

## Reviewer's report

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

**Version:** 1 **Date:** 7 February 2009

**Reviewer:** eman noaman

### Reviewer's report:

Minor Essential Revisions

This investigation includes some flaws in experimental design and results, incorrect interpretation of data and correction of some parameters is required.

Abstract

Page 2

In the methods

Line # 9 "trypan blue exclusion"

Instead of "blue exclusion"

In the results:

Line#13&14 "Bcl-2/Bax"

Instead of "Bax/Bcl-2"

1-Introduction

Page 3

Line#8 &9 (2nd paragraph)

- Iron has the capacity to accept and donate electrons, and this characteristic makes it useful.....

Instead of

- Iron has the capacity to accept and donate electrons, and it is this characteristic.....

Line#15

- From bivalent and trivalent ferrates and it is sold .....

Instead of

- From bivalent and tervalent ferrates and is sold .....

3-Material and Methods

Page 3

Line#17

- as determined by trypan blue.....

Instead of

- as determined by the-blue.....

Page 4

The actual use of trypan blue exclusion method is to determine cell mortality percentage. So, the title of the second paragraph should as follows:

- Cell mortality percentage detected by trypan blue exclusion.

Instead of

- Apoptosis by blue exclusion.

Line#10

- Word trypan must be added before blue stain.

Page 5

The first paragraph under title of "Intracellular calcium (Ca<sup>+</sup>) flux", includes serious mistakes, incorrect description of the material applied and cell line used.

This paragraph should be rewritten with the proper material.

Page 7

Line#4

- Data are presented as mean  $\pm$ SEM

Instead of

- Data are presented as mean +SEM

3-Results

Page 8

Title of second paragraph should be as follows:

"Mortality percentage as examined by trypan blue"

Line #9

- The protective effect of MRN-100 against H<sub>2</sub>O<sub>2</sub> induced mortality ....

Instead of

- The protective effect of MRN-100 against H<sub>2</sub>O<sub>2</sub> induced apoptosis.....

Line #12

- MRN-100 reversed H<sub>2</sub>O<sub>2</sub> induced mortality ...

Instead of

- MRN-100 reversed H<sub>2</sub>O<sub>2</sub> induced apoptosis....

Line#21

- Raw-264-7

Instead of

- Raw

Page 9

Intracellular calcium results which was described in page 8&9 are not presented neither in figures nor in tables

In Bcl-2 protein level

In the description of the results, Bcl-2 were presented in three figures 3A, 3B, 3C, while in the figure legends, it was written in figures 3, 4 and 5 so this conflict should be corrected in figure legends.

Line#11

- Ratio of Bcl-2/Bax

Instead of

- Bax/Bcl-2

Page 9 "second paragraph"

Line #6

- Percent of mortality of splenic lymphocytes by trypan blue .....

Instead of

- Apoptosis of splenic lymphocytes by blue .....

Line #8

Ward trypan must be added before blue stain.

"Third paragraph"

- there is no legend of Figure 3C.

4-Discussion

Page 11

Line#2

- Bcl-2/Bax

Instead to

- Bax/Bcl-2

Line#7

- trivalent

Instead of

- tervalent

5- Conclusions

Page 12

Line# 12

- Bcl-2/Bax

Instead to

- Bax/Bcl-2

Figure Legends

Page 16

Line #6

- Mortality percentage of splenic lymphocytes by trypan blue assay...

Instead of

- Apoptosis of Splenic lymphocytes by blue assay.....

Line #8

- Word trypan must be added before blue stain.

"Third paragraph"

Legend of figure 3C is missing.

Page 19

The figure must be named as Figure 3A

Page 20

The figure must be named as Figure 3B

And the second column named

Page 21

The figure must be named as Figure 3C

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests' below