

Author's Response to Decision Letter for (Manuscript NO.: 69646, Basic Study)

Dear Editor Lian-Sheng Ma,

Thank you for giving us the opportunity to submit a revised draft of our manuscript for publication in the World Journal of Gastrointestinal Pharmacology and Therapeutics.

We appreciate the time and effort you and the reviewers have taken to provide feedback on our manuscript and we are grateful for insightful comments and valuable improvements to our article. We incorporate the suggestions made by the reviewer.

Below is a point-by-point response to the comments and concerns raised after the review.

Response to reviewer:

The original finding is completely lacking in the study design, as many research have already proved this research question and provide enough mechanism evidence. You need to provide adequate reasoning as how this study is different and have novelty in providing new scientific information.

Thank you for your comment.

The original aim of this study was to evaluate the effect of MLT on CCL₄-induced liver injury in rats in terms of oxidative stress, reticular stress, and cell damage.

Our results demonstrate that the effect of CCL₄ induced liver damage, with accumulation of free radicals, damage to lipid membranes, imbalance in antioxidant enzymes, reticulum stress, cell redox imbalance and zinc deficiency, according to the proposed objective. Administration of MLT reversed the damage observed in all parameters studied. In the current literature, there are few studies

that use the CCL₄ model and evaluate the action of MLT on reticulum stress (ATF6, GRP78), on antioxidant enzyme promoters (Keap1-Nrf2), on cellular redox homeostasis (HSP70 and HSF1) and in blood levels of zinc, as we have shown.

Hu,C., Zhao L., Tao J.and Li, L. – Protective role of melatonina in Early-stage and end-stage liver cirrhosis J. Cell Mol Med.2019;23:7151-7162

Wu Y, Li Z. Xiu Ai-Yuan,Meng D, Wang Si, Zang C. Carvedilol attenuates carbon tetrachloride-induced liver fibrosis and hepatic sinusoidal capillarization in mice. Drug Desing, Development and Therapy ,2019;13.2667-2676

Ustuner D,Kolac U, Ustuner M et al. Naringerin Ameliorate carbon tetrachloride- induced Hepati damage through inhibition of endoplasmic reticulum stress and autophagy in rats. Journal of Medicinal Food ,;23 (11), 2020

Kopustinskiene ,D and Bernatoniene, J. Molecular Mechanism of Melatonin -mediated Cell Protection and Signaling in Health and Disease. Pharmaceutics 2021,13,129.

This work is part of a line of research with this experimental model, and complements a set of evaluations of pathophysiological pathways, already demonstrated (oxidative stress [damage to lipids and proteins], antioxidant enzymes, inflammatory process, fibrogenesis, angiogenesis, histological evaluations of the liver and DNA damage): Bona, S. et al. Antifibrogenic effect of melatonina in rats with experimental liver cirrhosis induced by carbon tetrachloride . JGH , 2018.

We have experience in works evaluating cirrhosis through different experimental models and also with the use of melatonin in several study models: Rosa, Carlos Gustavo Sakuno ; Colares, Josieli Raskopf ; Da Fonseca, Sandielly Rebeca Benitez; Martins, Gabriela Dos Santos ; Miguel, Fabiano Moraes ; Dias, Alexandre Simões; Marroni, Cláudio Augusto ; Picada, Jaqueline Nascimento ; Lehmann, Maurício ; Marroni, Norma Anair Possa . **Sarcopenia, oxidative stress and inflammatory process in muscle of cirrhotic rats - Action of melatonin and physical exercise.** *Experimental And Molecular Pathology*, v. 121, p. 104662, 2021.

Salvi, Jeferson De Oliveira ; Schemitt, Elizangela ; Fonseca, S. R. ; Hartmann, R. M. ; Colares, J. R. ; Marroni, Cláudio Augusto ; Picada, J. N. ; Marroni, N.A.P. . **Melatonin modulates antioxidant response and protects hepatocytes in rats with severe acute liver failure.** *South American Journal of Basic Education, Technical and Technological*, v. 7, p. 280-312, 2020.

Ostjen, Cristian Augusto ; Rosa, Carlos Gustavo Sakuno ; Hartmann, Renata Minuzzo ; Schemitt, Elizângela Gonçalves ; Colares, Josieli Raskopf ; Marroni, Norma Possa . **Anti-inflammatory**

and antioxidant effect of melatonin on recovery from muscular trauma induced in rats. *Experimental and Molecular Pathology*, v. 106, p. 52-59, 2019.

Marroni, N.A.P.; Bosco, A. D. ; Schedler, F. B. ; Colares, J. R. ; Schemitt, E.G. ; Hartmann, R. M. ; Forgiarini Júnior, L. A. ; Dias, A. S. . **Melatonin effects on pulmonary tissue in the experimental model of Hepatopulmonary Syndrome.** *Jornal Brasileiro de Pneumologia*, v. 45, p. 1-6, 2019.

Salvi, Jeferson De Oliveira ; Schemitt, Elizângela Gonçalves ; Colares, Josieli Raskopf ; Hartmann, Renata Minuzzo ; Marroni, Cláudio Augusto ; Marroni, Norma Anair Possa . **Action of Melatonin on Severe Acute Liver Failure in Rats.** *Journal of Pharmacy and Biological Sciences*, v. 12, p. 62-75, 2017.

