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OPEN PEER REVIEW

Protective Effects of Vitamin E on Heart and Testis Histology Following MDMA (Ecstasy) Exposure in Mice

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

3 comments · 9 Nov 2018 – 10 Jan 2019

Referee 1 Reviewer

MAJOR REVISION 9 Nov 2018

Dear AE,
The current study evaluated the effects of MDMA on testis and heart tissues of mice and the protective effects of Vitamin D on this process. There are several comments for authors as below:
Abstract
1- Line 1, what is the mean of "some adverse health effects on…", MDMA has health effect?
2- Is the olive oil group named the sham group? If yes, please define it.
3- Are these abbreviations correct? germinal epithelium (SE)?? . diameter of seminiferous tu¬bules (STD)????
Introduction
1- What is the mean of " in reproductive and cardiovascular stems "?
Objectives
1- The objective is not correct, because the authors assayed the synergic effects of
MDMA and Vitamin. They did not assay the effect of Vitamin E after damage induced by MDMA. And this is the main deficit of the study.
Methods
1- Another insufficiency of the study is the lack of different dosages of Vitamin E. It is obvious that the Vitamin D can decrease the MDMA effect, however, using the different dosages could lead to determining the effective dose of the vitamin.
2- Please explain, the MDMA preparation
3- Please define "H&E" the first time.
4- Please explain why the authors use olive oil for the sham group.
5- Please delete "The elevation of Lactate dehydrogenase (LDH) enzyme which is found in many body tissues including heart may cause cardiac damages Furthermore, to estimate the heart function and help the diagnoses of acute myocardial infarction, a cardiac index namely creatine phosphokinase (CPK) is used.". It is not suitable for methods, the authors should explain these in the introduction.
Statistical analysis
1- Is the one-way ANOVA test suitable for comparison in a low sample size(n=7). I think no and you should use non-parametric tests.
Results
2- In tunnel staining, what is the relation between these results and table2?
3- What is the mean of Biochemical indices?
4- In the Biochemical indices section, please delete the results of your previous finding. You should insert the results of this study in the results section.
Discussion
1- The first paragraph is not related to your study." In this study, we explore the feasibility of chronic effects on the heart and testis structures of MDMA consumers, mainly youth, and vitamin E protective effects as well. For this aim, we mimic the pattern and the doses of administration of human chronic intake according to Barenys et al(7).
2- In this section, the authors explain the results of previous studies about the effect of MDMA on various tissues only, however; they did not discuss the protective effects of Vitamin D in similar studies.
Tables
1- Please check the "SE: epithelial height", Is this abbreviation correct.
2- Please define a, b, and in tables.

3- Please improve the title of table1 and 2.
Finally, the manuscript needs major revision regarding the English language.
Best regards

Associate Editor

MAJOR REVISION 10 Jan 2019

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author.
According to the comments, the document needs major revision.
Kind regards,

Editor-in-Chief

MAJOR REVISION 10 Jan 2019

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor in chief

Thanks for revising my article in your esteem journalReferring to your comments:

Abstract

1-the word health was deleted.

2-No the olive oil group is not a sham group. We dissolved vit E in olive oil to obtain a proper liquid to gavage the mice easily. Since the olive oil is a weak antioxidant, we also gavage olive oil separately to ensure that the positive effects is due to vit E or olive oil itself.

3-STD stands for seminiferous tubules diameters and SE for seminiferous epithelial height.

Introduction

1-stems changed into systems.

Objectives

1- The “objectives” was changed according to the comment.

Methods

1-vit E dosage was based on previous studies and dosimeter repetition seems not to be necessary. Ref: Ayinde OC, Ogunnowo S, Ogedegbe RA. Influence of Vitamin C and Vitamin E on testicular zinc content and testicular toxicity in lead exposed albino rats. *BMC Pharmacology and Toxicology*. 2012;**13**(1):17.

2- tne purified MDMA was perchuased from Biotechnology Unit of Iran Medical Sciences University, Tehran, and dissolved in saline according to previous study. Ref 13 in this article.

3-correction done according to the comment.

4- We dissolved vit E in olive oil to obtain a proper liquid to gavage the mice easily. Since the olive oil is a weak antioxidant, we also gavage olive oil separately to ensure that the positive effects is due to vit E or olive oil itself.

