OPEN PEER REVIEW

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As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

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- More motivations for all involved roles in the review process
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- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

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Peer Review Report for "Effects of Aminoguanidine on Apoptosis in the Hippocampus and Memory Disorder in a Rat Model of Alzheimer's Disease"

Author(s): Hamid -Reza Sameni, Abbas Ali Vafaei, sam zarbakhsh, Afsaneh Talebian, Mohammad Reza Aldaghi

Review Timeline:

Submit Date: 10 Apr 2021
Revised Date: 16 Sep 2021
Accepted Date: 28 Sep 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

behpour yousefi: Reviewer | **Revision** (0) 19 May 2021

Dear AE,
Comments for the authors:
1- In the section of the abstract and results specific values of experimental and control groups need to be given for important results as brackets.
2- Please explain why the Cresyl violet staining method was used in the histopathological study?
3 In the histopathological study, the author should mention the sections are randomly selected or not?
4- How did you distinguish between neurons and neuroglia and also normal and damaged pyramidal cells?
5- Why did not use immunohistochemistry staining in your research?
Sincerely,
Dr.Behpour Yousefi
Departments of Anatomical Sciences, and fertility ward, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

REFEREE: Associate Editor | **Revision** (0) 22 May 2021

Dear EIC,
This manuscript has several major problems as follows:
1. Novelty of this work is not clear. This work does not cover previous similar studies in the introduction part.
2. Introduction is not cover previous similar studies
3. There are serious problems and ambiguities in the material and methods of this work, for example, The tunnel staining method is not correct on the base of the image fig.4. TUNEL-positive cells are determined by fluorescence technique, not DAB.
Sections with 5-10µm of thickness were used for histopathological study. It is not acceptable variation in thickness that used in this study can distort the results of the study. The histopathological part was completely rewritten and revised.
4. Discussion part is repetitive of previous study and introduction.
5. This manuscript is not accepted for publishes in this condition
Minor comments:
1. The words "rat and Alzheimer's and scopolamine added to keywords
2. Grammatically need to be checked
3. In lines 14-17 of the introduction authors mentioned" The pro-inflammatory elements such as nitric oxide (NO) and ROS releasing……" this sentence is not scientifically correct. These agents are free radical not pro-inflammatory cytokines
4. Code number of an agent should add to the manuscript (kit and ……..)
5. Ethical code number should be added to the manuscript.
6. Volume of saline or AG that injected into the animal should be added
7. Experimental groups in result part presented as:
scop+ag, saline+ag, saline+vehicle durg
8- Why author uses a parametric test. Normality test of data should be checked
9- This sentence " In the Scop-Amg group significantly reduced, the spent time compared to the Scop group ($P<0.05$) (not shown)" is completely ambiguous.
Author all of the results of spatial memory including Time days training (escape latency), time to find the location of the platform, time spent in the target zone and Thigmotaxis added to the manuscript.
10 - It is not clear how many fields of the hippocampus were used to count positive tunnel cells or pyramidal cells in the tunnel and dark cells in the histopathological study.
11. Hippocampal position should give based on the Paxion Atlas
12. BDNF is not measured in this study (lines 148 to 158).
Thanks.

Fatemeh Ehsani: EIC | **Revision** (0) 23 May 2021

Dear Authors
According to the reviewers and associate editor's comments, your article needs to be revised major, especially the method and material section. Please respond to the reviewers' comments and submit the required files for up to two months.
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Response to editor

Manuscript entitled **"Effects of Aminoguanidine on apoptosis in hippocampus and memory disorder in a rat model of Alzheimer's disease"**
We appreciate your constructive comments that define both the strengths and limitations of our manuscript. We have modified the manuscript according to your suggestions and have done our best to provide a more transparent presentation by implementing all of your concerns and observations. Below, please find a detailed response to your comments. The manuscript has also been revised to address your concerns.

Associate Editor's Comments:

1. *Novelty of this work is not clear. This work does not cover previous similar studies in introduction part.*

With regard to the previous studies, we believe that our research is different from the others studies. The previous studies mainly focus on the effects of Aminoguanidine only on memory disorders, but in our study in addition to memory impairments, we investigated histopathological changes and also apoptosis in the hippocampus. We used the Cresyl violet staining (that is a well-known histological method to detect of cell damage in the central nervous system (Alvarez-Buylla et al. 1990; Kosaraju et al. 2013) and also the Tunel assay was used for investigation of apoptosis in the hippocampus. In the similar previous study were used of another method for hippocampal investigation. Also, methods of administration were different and other doses of Aminoguanidine were used.
As suggested by the editor, previous similar studies added in introduction part.

