

January 8, 2018, México, D.F.

Dear Editor, Li-Jun Cui,
Science Editor, Editorial Office
Baishideng Publishing Group Inc
World Journal of Hepatology

Dear Dr. Li-Jun Cui,

We are very pleased with the results of the evaluation conducted by the Scientific Committee of your renowned journal of our manuscript entitled "*Morphological and biochemical effects of weekend alcohol consumption in rats. Role of concentration and gender*", and have carried out the task of responding to the well-targeted and greatly appreciated observations provided by the reviewers.

In this document, please find, in yellow, the changes kindly suggested by the Reviewers, which now appear as changes in the new version of the manuscript. Many thanks.

We follow here with the corrections and suggestions that we carried out on the article, taking into account the reviewer commentary:

Responses to the observations of the Editor:

1. Information has now been added regarding the following: Animal care and use statement and Biostatistics statement.
- 2.- The section was completed article highlights
- 3.- We verified the bibliographical references, put these into the journal's format, and PMID and DOI were added, where pertinent.
- 4.- We carry out and add the requested files

REVIEWER 1:

Major comments Morales-González JA et al. performed an animal experiments to investigate the risk of weekend alcohol consumption This study encompasses an interesting topic, but some critical problems are involved in this study. The manuscript may need some language editing. Major comments 1. The title looks like a review. Please indicate the result of the study and that this manuscript is an animal experiment in the title

Response: Thank you for your suggestion. The title has now been modified according to the commentaries of the Reviewer, in the following manner: *Morphological and biochemical effects of weekend alcohol consumption in rats. Role of concentration and gender.*

2. The introduction looks like a mini-review. Authors should focus the background and aim in the introduction section. Please summarize the background of the study and rewrite the introduction section.

Response: We have shortened the Introduction section, which is now less than two pages long, highlighting the experimental groups. Thank you.

3. Authors should separate males and females in the control group. I think the body weights were different among males and females in the control group.

Response: Thanks for the observation. The body weights of the control groups, both in females and males, were very similar; at the beginning as at the end of the experiment. Not having statistically significant differences. Therefore, the authors decided to leave table 1, without modifications. Thank you

4. The concentration of cholesterol and triacylglycerols in the liver should be measured after lipid extraction. FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957 May;226(1):497-509.

Response: Thank you for your suggestion. We are planning this type of determination for future investigations. One way of correlating serum levels of triglycerides and cholesterol with their levels in the liver is the histological study, where we reported good correlation in these two compartments (blood and liver). This is explained in the Discussion section, in which the findings of Tables 2 and 4 are correlated. In addition, the latter is supported by previous reports by Bhopale et al. (2017), Parra Vizuet et al. (2009), Morales-González et al. (2001), and Hernández-Muñoz et al. (1978). Thank you.

Bhopale KK, Amer SM, Kaphalia L, Soman KV, Wiktorowicz JE, Shakeel Ansari GA, Kaphalia BS. Proteomic Profiling of Liver and Plasma in Chronic Ethanol Feeding Model of Hepatic Alcohol Dehydrogenase-Deficient Deer Mice. *Alcohol Clin Exp Res* 2017; **41**: 1675-1685.

Parra-Vizuet J, Camacho LA, Madrigal-Santillán E, Bautista-Ávila M, Esquivel-Soto J, Esquivel-Chirino C, García-Luna-y- González-Rubio M, Mendoza-Pérez JA, Chanona-Pérez J and Morales-González JA. Hepatoprotective effects of glycine and vitamin E, during the early phase of liver regeneration in the rat. *Afr J Pharm Pharmacol* 2009; **3**: 384-390

Morales-González JA, Jiménez-García LF, Guitérrez-Salinas J, Sepúlveda J, Leija-Salas A, Hernández-Muñoz R. Effects of ethanol administration on hepatocellular ultrastructure of regenerating liver induced by partial hepatectomy. *Dig Dis Sci* 2001; **46** :360-9

