RESPONSE TO REVIEWERS

RE: Editorial decision *World Journal of Gastroenterology* NO: 81658, entitled "New uses for an old remedy: digoxin as a potential treatment for steatohepatitis and other disorders"

We thank the editor and reviewers for their insightful and constructive critique of our work. We took the comments very seriously and have substantially revised the manuscript in the light of the comments received. Overall, we believe we have responded to all of the comments and criticisms and hope the reviewers and editor will agree. Our edits to the manuscript are noted with Track Changes in the revised manuscript attached to this document, and our responses to individual reviewers are noted below:

Reviewer #1:

Comment

Jamshed et al. present a review article suggesting the use of cardenolide, digoxin as a potential drug in the treatment of steatohepatitis characterized by inflammation of the liver with concurrent fat accumulation. The article reads well, but often one has the impression of superficial treatment of some aspects of the topic. This also applies to the omission of some citations, which is astonishing in some cases.

Response: We thank the reviewer for their input and thorough review of our manuscript. We have revised the article extensively and included additional citations as appropriate.

1. Digoxin in a Nutshell:an overview of 200 years.
Line 78-84.
The authors should cite the following papers:
[Am. J. Cardiol., 65 (1990), pp. 10E-16E. doi: 10.1016/0002-9149(90)90245-v];
[Prog. Cardiovasc. Dis., 21 (1978), pp. 141-158. doi: 10.1016/0033-0620(78)90020-8];
[J. Am. Coll. Cardiol., 5 (1985), pp. 16A-21A. doi: 10.1016/s0735-1097(85)80459-9]
Response: We thank the reviewer for suggesting these landmark publications; we have included them in the manuscript.

2. Digoxin in steatohepatitis section:
It has been recently reported that lack of hepatic glucose-6 phosphatase results in liver damage [Mol Metab. 2021 Jan;43:101108.doi: 10.1016/j.molmet.2020.101108]. This gene is directly regulated by the RORgamma isoform of the RORC gene. Karas et al. showed that at non-toxic concentrations digoxin and other cardiac glycosides are agonists of this receptor [Front Pharmacol. 2019 Jan 7;9:1460. doi: 10.3389/fphar.2018.01460]; [Toxicol Lett. 2018 Oct 1;295:314-324. doi: 10.1016/j.toxlet.2018.07.002] being able to upregulate G6PC expression and thus might improve liver cell functions.

Response: We have made extensive changes to the steatohepatitis section to discuss digoxin as an inhibitor of RORγT in high doses, and as an activator of RORγT in low doses. We have also included in this section the ability of RORγT to modulate hepatic glucose-6 phosphatase potentially improving liver cell functions.

3. Digoxin in obesity and metabolic disorders:
The authors mentioned RORgammaT and IL17A, however, they did not cite the first identification of digoxin as an inverse agonist of this receptor [Nature. 2011 Apr 28;472(7344):486-90. doi: 10.1038/nature09978.]! This is not ok in my opinion. The authors should put more attention the fact that digoxin is considered as an endogenous cardiac glycoside and a modulator of many nuclear receptors' activity Please see and cite: [Biomed Pharmacother. 2020 Jul;127:110106. doi: 10.1016/j.biopha.2020]
Response: As mentioned in our response above, we have extensively revised the manuscript in regards to explaining the multifunctional role of RORγT. We have included the Nature paper by Hull et al noting the first identification of RORγT, and digoxin modulation the nuclear receptors. We have also noted digoxin and other cardiac glycosides as modulators of nuclear receptor activity, citing the suggested reference, in the section entitled "Digoxin in a Nutshell: overview of 200 years".

4. Digoxin in cancer:

The authors should definitively read and cite [Biomed. Pharmacother., 84 (2016), pp. 1036-1041 doi: 10.1016/j.biopha.2016.10.030]. As Th17 cells are promising in adoptive therapy [Blood 112, 362–373. Doi: 10.1182/blood-2007-11-120998]; [Immunotherapy 2, 21–24. Doi: 10.2217/imt.09.83] digoxin was suggested to be an effective compound that could improve the Th17 phenotype [Front Pharmacol. 2019 Jan 7;9:1460. Doi: 10.3389/fphar.2018.01460]
Response: We thank the reviewer for their suggested references, which have all been discussed and appropriately cited in the manuscript in the section entitled "Digoxin in a Nutshell: overview of 200 years" and/or in the section on "Digoxin and cancer".

5. Digoxin in viral infection:
In line 336, the authors claim "Digoxin inhibits coronavirus and other viruses [46]". What does it mean? Which coronavirus (there are many) and what it inhibits? Replication, infectivity, entry? The authors should also write on which concentrations of digoxin show its antiviral activity and how it is related to the toxicity of this drug.
Response: We have clarified that digoxin interferes with endocytosis through a non-elucidated pathway thus inhibiting cells entry. In the post-entry stage, digoxin significantly inhibits viral replication and viral protein expression, and provided the dose at which this viral replication occurs.

6. In general, the authors avoid indicating digoxin concentrations when describing various studies, which is crucial to whether digoxin can be considered a drug for a given condition at all. Please correct it.
Response: We thank the reviewer for their comment. In most of the studies, a range of digoxin doses were used; we have added digoxin dose wherever feasible.