2. Introduction is not cover pervious similar studies.

As suggested by the editor, previous similar studies added in introduction part.

3 Tunnel staining method is not correct on the base of image fig.4. TUNEL-positive cells determine by fluorescence technique not DAB. Sections with 5-10μm of thickness were used for histopathological study. It is not acceptable variation in thickness that used in this study can be distort the results of the study. Histopathological part completely rewrite and revised

Tunnel technique was modified in material and methods. Histopathological study modified and revised.

4 *Discussion part is repetitive of pervious study and introduction.*

Discussion completely revised.

5. This manuscript is not accepted for publishes in this condition

Manuscript has been revised to be clearer.

Minor comments:

1. The words "rat and Alzheimer's and scopolamine added to keywords

Rat, Alzheimer and Scopolamine added to keywords part in abstract.

2. Grammatically need to checked

The English of the manuscript was improved.

3. In the line 14-17 of introduction authors mentioned" The pro-inflammatory elements such as nitric oxide (NO) and ROS releasing……" this sentence is not scientifically correct. These agents are free radical not pro-inflammatory cytokines

As suggested by the editor was modified and revised.

4. Code number of agent should added to manuscript (kit and …….)

Code numbers of Scopolamine, Aminoguanidine and Tunel kit were added in manuscript.

5. Ethical code number should added to manuscript

Ethical code number was added in manuscript.

6. Volume of saline or AG that injected to animal should be added

Volume of injections added to materials and methods part.

7. Experimental groups in result part presented as: scop+ag, saline+ag, saline+vehicle durg

Experimental group was modified and revised.

8. Why author used parametric test. Normality test of data should checked

The Shapiro-Wilk test used to test the normality of the data.

9- This sentence " In the Scop-Amg group significantly reduced, the spent time compared to the Scop group (P<0.05) (not shown)" completely ambiguous. Author all of results of spatial memory including Time days training (escape latency), time to find location of the platform, time spent in the target zone and Thigmotaxis added to manuscript.

All of results of spatial memory were added to manuscript.

10 - It is not clear how many fields of the hippocampus were used to count positive tunnel cells or pyramidal cells in the tunnel and dark cells in histopathological study.

As suggested by the editor was modified and revised:

Hippocampal tissues sections with 7 μm of thickness were prepared according to Paxinos and Watson atlas that were cut from the dorsal surface of the hippocampus, 3 mm to 4 mm posterior to the Bregma (6 Slices of each rat with 150 μm interval) and finally were stained according to the cresyl violet staining protocol. The CA1 regions of hippocampus were selected and the normal pyramidal cells densities were determined by using a microscope (Nikon) with a grid eyepiece, a digital camera (Sony, Japan). The number of surviving or degenerating neurons was counted in the CA1 hippocampal area on upper quadrant of microscopic fields. Then, the results from 6 sections per animal were calculated.

11. Hippocampal position should give based on the Paxion Atlas

Hippocampal position has been revised based on the Paxinos Atlas.

12. BDNF is not measured in this study (line 148 to 158).

As suggested by the editor, discussion part has been revised completely.

Response to reviewer:

Manuscript entitled **"Effects of Aminoguanidine on apoptosis in hippocampus and memory disorder in a rat model of Alzheimer's disease"**

We appreciate your constructive comments that define both the strengths and limitations of our manuscript. We have modified the manuscript according to your suggestions and have done our best to provide a more transparent presentation by implementing all of your concerns and observations. Below, please find a detailed response to your comments. The manuscript has also been revised to address your concerns.

1- *In section of the abstract and results specific values of experimental and control groups need to be given for important results as brackets.*

As suggested by the reviewer was modified.

2- *Please explain why the Cresyl violet staining method was used in the histopathological study?*

The Cresyl violet is a standard histological stain for neurons. It is a well-known histological method to detect of cell damage in the central nervous system (Alvarez-Buylla et al. 1990; Kosaraju et al. 2013). Cresyl Violet staining method is designed for staining Nissl substance in neurons on formalin fixed, paraffin-embedded tissue and it was completely suitable for our sections.