Hernández-Muñoz R, Santamaría A, García-Sáinz JA, Piña E, Chagoya de Sánchez V. On the mechanism of ethanol-induced fatty liver and its reversibility by adenosine. *Arch Biochem Biophys* 1978; **190**: 155-62

5. Authors presented the continuous variables as the mean \pm SEM. I think the continuous variables are non-parametric data. If so, Student t test is not suitable for

non-parametric data. Please reanalysis non-parametric data using non-parametric analysis, e.g. u test. When authors compare variables among more than 3 groups, authors used ANOVA. Please use non-parametric analysis as a post-hoc analysis, e.g. Steel Dwass test.

Response: Thank you for your commentary and suggestion. According to Dr. Tomás Fregoso-Aguilar, it is considered appropriate to analyze the data with the parametric Student *t* test because knowledge is desired concerning whether there are differences between the average values of the different groups only at the beginning and at the end of the experiment, comparing these with the average values of the independent group that served as control, and not against the groups themselves; this way of analyzing is adjusted to that established for a comparison test of two populational means (Gosset, 1908).

In the same manner, the data of the measurements of the different metabolic parameters were analyzed by means of the unifactorial Analysis Of VAriance (ANOVA) parametric test because these were groups that were independent of each other, for which we determined the serum values of glucose, triglycerides, triacylglycerides, bilirubin, and albumin; these values are quantitative and, normally, their measurement in scientific articles is the value of the mean \pm standard error of the mean (quantitative variables and not qualitative). This form of analysis is adjusted to the criteria established for this test (Fisher, 1966) in the design of an experiment using quantitative variables and the Student-Newman-Keuls method as post-hoc evaluation for multiple comparisons.

José Antonio Morales González, Jorge Alberto Mendoza Pérez, Jorge Chanona Pérez, Sergio Odín Flores Valle, Eduardo Osiris Madrigal Santillán, Pablo Ligerio Martínez Risco, Ángel Morales González. *Medio ambiente y salud*. (2009). 400 pp. Ed. UAEH ISBN-978-607-482-063-8. NLM ID: 101621056 [Book]

Fisher R.A. (1966). The desing of experiments. 8th ed. Oliver and Boyd, Edinburgh. pags. 3 – 18-

Gosset W.S. ("Student"). (1908). The probable error of a mean. *Biometrika*. 6 (1): 1 – 25.

6. Authors should separate males and females in the control group. I think the body weights were different among males and females in the control group.

Response: Thanks for the observation. The body weights of the control groups, both in females and males, were very similar; at the beginning as at the end of the experiment. Not having statistically significant differences. Therefore, the authors decided to leave table 1, without modifications. Thank you.

7. The principal finding of the study was that the weekend alcohol consumption at 5% caused the body weight gain. Authors explained that the metabolism of the first pass of ethanol increased the body weight. However, the appetite may be increased by the weekend alcohol consumption at 5%. Did authors measure the daily volume of diet in each group? If so, please indicate it. If not so, please discuss the possible relationship between appetite and weekend alcohol consumption.

Response: This is very important commentary for us, many thanks. We quantified the food consumption in each group and did not find significant differences in the food consumption in any group. Our results are in agreement with that reported by Nu Rocha et al. wherein, after 10 weeks of alcohol consumption (3 days per week at 30% ad libitum), the authors found very similar food consumption between the control group and the group with alcohol consumption. However, the group with ethanol consumption exhibited a greater weight gain than the control. Therefore, we think that it is the alcohol and its metabolism that is the cause of the increase in weight, in that fat levels are altered in the liver, as mentioned in the Discussion section, and as follows: "It has been reported that ethanol favors hepatic stenosis, probably due to the increase of lipogenesis, diminution of the transport of lipids of the liver, and alteration of oxidation of the fattyacids (Bhopale et al.)", the latter in agreement with prior reports of our investigation group (Piña et al., 2003; Morales-González, et al., 1999; 2001).