5-the mentioned phrase was moved to introduction.

Statistical analysis

1-according to ethical committee of animals we must use at least 5 and utmost 8 samples.

Results

2-corrected according to comment.

3-changed to Biochemical analysis

4-the mentioned part was deleted as wanted.

Discussion

1- Done as mentioned.
2- The results of the previous studies have been explained in discussion section, ref: 8,12 22,26 and 27.

Tables

1- SE is abbreviation of epithelial height according to the literature. For example: MOVAHED, Elham, et al. Toxic effect of acyclovir on testicular tissue in rats. *Iranian journal of reproductive medicine*, 2013, 11.2: 111.

2-. *In each row, all groups were compared with each other. Different letters in each row indicate significant difference ($p<0.05$). a letters do not have significant difference with each other while they have significant difference with b letters. This is done based on previous articles for instance:* Ghafori SS, Javanmard MZ, Ahmadi A, Peirouvi T, Karimipour M, Modirkhamene S. The Effects of Vitamin E on Prevention of Damages Due to MDMA (Ecstasy) Exposure on Sperm Parameters in Mice. *International Journal of High Risk Behaviors and Addiction*. 2017(In Press).

3-Table titles improved as mentioned.

Finally thanks alot for reviewing and commenting.

Sinscerely

2 Revision 1

3 comments · 10 Feb 2019 – 24 Feb 2019

Referee 2 Reviewer

MAJOR REVISION 10 Feb 2019

Dear AE,
I review the manuscript " Protective effects of Vitamin E on heart and testis histology following MDMA (ecstasy) exposure in mice" After proper revision and corrections it should be considered again for review.
1- Testis and heart have not objective relationship with each other, heart issues should be removed.
2- Research Ethics Committee's license should be noted
3- In the first paragraph of discussion authors has written that " we mimic the pattern and the doses of administration according to Barenys et al" but in the current study has worked on mice instead of rat and for 5 weeks instead of 12 weeks
4- In materials and method referred to previous article that is in press in JHRBA now! and shorten content.
5- The counting method of Sertoli and Leydig cells has biased. basically mentioned valid references for this part of the experience.
6- TUNEL cannot alone put any difference among apoptosis, necrosis, autolysis, and other cell death.
7- For confirmation and more information FSH, LH and testosterone should be assayed, If it done it would be pleased to add to the manuscript
8- A histomorphometry study should include accurate proper high-resolution figures.
8- Rewrite discussion with emphasis on testis morphology and with more coherence.
Thanks.

Associate Editor

MAJOR REVISION 20 Feb 2019

Dear EIC,
This manuscript is the first revise, but I have no access to the comments of the first reviewers.
In addition, there are the comments of a reviewer as attachments.
Best regards

Editor-in-Chief

MAJOR REVISION 24 Feb 2019

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Author(s)
I review manuscript "
Protective effects of Vitamin E on heart and testis histology following MDMA (ecstasy) exposure in mice"
After proper revision and corrections it should be consider again for review.
1-Testis and heart has not objective relationship with each other , heart issues should be removed.
2- Research Ethics Committee's license should be noted
3- In first paragraph of discussion authors has written that " we mimic the pattern and the doses of administration according to Barenys et al" but in current study has worked on mice instead of rat and for 5 weeks instead of 12 weeks
4- In materials and method referred to previous article that is in press in JHRBA now! and shorten content.
5- Counting method of Sertoli and Leydig cells has biased. basically mentioned valid references for this part of experience.
6- TUNEL cannot alone put any difference among apoptosis, necrosis, autolysis and other cell death.
7- For confirmation and more information FSH, LH and testosterone should be assayed, If it done it would be pleased to add to manuscript
8- A histomorphometry study should include accurate proper high resolution figures.
8- Rewrite discussion with emphasis on testis morphology and with more coherence.

Respected reviewer

Thanks for revising my article in your esteem journal.

Referring to your comments:

1- This study was based on an approved University proposal. The major part on which the effects of MDMA was studied includes Central Nervous System and less on other organs such as testis and heart.as abusing MDMA results in the production of free radicals and hyperthermia and they have adverse effects on testis and heart tissues according to previous studies, in this article for the first time, the protective effects of vitamin E against MDMA abusement have been studied.