3- In the histopathological study, the author should mention the sections are randomly selected or not?

In our study, Hippocampal sections were randomly selected. Briefly, hippocampal tissues sections were prepared according to Paxinos and Watson atlas that were cut from the dorsal surface of the hippocampus, 3 mm to 4 mm posterior to the Bregma (6 Slices of each rat with 150 µm interval) and each of them is embedded in a paraffin block and finally the sections with 7 µm thickness of the hippocampus of rats were mounted on slides and were stained according to the cresyl violet staining protocol.

4- How did you distinguish between neurons and neuroglia and also normal and damaged pyramidal cells?

The Cresyl violet labels the neurons that contain Nissll substance. Cresyl violet is a dye that is widely utilized to stain neuronal tissues. Alternatively, when nerve tissue is stained using the cresyl fast violet technique, the Nissl bodies, nuclear membranes and nucleoli stain intensely blue or violet, while the cytoplasm of ganglia cells and glias stain weakly blue. Also distinguish between normal and damaged pyramidal cells were based on the nuclei of the normal pyramidal cells with clearly visible nucleoli, well-defined nuclear membrane and lightly colored cytoplasm were taken into consideration for measurement. The shrunken pyramidal cells with a dark cytoplasm, irregular boundaries and pyknotic nucleus were considered degenerated cells.

5- Why did not use immunohistochemistry staining in your research?

Immunohistochemistry is used in histology to detect the presence of a specific protein marker and differentially expressed proteins in different parts of a biological tissue and also is widely used in the diagnosis of abnormal cells, but the cost of this method is relatively expensive and we have not enough money for use of this procedure in our study.

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Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (1) 27 Jul 2021

Dear EIC,
Comments for the authors:
1. Fig. 3 and the legend of Fig. 3 should corrected. The scale of the vertical line corrected and data of the target zone of WM was only presented. What do you mean: SW and NW, NE and SE in Fig. 3. Please define these abstracts in the legend of Fig. 3. This issue should be presented in the martial method " Learning and spatial memory test" and result in part of the manuscript.
2. Why not present data of DAPI in fig.4. While in material method "TUNEL reaction mixture, rinsed with PBS and subjected to fluorescent counterstaining with DAPI"
3. The vertical line of Fig. 5 changes to the number of normal pyramidal cells
4. In Figure 4 normal cells and dark cells are demonstrated by an arrow in the image.
5. In Figure 4 TUNEL positive cell is demonstrated by the arrow in the image.
6. The English in the manuscript needs to be improved.
Thanks.

Fatemeh Ehsani: EIC | **Revision** (1) 28 Jul 2021

Dear Authors
According to the reviewers and associated editor's comments, your article needs to be revised minor. Please respond the reviewers' comments and submit the required files up to two months. In addition, some grammatical problems are observed in the manuscript. It is suggested that the article is edited by native editor and send the confirmation letter for journal.
Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Associate Editor:

1. The fig. 3 and legend of fig.3 should corrected. Scale of vertical line corrected and data of target zone of WM only presented. What do you mean: SW and NW, NE and SE in fig.3. Please defined these abstract in legend of fig.3. This issue should be presented in martial method " Learning and spatial memory test" and result part of manuscript.

As suggested by the editor the fig. 3 was corrected.

2. Why not present data of DAPI in fig.4. While in material method "TUNEL reaction mixture, rinsed with PBS and subjected to fluorescent counterstaining with DAPI"

As suggested by the editor data of DAPI was added in fig.6.

3. Vertical line of fig. 5 change to Number of normal pyramidal cells.

As suggested by the editor vertical line of fig. 5 was modified.

4. In figure 4 normal cell and dark cell demonstrated by arrow in image.

As suggested by the editor normal pyramidal cells and dark (shrunken) cells were demonstrated by arrows and arrowheads in figure 4.

5. In figure 4 TUNEL positive cell demonstrated by arrow in image.

As suggested by the editor Tunel positive cells were demonstrated by arrows in the image.

6. The English in the manuscript needs improved.

The English of the manuscript was improved.

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (2) 21 Sep 2021

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

Fatemeh Ehsani: EIC | **Revision** (2) 28 Sep 2021

Dear Authors
We are pleased to inform you that your article is accepted for publication in the MEJRHS.
We hope to receive the other qualified articles from your group.
Thanks.