Reviewer's report

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

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Reviewer: Nariman Badr El-Din

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Evaluation of the manuscript

An iron-based beverage, HydroFerrate Fluid (MRN-100), alleviates

oxidative stress in murine lymphocytes in vitro

Mamdooh Ghoneum, Ph.D. 1, Motohiro Matsuura, Ph.D.2, and Sastry Gollapudi, Ph.D.3

1Drew University of Medicine and Science, Department of Otolaryngology

Los Angeles, CA 90059 2Jichi Medical School, Department of Infection and Immunity,

Tochigi, 329-0498, Japan.3University California, Irvine, Division of Basic and Clinical

Immunology, Irvine, CA 92718

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In this investigation, authors aimed to in vitro investigate the protective effects of water (MRN-100) produced by the ACM water purification system that transform tap water into purified and energized water using micro amounts of iron, against H2O2 - induced apoptosis in murine splenic cells as a result of oxidative stress.

The results suggested that iron based compounds may provide an effective complementary and alternative treatment against oxidative stress.

This study reports a very interesting data . The design of the study is appropriate, enabling treatment and control groups to be compared. The topic is well presented in the introduction and the manuscript is globally well written.

The methods used are appropriate but some are not well described and lake details. Also some important details of the results are lacking from its description. The discussion and conclusions are well balanced and adequately supported by the data.

In general, the manuscript adheres to the relevant standards.

I recommend the acceptance of the submitted manuscript for publication after minor essential revisions concerning the following:

Abstract

Line # 8: mention the full name of the dye

Line # 12: correct: and increased the ratio of Bax to Bcl-2 to match the results presented in table 3C.

Introduction

Page# 2 line11: correct to: ferritin as a protectant against

Materials and Methods

2.4 Preparation of splenic lymphocytes

Line # 4: mention the full name of the dye

2.5 Experimental protocol

What is the concentration of lymphocytes/MRN-100 solution?

2.7 Apoptosis by blue exclusion

•Mention the full name of the stain.

•the 4th group (MRN-100+H2O2) is not mentioned.

2.8 Determination of nitric oxide (NO) production

Line#3: What does LPS stand for?

2.9 Intracellular calcium (Ca2+) flux

This part should be checked and corrected as it includes materials away from this investigation such as MDA-MB-231 cells and S. cerevisiae, with no mention for the materials of this experiment: the splenic lymphocytes, MRN-100 and H2O2.

3.0 Western blot: •change to Western blot analysis.

•There is no mention about Bax protein detection?

3. Results

3.2 Apoptosis as examined by trypan blue

Line#1: correct -indcued to induced.

3.5 Bcl-2 protein level: •change to Bcl-2 and Bax protein levels

•there is no mention about the detection of the proapoptotic protein Bax.

•The result of the ratio of Bcl2/ Bax is not clear and confusing.

According to the 5th figure (3C), pretreatment with MRN-100 has attenuated oxidative stress in immune cells by increasing the Bcl2/Bax ratio and not the Bax/Bcl2 ratio .

4. Discussion

Page#11, check the upper paragraph and correct the description regarding Bcl-2/Bax ratio.

[In this study we have shown that MRN-100 prevented H2O2-induced upregulation of Bax and down regulation of Bcl-2 resulting in an increase of the Bax/Bcl-2 ratio]

5. Conclusions

Correct the description about Bcl-2/Bax ratio.

Figure Legends

•Figure 2. Mention the full name of the stain

.•Figure 3.

.Line#3: correct Figure 1A to 3A

Line#5: correct . Fig 1B to 3B & correct the labels of the figure.

The last figure(3C) is not mentioned in the figure legends.

Nariman K. Badr El-Din, Ph.D.

Professor, Biological Chemistry

Department of Zoology, Faculty of Science. University of Mansoura, Egypt

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'