Author's response to reviews

Title: An iron-based beverage, HydroFerrate Fluid (MRN-100), alleviates oxidative stress in murine lymphocytes in vitro

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Author's response to reviews: see over
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Dear Dr. Nehme Gabriel,

Thank you very much for sending the article to us with the reviewer comments. We found that the comments were very constructive and we believe that the quality of the article has been significantly improved as a result. I hope the article is now acceptable for publication. If you have any further comments or questions, please let us know.

Thank you,

Dr. Gollapudi

Our response to the reviewer comments are as follows:

**Reviewer 1 – Steven B. Oppenheimer**

1. The article has 3 figures. Figure 3 previously had three subfigures, A, B, and C, but has been expanded to contain four subfigures, A, B, C, and D. There is no Figure 4 or 5.
2. All figures now appear to be large enough to see all details of the figure.
3. Fig. 3 legend has been changed to indicate that the legend is referring to Figure 3, not Figure 1.
4. The Western Blot analysis has been expanded.
5. The *in vivo* effect of MRN-100 on the antioxidant potential is currently under investigation.
6. The number of repeats has been added to the figure legends.

**Reviewer 2 – Eman Noaman**
1. Page 2, line #9 has been corrected to read “Trypan blue exclusion”

2. In the results, the “Bax/Bcl-2” has been corrected to read “Bcl-2/Bax.”

3. In the introduction, line #8-9, the words “it is” have been removed, to read “capacity to accept and donate electrons, and this characteristic makes it useful.”

4. Line 15 has been corrected from “bivalent and tervalent ferrates and is sold” to read “bivalent and tervalent ferrates and it is sold.” We prefer the use of the term “tervalent”, and this term is equivalent to “trivalent.”

5. In Materials and Methods, line #15, the word “trypan” has been added to read “as determined by the trypan blue”

6. The title of the second section of Materials and Methods has been changed from “Apoptosis by blue exclusion” to “Cell death detected by trypan blue exclusion.”

7. The word trypan has been added all over the manuscript to correct “blue exclusion” to read “trypan blue exclusion”

8. The method for intracellular Ca$^{2+}$ has been revised to reflect the materials used in this study.

9. On page 7, “data are presented as mean + SEM” to read “data are presented as mean ±SEM”

10. On page 8, the title of the second paragraph has been corrected to read “cell death percentage as examined by trypan blue.”

11. Page 9, line #9, “induced apoptosis” has been changed to “induced cell death”

12. Page 9, line #12, “induced apoptosis” has been changed to “induced cell death”

13. Page 9, line #21, “Raw” has been corrected to read “Raw-264.7”

14. Page 9, the intracellular calcium section has been clarified by adding “data not shown.”
15. In the Bcl-2 protein figure legend, the figure numbers have been corrected. There is no figure 4 or 5. In addition, Figure 3 has been expanded to contain four subfigures, A-D.

16. Page 9, line 11, “Bax/Bcl-2” has been corrected to read “Bcl-2/Bax”

17. The word “trypan” has been added to line 8

18. A legend has been added for all subfigures A-D, including the new subfigure.

19. Discussion, page 11, line 2, “Bax/Bcl-2” has been corrected to read “Bcl-2/Bax”

20. We prefer the use of the term “tervalent”, and this term is equivalent to “trivalent.”

21. Conclusions, page 12, line 12, “Bax/Bcl-2” has been corrected to read “Bcl-2/Bax”

22. Figure legends, page 16: “cell death” has been used instead of “apoptosis”

23. Line 8, the word “trypan” has been added before “blue stain”

24. Figure 3 legend is now complete

25. In the figure legend for Figure 3, the numbers have been corrected, and now all refer to Figure 3, and not Figure 1, 4 or 5.

Reviewer 3 – Nariman Badr El-Din

1. Abstract, line 8, the full name of the dye “trypan blue” has been added

2. Abstract, line 12, the “ratio of Bax to Bcl-2” has been corrected to “ratio of Bcl-2 to Bax”

3. Introduction, page 2, line 11: has been corrected to read “ferritin as a protectant against”

4. Materials and methods: preparation of splenic lymphocytes – the full name of the dye “trypan blue” has been added

5. Materials and methods: experimental protocol – the concentrations of MRN-100 and splenic lymphocytes have been added
6. Materials and methods: apoptosis by blue exclusion - the full name of the dye “trypan blue” has been added

7. Materials and methods: the 4th group (MRN-100+H2O2) has been added.

8. The description of LPS “lipopolysaccharide” has been added.

9. Intracellular calcium flux section has been corrected.

10. Materials and methods: “Western blot” has been changed to “Western blot analysis”
    a. The Bax protein detection details have been added

11. Results: Apoptosis by trypan blue – “indcued” has been corrected to “induced”

12. Results: Bcl-2 protein level – title has been changed to “Bcl-2 and Bax protein level”
    a. The detection of Bax has been added
    b. The result of Bcl-2/Bax has been expanded.

13. The “Bax/Bcl-2” ratio has been corrected to read “Bcl-2/Bax ratio” as the figure 3 describes.

14. Discussion: page 11 – the description regarding the Bcl-2/Bax ratio has been corrected and clarified.

15. Conclusions: the description regarding Bcl-2/Bax ratio has been corrected

16. Figure legends: Figure 2 – the full name of the stain “trypan blue” has been added.

17. Figure 3 – the descriptions of A and B have been corrected to read “3A” and “3B”

18. Figure 3 – A legend has been added for all of figure 3, including the new subfigures.