7. Why in the last paragraph the authors do not cite relevant articles? This is completely incomprehensible. In this paragraph, the authors should focus on the rationale for the use of digoxin having in mind that this drug exerts high toxicity and is dangerous to patients. Lines 430-433 – statements like that require relevant citations and based on the [Front Pharmacol. 2019 Jan 7;9:1460. Doi: 10.3389/fphar.2018.01460.] there are not fully true.
Response: We have included the relevant references in the conclusion and future directions paragraph, and also expanded on the paragraph to clarify the effects of high versus low digoxin concentrations. In light of the potential toxicity of high dose digoxin above a certain threshold, we have highlighted the biological effects hitherto known regarding high versus low digoxin, and emphasized the need for more studies clarifying the biological mechanisms and potential therapeutic effects of low dose digoxin.

8. Lines 426-430 “At relatively high concentrations, digoxin and other cardiac glycosides inhibit the Na-K ATPase pump, leading to accumulation of sodium ions in the cytosol that drives an influx of calcium into the heart, increasing contractility. At lower doses, digoxin induces the Na-K ATPase to act as a receptor that can modulate a variety of pathways.” Please include these concentrations and cite relevant articles.
Response: We have included the references as requested and have also provided a concentrations for high versus low digoxin.

9. Figure 1. The authors should improve the figure. First, please include the structure of digoxin, include names of the reactive oxygen species, etc. Include also the figure showing other digoxin activities.
Response: We thank the reviewer for their comment. We have included the structure of digoxin as suggested, as well as the names of the reactive oxygen species shown to be modulated by digoxin. Given our emphasis on GI-related digoxin applications for the benefit of WJG readership, we have focused Figure 1 on GI-related activities, and in addition, we have put together an additional Table (Table 2) that summarizes digoxin activities in other organ systems.

10. Table 1. Please include digoxin doses that are planned to be examined in the clinical trials.
Response: We have edited the table to include the dose of digoxin and other medications being studied in these ongoing clinical trials.

11. Minor concerns 1. Line 431. Replace RORt with RORγT.
Response: We thank the reviewer for their thorough review of the article. We have made this correction.

Reviewer #2:

Comment: The writing and organize of the manuscript need to improve. There are also some issues in structure and scientific writing, need to be noted:

Response: We thank the reviewer for their comments. We have extensively revised and edited the manuscript to improve the writing and organization.

1. Review papers must provide a comprehensive critical review of recent developments in a specific area or theme that is within the journal's scope and not only a list of published studies.
Response: We have revised the manuscript to provide an extensive review of recent developments pertaining to the non-cardiac application of digoxin.

2. Reviews are expected to have an extensive literature review followed by an indepth and critical analysis of the state of the art, and identify challenges for future research.
Response: We have revised the manuscript to review the available literature even further, and to identify challenges for future research in the field. In this regard, we have substantial increased the number of primary articles and total references reviewed and cited, from 59 references cited in our initial submission to 96 references cited in this revised version.

3. It is better to cite more original studies.
Response: We thank the reviewer for their comment. We have edited the article to include citations of original papers wherever necessary, and have also increased the number of original papers reviewed and cited.

4. The content of the selected original articles used in this review should be prepared in the form of a table.
Response: We have created a new Table (ie Table 2) which summarizes the main findings, including molecular/biochemical and histological findings, from the original articles reviewed in the manuscript.

5. It would be helpful to add explanations in more detail about therapeutic results of digoxin and discuss subsequent pathological and also therapeutic outcomes in every research. therefore, in order to make the article more informative, each therapeutic case of digoxin should be accompanied by their biochemical, histological, and molecular outcomes at least included in the table.
Response: We thank the reviewer for their comment. Please refer to response above for question #4.

6. In text: When “CGs”, “RORγt”, “IL”, ... were first written, their full names were not mentioned and direct abbreviations were written in the text.
Response: We have checked the article and added the full names of each abbreviation when they are mentioned for the first time.

7. Unfortunately, the manuscript has revealed some grammatical and typographical problems that needs to be addressed. So, the English language of manuscript should carefully check and edit.
Response: We have extensively reviewed and corrected all grammatical and typographical errors.

8. The authors should add related references to the results using the table.

Response: Table 1 contains ongoing studies listed on clinicaltrials.gov. We have provided the clinical trial registration number (NCT) for easy reference for the readership.

9. It would be nice to add more recent articles in references.

Response: We thank the reviewer for their comment. We have revised the manuscript to include pivotal and recent references on this important subject.

ROUND 2

The authors improved the manuscript significantly however, they made a substantial error: “More recently, Karas et al. reported opposing findings with cardiac glycosides activating RORγT in HepG2 cells (…) RORγ is broadly expressed so in HepG2 this isoform is present, and Th17 express exclusively RORγT, thus the authors should correct this sentence accordingly: “More recently, Karas et al. reported opposing findings with cardiac glycosides activating RORγ in HepG2 cells and RORγT in Th17 lymphocytes” And further: “RORγ directly regulates glucose-6 phosphatase (G6Pase) and a number of genes involved in glucose regulation and insulin sensitivity **. G6Pase facilitates glucose-6 phosphate (G6P) hydrolysis into inorganic phosphate and free glucose [50-52], with suppression of hepatic G6Pase resulting in accumulation of G6P and metabolic reprogramming involving increased carbohydrate response element binding protein (ChREBP) activity and gene expression that lead to hepatic steatosis [53-56]. Digoxin-mediated activation of RORγ upregulates G6Pase, resulting in improved glucose homeostasis and decreased NAFLD phenotype”

Response: All comments from the reviewers have been addressed.