Marroni, N.A.P.; Bona, S. ; Janz Moreira, A. C. ; Rodrigues, G. ; Di Naso, F. ; Noda, J. M. ; Silveira, T. R. ; Marroni, C.A. . . **Protective effect of melatonin on carbon tetrachloride-induced chronic hepatotoxicity.** *Journal of Hepatology*, v. 66, p. s585-s585, 2017

Bona, S. ; Filippin, L.I. ; Naso, F.C. ; David, C. ; Valiatti, B. B. ; Schaun, M. ; Xavier, R. M. ; Marroni, N.A.P. . **Effect of antioxidant treatment on fibrogenesis in rats with carbon tetrachloride-induced cirrhosis.** *ISRN Gastroenterology*, v. 2012, p. 1-7, 2012.

Rosa, D.P. ; Bona, S. ; Simonetto, D.A. ; Zettler, C.G. ; Marroni, C.A. ; Marroni, N.A.P. . **Melatonin protects the liver and erythrocytes against oxidative stress in cirrhotic rats.** *Arquivos de Gastroenterologia (Impresso)*, v. 47, p. 1-6, 2010.

Pavanato, M. A. ; Marroni, N.A.P. ; Marroni, C.A. ; Llesuy, S. . **Quercetin prevents oxidative stress in cirrhotic rats.** *Digestive Diseases and Sciences*, v. 00, p. 10, 2007.

Pavanato, M. A. ; Tuñón, M. J. ; Campos, S. S. ; Marroni, C.A. ; Llesuy, S. ; González-Gallego, J. ; Marroni, N.A.P. **Effects of quercetin on liver damage in rats with carbon tetrachloride-induced cirrhosis.** *Digestive Diseases and Sciences*, v. 48, n.4, p. 824-829, 2003.

Cremonese, Ricardo Viégas ; Pereira-Filho, Arthur Azambuja ; Magalhães, Richard ; Mattos, Angelo Alves De ; Marroni, Claudio Augusto ; Zettler, Cláudio Galeano ; Marroni, Norma Possa . **Cirrose experimental induzida pela inalação de tetracloreto de carbono:** adaptação da técnica e avaliação da peroxidação lipídica. *Arquivos De Gastroenterologia (Impresso)*, v. 38, p. 40-47, 2001.

The methods are old and now provide any significance means of using technology to find more evidence/details in the data.

The cirrhosis experimental model with CCL4 is a classic method, confiável, reprodutível, used until now in the most different research laboratories worldwide, by similarity to study the human cirrhosis. CCL4 is free radical source and a strong toxic substance, particularly in the liver, with oxidative damage caused by lipid peroxidation, which starts after the metabolization of CCL4 to free radicals highly toxic via cytochrome P450 enzyme. These toxic free radicals induce a

chain reaction and lipid peroxidation in membrane-like structures rich in phospholipids (mitochondria, endoplasmic reticulum) inducing oxidative stress, mitochondrial stress, endoplasmic reticulum stress. Free radicals play a role in various pathological conditions including liver disease. Free radicals are antagonized and removed by antioxidants. Antioxidant compounds react with free radicals from CCL4 and are involved in reducing cell damage and can help maintain normal physiological function. (Unsal V. et al 2020, Reviews on Environmental Health. Toxicity of carbon tetrachloride, free radicals and role of antioxidants).

Melatonin, an endogenously synthesized indolamine, is a powerful antioxidant exerting beneficial action in many pathological conditions, protects from oxidative stress, decreases inflammation, modulates the immune system, inhibits proliferation, promotes apoptosis. Melatonin stimulates antioxidant enzymes in the cells, protects membrane phospholipids from oxidation thus preserving integrity of the membranes. (Kopustinskiene D. M., et al 2021, 13, 129; Pharmaceutics; Molecular Mechanisms of Melatonin-Mediated Cell Protection and Signaling in Health and Disease).

The use of Melatonin as an anti-inflammatory, antioxidant, in experimental cirrhosis is very rare; the literature review shows very few publications under these circumstances, which is why there is a need to observe its actions in inflammatory pathways, in addition to the work recently published by our group, where we observed its anti-fibrogenic action. The conclusions presented in this work, where the beneficial effects of the use of Melatonin in the experimental model of cirrhosis caused by CCL4 are observed, are new and we did not find similarities under these circumstances.

The conclusion part is not written well. You need to adequately write the conclusion with study gaps, and recommendation for future work.

We rewrite the conclusions as requested and insert them into the work.

The language throughout the test needs to be revised with grammatical, syntax, paragraph corrections. Also, the writing structure need to be revised.

The article was sent for review in English to an accredited specialist, and it was again evaluated for language after the reviewer's indication.

The statistical analysis in figures is not correct. Check it and correct it. Also, you need to change the discussion accordingly.

Statistical analysis was corrected, as well as adequate discussion.

The SI unit need to be written perfectly. nmoles/mg protein?

The unit considered for this evaluation and presented in the literature is nmoles/mg protein.

Table 1 values need to be corrected. You have used, in zinc values.

Have been corrected