiz, M.Sc.
IJERPH Editorial Office

Dear Ruiz:

Please find the revised version of our manuscript ID: ijerph-434373 entitled “Combined Effect of Diosgenin Along with Ezetimibe or Atorvastatin on the Fate of Labelled Bile Acid and Cholesterol in Hypercholesterolemic Rats” by Alejandro Marín-Medina, Gonzalo Ruíz-Hidalgo, Jorge L. Blé-Castillo, Alma M. Zetina-Esquível, Rodrigo Miranda-Zamora, Isela E. Juárez-Rojop *, Juan C. Díaz-Zagoya *, which we are re-submitting to be considered for possible publication in Int. J. Environ. Res. Public Health; section: Health Behavior, Chronic Disease and Health Promotion.

We have carefully considered the reviewers comments and Editor´s remarks as follows:

Reviewer 2

Comments and Suggestions for Authors: This manuscript needs a very deep revision to be reconsidered: Minor revision:

any imprecisions as for example line 67-68: “The aim of this study was to analyze the combined effect of DG along with ATV or EZT”, but authors analyze the effect of the single drugs and various combinations among them. Correct also legends adding the effect of combinations of drugs.

1. line 78, 0.5% or 5% as then reported in line 80? We have modified this part. (pag. 2; line 80)

2. Line 147, add” or normal diet”. Corrected

3. Table 2, “a” underlined, what does it mean? Or”,” before a letter? We have changed a for * and b for # in all manuscript tables.
Major revision:

Several mistakes about statistics if you consider what is written in the text respect to what is reported in tables. This makes very difficult to evaluate this manuscript. Just some example:

4. line 114, for HD+EZT group no statistics is reported in table 1. Corrected (pag. 3; table 1)

5. line 116, (p<0.0001, p<0.05 and p<0.0001; respectively) data not correct if I look at the table (b for all the groups)? We have changed (p<0.0001, p<0.05 and p<0.0001; respectively) for (p<0.05) in all manuscript.

6. line 128, p<0.001 or 0.0001? it is not written in table what value is “a”; somewhere in the text it is 0.0001. Corrected. We have improved the results section

7. line 177, (p< 0.05) but in table there it is not “b”. Done

- etc, etc, etc…….

About Discussion:

8. too long because authors report several information which should be in introduction, not in this section. We have improved the discussion section

9. line 213, authors refer to fecal excretion in a wrong context (Biochemical parameters) and they talk about stimulation by diosgenin of cholesterol excretion but in their results I don’t find this (table 4). we correct this paragraph (pag. 6; line 207-211)

10.There are a lot of data not discussed. For example, authors don’t discuss epididymis values that in some cases seem statistically significant. Then, line 229, “HD rats had 50% more activity in the liver, kidney and spinal cord than the same tissues in ND” …kidney? It doesn’t seem from table 2. We have modified this part and the results in epididymis and kidney were commented in the discussion (pag. 7; line 238-251)

11.Then, I would compare data obtained for not labeled and labeled cholesterol that means discuss about the effect of drugs on cholesterol coming from synthesis and absorption (not labeled) with respect to the effect on cholesterol from just diet(labeled). For example, line 232-233, “DG diminished 26-14C-
cholesterol activity in the liver and spinal cord”, compare this with data in table 1 and you will not find the same, it would be nice to discuss this. Moreover, data from spinal cord is not statistically relevant (see table 2). We have improved the discussion section.

12. Line 234 “ATV increased 26-14C-cholesterol activity detected in spinal cord”, please discuss why, in your opinion it is not the same for the combination ATV-EZT, considering also the effect of EZT alone. We have included in the discussion section (page 7; line: 237 and 238).

However, ATV significantly increases spinal cord activity possibly due to modification in the expression of the genes mentioned in the discussion section. Analyzing the results, we observed that the group with EZT has a similar effect on the kidney; the effect of certain drugs on the expression of proteins is known, in the case for example of CYP genes it can be observed that one substance increases its expression and another decreases it. Considering that effect and since there is no work that studies that effect in relation to ATV, EZT and cholesterol transport, we consider that when combining ATV with EZT somehow this effect is lost on the transporters of cholesterol in the spinal cord and the kidney, however further studies are needed in order to answer this question more appropriately. We appreciate your observation that helps enrich future goals in the study of cholesterol metabolism.

13. Line 252, “we observed increased fecal excretion of neutral sterols in DG treated rats”, it doesn’t seem from table 4. Corrected. We referred to the increase in the concentration of labelled taurocholic acid in the intestine of DG rats.

Best regards

Juan C. Diaz-Zagoya