Rocha KK, Souza GA, Seiva FR, Ebaid GX, Novelli EL. Weekend ethanol consumption and high-sucrose diet: resveratrol effects on energy expenditure, substrate oxidation, lipid profile, oxidative stress and hepatic energy metabolism. *Alcohol Alcohol* 2011; **46**: 10-6.

Bhopale KK, Amer SM, Kaphalia L, Soman KV, Wiktorowicz JE, Shakeel Ansari GA, Kaphalia BS. Proteomic Profiling of Liver and Plasma in Chronic Ethanol Feeding Model of Hepatic Alcohol Dehydrogenase-Deficient Deer Mice. *Alcohol Clin Exp Res* 2017; **41**: 1675-1685.

Piña Garza E, Gutiérrez Salinas J, Morales González JA, Zentella de Piña M. ¿Es tóxico el alcohol? In: *Temas Bioquímicos de vanguardia*. Riveros Rosas, H.; Flores Herrera, O.; Sosa Peinado, A.; Vázquez Contreras, E. Eds. Facultad de Medicina UNAM. México, **2003**; pp 121-146.

Morales-González JA, Gutiérrez-Salinas J, Yáñez L, Villagómez-Rico C, Badillo-Romero J, Hernández-Muñoz R. Morphological and biochemical effects of a low ethanol dose on rat liver regeneration: role of route and timing of administration. *Dig. Dis. Sci.* 1999; **44**: 1963-1974. [PMID: 10548344]

Morales-González JA, Jiménez-García LF, Gutiérrez-Salinas J, Sepúlveda J, Leija-Salas A, Hernández-Muñoz R. Effects of ethanol administration on hepatocellular ultrastructure of regenerating liver induced by partial hepatectomy. *Dig Dis Sci* 2001; **46** :360-9. [PMID: 11281186]

Minor comments 8. Page 6; "a average alcohol consumption of 4.56 g per day" → "an average alcohol consumption of 4.56 g per day"

Response: Thank you for your observation. This change has been made. Thank you very much.

9. Page 7; "Some reports describe the histologic damage that alcohol causes to the liver[15-17] but, to our knowledge, there are no reports on the histologic changes caused by weekend alcohol consumption to the liver. " → "Some reports describe the histologic damage that alcohol causes to the liver[15-17] , but, to our knowledge, there are no reports on the histologic changes caused by weekend alcohol consumption to the liver. "

Response: Thank you for your observation. This change has been made. Thank you very much.

10. Page 7; "Reagents All other chemical reagents were obtained from Merck (Merck de México, S.A.) and were of the best quality available." What does "all other chemical reagents" mean?

Response: Thank you for your comment. You are correct. The correct way is the following: Reagents all chemical reagents were obtained from Merck (Merck de México, S.A.) and were of the best quality available.
This has now been corrected. Thank you.

REVIEWER 2:

Since this is a preclinical study, please modify the discussion and discuss mostly preclinical studies rather than clinical studies. The introduction should also be shortened.

Response: Thank you for your commentary. In the Introduction section, we summarized and annotated the clinical reports in a sole paragraph. These are mentioned in order to understand the importance of the study, leaving the Introduction section at less than two pages in length. In the Discussion section, there is a predominance of pre-clinical (experimental) studies, which are employed to discuss our findings. We have indeed mentioned some clinical reports, but only to understand the importance of this study, in the Initial paragraph, while the pre-clinical (experimental) reports are those utilized to compare and explain our results and these occupy more than 90% of the Discussion section, the extension of which is less than three pages. Thank you.

As a general point, we wish to mention that the observation on the editing of the article in English has been punctiliously attended to, and a new review of the language of the entire manuscript has been carried out in order for this to be adequate.

We hope that our responses to the reviewers' observations are satisfactory and are available at any time for doubts and concerns related with this new version of our manuscript.

Sincerely yours,

José Antonio Morales, MD, PhD.

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