Example: Cytoprotective effects of DL-alpha-lipoic acid or squalene on cyclophosphamide-induced oxidative injury: An experimental study on rat myocardium, testicles and urinary bladder. Food and Chemical Toxicology.Volume 48, Issues 8–9, August–September 2010, Pages 2326-2336

2-research ethics committee's license noted as mentioned.(yellow color highlighted)

3- We mimic pattern (3 sequential weekend days) and dose administration (10mg/kg) of Barenys and et al. and also we chose 5 week because the spermatogenesis period of mice is 5 weeks.

4- your mentioned study also has been done by our team and since they include the same mice, same number, same groups, drug dose administration and same period, the Materials and Method structurally are the same in first parts inevitably.

5-the requested reference for sertoli and lydig cells counting was inserted. (yellow color highlighted)R17

6- TUNEL cannot put any difference by itself and there are some other methods to do fulfill this. But we chose TUNEL based on our financial limits.

7-of course, in that condition the study would be much more comprehensive but our available serum was not enough to fulfill the FSH, LH and testosterone.

8-preparing mice testis tissue is somehow more difficult than other tissues and we tried our best for many times to obtain a good and high resolution images.

Thanks again for your efficacious comments

Sincerely

3 Revision 2

4 comments · 7 May 2019 – 6 Jul 2019

Referee 2 Reviewer

REJECT 7 May 2019

Dear AE,
Unfortunately, the Author(s) refuse to refine the manuscript moreover they have done merely justify the manuscript and their research.
Thanks.

Referee 3 Reviewer

MINOR REVISION 19 May 2019

Dear AE,
Comments to the Author
The presented scientific article is interesting but some points can be improved for publishing in the journal as follow:
1- The authors mentioned in statistical analysis Mean± S.E.M was used to present Summary statistics but in Tables 1 & 2 legend Data are presented as mean ±SD, please correct it.
2- Because TUNEL staining cannot determine the difference between apoptosis and necrosis and other cell death it should be better to use TUNNEL positive cells instead of apoptotic cells.
3- It should be better to add a reference for TUNNEL positive cells counting.
4- The authors should compare their findings about histological alteration with other researchers finding (in your opinion, what is the reason for the discrepancy in your finding with others? You did not mention).
Thanks.

Associate Editor

MAJOR REVISION 14 Jun 2019

Dear EIC,
The authors corrected some parts of the manuscript according to reviewers' suggestions, but the manuscript needs re-revise according to the following comments:
- There is no answer to the comments of the second reviewers.
- The authors answer to most questions instead of correction according to comments
- They should re-revise according to previous and new comments.
Thanks.

Editor-in-Chief

MINOR REVISION 6 Jul 2019

Dear Author,
Please revise the article according to the present and previous comments and add which comments that you cant do it to the limitation.
regards,

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Respected reviewer

Thanks for revising my article in your esteem journal.

According to EIC Decision manuscript corrected according previous comments (Red color)-present comments(yellow highlighted) and English polish(purple).

Abstract

1- It is very common term in health and means promotion of a structural or functional abnormality which reduces the quality of life.

2-defined

3- Corrected

Background

1- System is correct

Objectives

1- Corrected

Methods

1-Ofcourse, but at the research meeting of university only a single dose of vitamin E was approved.

2--added

3-full term of H& E was added

4-olive oil is a solvent of vitamin E, it is not sham group.

5-explained in introduction

Statistical analysis

1- Analysis with "Shapiro-wilk test" revealed that data has normal distribution. After this test using of ANOVA (N=7) is allowed.

Results

2-There is relation between Tunnel assay and Table 1

3-It replaced by Biochemical markers

4- Results of previous finding have been deleted

Discussion

1- Ref.7 and first paragraph deleted

2-some paragraphs elated to role of vit. E with Ref. numbers: 8-12-22-26-27 were added to discussion.

Tables

1- It changed to SEH: seminiferous epithelial height

2-a and b “have been defined

3-They changed

Present comments:

1- Corrected

2-Tunnel positive has been replaced

3-Ref.18 added

4-The reason of difference between present and past research is” Dose of exposure 10mg/kg) but in some studies they used 40mg/kg MDMA.

Thanks again for your efficacious comments

Sincerely

4 Revision 3

1 comment · 28 Sep 2019

Editor-in-Chief

ACCEPT 28 Sep 2019

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.