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OPEN PEER REVIEW

The Protective Effects of the *Echium amoenum* Seed Oil Against Seizures Induced by Pentylenetetrazole

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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

2 comments · 13 Jul 2023 – 16 Jul 2023

Referee 1 Reviewer

MAJOR REVISION 13 Jul 2023

1. Did you receive the herbarium code for the Echium amoenum seed?
2. Why is the positive control group not considered in this study? With which drug has the effect of EO on the threshold of seizures induced by PTZ been compared? Can a significant increase in the seizure threshold compared to the vehicle group or the untreated group be considered evidence of the effectiveness of this oil? It would have been better to consider a positive control group (use of a standard anticonvulsant drug) in this study.
3. The abbreviation for gram must be written as "g" in the article.
4. The method states that "24 hours after PTZ injection, the performance of the animals was monitored in terms of abnormal behaviors such as sensitivity to external stimuli such as sound and light, cleaning body hair, muscle tremors, hyperactivity, lack of exploratory activity, isolation, and death", but this is not mentioned in the results and discussion section.
5. Some abbreviations, such as GSH, TBARS, and LPS, are not defined.

Referee 2 Reviewer

MAJOR REVISION 16 Jul 2023

Dear authors,
The manuscript entitled “The protective effects of the Echium amoenum seed oil against seizure induced by pentylenetetrazole” needs major revision to be published in Jundishapur Journal of Natural Pharmaceutical Products. However, some points are mentioned as follows:

1. Please read your manuscript again to find the grammatical and punctuation errors, some of which are highlighted or mentioned in the comments.
2. All abbreviations have to be written in the correct form at first use. Also, please check the correct form of degrees Celsius in other articles.
3. “g” is the correct abbreviation for gram instead of “gr”. Please revise “gr” in the manuscript.
4. The figures and table are unclear because they lack numbering and subtitles. In addition, it is necessary to mention the limits of the p-value.
5. The Echium amoenum seeds used should be identified by a herbarium center, and the voucher number ought to be reported.
6. The conclusion in the abstract section is different from your results. Check it again, please.

Best regards

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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.

Reply to the reviewer's comments:

Dear Reviewer #1, thanks for your insightful reviews. All the issues you mentioned have been corrected in the revision.

Highlights:

1. Read your manuscript again to find the grammatically and punctuation errors, which some are highlighted or mentioned in comments.

   Reply: it was edited.

2. All abbreviation words have to be written in correct form for the first time. Also, please check the right form of Celsius degree in other articles.
   Reply: They were corrected and highlighted in the text.

3. "g" is the correct abbreviation of gram instead of "gr". Please revise "gr" in manuscript.
   Reply: it was corrected.

4. The figures and table are unclear because there is no numbering and subtitle. Another point, it is necessary to mention the limits of p-value.
   Reply: the figures and table had numbering and subtitles. Also, the p-value was mentioned in each figure caption below:

"Figure. 1. Chromatograms of Echium oil obtained by Gas chromatography (GC).

Figure. 2. Effects of Echium oil (EO) on the clonic seizure threshold (A) and superoxide dismutase activity in serum (B). The sub-chronic oral administration of EO increased **A**) seizure threshold, dose-dependently, and **B**) the SOD activity at doses of 1 and 3g/kg. Data represented the values of mean±SEM. * P value<0.05, ** P value <0.01, and *** P value<0.001 vs. vehicle.

Figure. 3. Effect of Echium oil (EO) on the serum level of the liver (A-C) and kidney parameters (D, E). The sub-chronic oral administration of EO did not change the **A**) ALT, **B**) AST, **C**) ALP, **D**) Creatinine, and **E**) Urea. Data represented the values of mean±SEM.

Figure. 4. Effect of Echium oil (EO) on the serum level of lipids (A-E). The sub-chronic oral administration of EO at doses 3 and 5g/kg decreased **A**) TG, **B**) cholesterol, and C**)** VLDL compared to the vehicle. **D**) LDL and **E**) HDL did not change. Data represented the values of mean±SEM. * P value<0.05 and ** P value <0.01 vs. vehicle.

Table 1. The fatty acid composition of Echium oil."

5. The used Echium amoenum seeds should be identified by a herbarium center and the voucher number ought to be reported.

Reply: "herbarium code: 2hc.1400.her" was added to the method.

6. The conclusion in abstract section is different from your results. Check it again.

Reply: it was corrected.

Thank you again and I am looking forward to receiving your reply.

Dear Reviewer #2, Thanks for your careful reviews. All the issues you mentioned have been corrected in the revision.

Highlights:

1. Did you receive the herbarium code for the Echium amoenum seed?

Reply: “herbarium code: 2hc.1400.her” was added to the method.

2. Why is the positive control group not considered in this study? The effect of EO on the threshold of seizure induced by PTZ, has been compared with which drug? Can a significant increase in the seizure threshold compared to the vehicle group or the untreated group be considered as the effectiveness of this oil? It would have been better to consider a positive control group (use of a standard anticonvulsant drug) in this study.

Reply: Thank you for your accurate and completely correct point. We excluded the positive control group in this study due to below reasons;

1. Reduce the number of animals used in this study comply with animal ethics,

2. Some studies conducted in the PTZ threshold model excluded the positive control group (standard drug), (DOI: 10.1016/j.brainresbull.2021.02.011, DOI: 10.1016/j.eplepsyres.2018.10.013),

3. Our goal was to investigate the effectiveness of this oil against convulsions induced by Pentylenetetrazol and we also intended to imitate convulsions in humans. Therefore, we compared the preventive effect of sub-chronic administration of Echium oil in all groups receiving the oil (supplement) with the group receiving the vehicle (placebo) as some human studies.

If we added the positive control group in this study, it will take a 1mounth at least. If it is not necessary in your opinion, we exclude it in the present study. I will consider your informative point in our future studies.

3. The abbreviation of the gram must be written as "g" in the article.

Reply: It was corrected in the text.

4. In the method, it was mentioned that "24 hours after PTZ injection, the performance of the animals was monitored in terms of abnormal behaviors such as sensitivity to external stimuli such as sound and light, cleaning body hair, muscle tremors, hyperactivity, lack of exploratory activity, isolation, and death"; but it is not mentioned in the results and discussion section.

Reply:

- “No abnormal behaviors were observed in the animals during the treatment.” was added to the results.
- “Furthermore, we did not observed any abnormal behaviors in the animals of all experimental groups during the study. So it could be proposed that the subchronic administration of EO is well tolerated.” was added to the discussion.

5. Some abbreviations are not defined such as GSH, TBARS, LPS, ...

Reply: They were corrected and highlighted in the text.

Thank you again and I am looking forward to receiving your reply.

2 Revision 1

0 comments

No public comments in this round.