Author’s response to reviews

Title: Investigation of Gastroprotective Effect of A Novel Dibromo Substituted Schiff Base Compound Against Ethanol-induced Acute Gastric Lesions in Rats.

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Author’s response to reviews:

7th November 2018

Dr. Simone Brogi,

Thank you for the very helpful reviewers’ comments on our manuscript. We have carefully modified the manuscript to fully address all comments. Please find below our point to point reply to the comments of reviewers. We hope that these changes meet both yours and the reviewer’s expectations. In addition, we have added Dr. Hamed Karimian in our list of authors for his significant contribution. Please do not hesitate to contact us for further clarifications.

Thank you in advance for your kind consideration.

Sincerely Yours,

Kamelia Saremi,

(Corresponding Author)
Reviewers’ Comments:

Reviewer #1
Neil Henney (Reviewer 1): This is an interesting study which I suspect would be of interest to scientists in the field. In my opinion, the manuscript submitted needs some further work to present the study to a good standard.

Comment # 1
Most importantly, the manuscript requires very careful and quite extensive proof reading, to resolve the many problems with the English grammar and syntax (and sometimes word choice) which currently detract quite substantially from the quality of the submission. Because of this, it is difficult in quite a lot of places to understand exactly what is meant by what is written.
Reply:
It has been revised accordingly.

Comment # 2
I'm not clear on how an MTT assay on fibroblasts in vitro allows proper evaluation of "wound healing activity". If you think it does, I suggest you provide your rationale for this in your discussion of the findings
Reply:
It was revised and highlighted in the MS by adding some references) (Page 16).

Comment # 3
All the findings (narrative and figures reported) need additional information on the statistics you have reportedly carried out. The value of n should be given in every case, the p value should be reported as the actual number calculated (and not just < 0.05), and ideally confidence intervals would be provided to demonstrate there was sufficient statistical power in your experiments to lead you to the conclusions you have reached.
Reply:
It was revised and highlighted in the MS (tables and figs)

Comment # 4:
You have used the terms "ulcer healing" and "gastroprotection" almost interchangeably. In my opinion these are not the same. The latter is about protection (prevention) and the former lends itself more to cure.
Reply:
It was revised and highlighted in the MS (Page 12).

Comment # 5:
Finally, please check you have shown the correct SEM bars on figures 2 and 3 as these look remarkably similar across all data values shown.
Reply:
It was revised in the Figs

Reviewer #2:

Comment # 1:
Please review the English in the manuscript, as well as typos (like the one in page 7, section 6.3
(umbers).
Reply:
it was revised accordingly.

Comment # 2:
Methods: it is not clear for how long the rats are exposed to the compound. The authors said orally, does it mean with water? With the food? Gavage?
Reply:
It is mentioned that the animals were fed orally by Gavage and it was revised (page 8).

Comment # 3:
Since the compound is schiff base, and could be reactive, how do they assure the stability of the compound while administering to the animals? The authors use different buffers for administration/in vitro assays (tween, DMSO), is the compound stable?
Reply:
DMSO are normally used for diluting such compounds and tween are used to feed orally to animals (Schiff based compounds with 2 Br), such as:


2. Novel indanyl-substituted imidazo[1,2-a]pyridines as potent reversible inhibitors of the gastric H+/K+-ATPase

3. Anti-Gastric Ulcer Activity of the Water Extract from Payawanorn (Pseuderanthemum Palatiferum)

Comment # 4:
Since some of the effects were not dose-dependent (same effect for both of the concentrations) can authors measure AUC of the compound in blood?
Reply:
Thanks for your interesting comment. The blood is already collected and analysed. We have no more blood left to analyse the AUC, however, our aim is to find the effects of the compound, later we will use your advice to study pharmacokinetics of this compound.

Comment # 5:
The compound contains 2 atoms of Br, but the toxic effects were measured only after 1 dose. Can authors measure long-term effect (since prophylaxis of ulcers is a long term treatment).
Reply:
Eventually we measured the toxicity effect of the compound in different concentrations (serial dilution) 100, 50, 25, 12.5 and 6.25 μg/mL). Because in this range of the concentrations of CNBP we did not detect toxicity effect of the compound, hence, the HD and LD of CNBP were used for further evaluations. Besides this analysis, we followed the procedure based on protocols that in some studies which evaluated the anti gastreic effect of Dibromo have been used (the articles are entitling:

2. Novel indanyl-substituted imidazo[1,2-a]pyridines as potent reversible inhibitors of the gastric H+/K+-ATPase,
3. Anti-Gastric Ulcer Activity of the Water Extract from Payawanorn (Pseuderanthemum Palatiferum)(Page 7).

Comment # 6:
The MTT assay contains too much DMSO (0.25%) and that concentration could compromise the membrane integrity.
Reply:
Thanks for your sharp comment. We used 25% of DMSO in control only to check any sign of DMSO toxicity in our treatments. The percentage of the DMSO in all treated cells were less than 0.1%. (Page 7).

Comment # 7:
The method of the gastric ulcer induction is confusion. For how long did the investigators treat the rats prior exposure to the acid? Or was it after acid exposure (It won't be preventive, but palliative then).
Reply:
The rats were fed via oral gavage with 5mL/kg of 10% Tween 20 (normal and ulcer control groups), 20 mg/kg omeprazole (5 mL/kg) (reference group) and low and high doses of CNBP (10 and 20 mg/kg) as experimental groups. After one hour, In order to induce stomach injury, the other five groups, except the normal group, were exposed to oral gavage of absolute alcohol. After additional hour, the rats were sacrificed followed by removing their stomachs, immediately for carrying out the further tests [20, 23] (this method is using as ulcer protective assay). (Page 8)

Comment # 8:
MTT assay is not clear if the DMSO was constant across the wells. Also, how many times the experiment was repeated? Or does the graph represent 1 experiment but using multiple wells?
Reply:
Answer: All vitro experiments were repeated 3 independent times. The percentage of DMSO in treated cells were less than 0.1%, and only in control with 0.25%. Its been corrected in Manuscript. (Page 7 & Page 28)

Comment # 9:
The authors list on results "high" antioxidant properties, but comparing with Ascorbic acid is not that high. Please refer for including the word high, since it is misleading. In addition, have the authors tested whether or not the compound could chelate Fe instead of doing a redox reaction?
Reply:
first question: It is mentioned in page 12, the activity is significant (Page 12). Due to our limitation, we don’t have enough budget at the moment to check Fe, but for sure in our continued project we will check.

Comment # 10:
Some of the histological sections are hard to see, could they provide an amplified copy?
Reply:
We have enhanced the quality of the images and now looks better. However, the results we have generated was the best that we could get from this instrument. We have tried several times again, but we could not get the better one.