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Peer Review Report for "Neuroprotective Effects of *Rosa damascena* Essential Oil Against Ethanol-Induced Toxicity in Neural Progenitor Cells"

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Review Timeline:

Submit Date:	8 Mar 2025
Revised Date:	31 May 2025
Accepted Date:	10 Jul 2025

Revision (0)

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

REFEREE : Reviewer

9 Mar 2025

Thank you for your valuable contribution to the field of neuroprotection. Below, I have outlined several questions and suggestions to further improve the manuscript.

1. Introduction

What was the rationale for selecting *Rosa damascena* essential oil for this study? Have comparisons been made with other plant-derived antioxidant compounds? Previous studies on *Rosa damascena* have primarily focused on its antioxidant properties. Are there any direct prior findings supporting its neuroprotective effects

in neural progenitor cells?

2. Methodology

How were the selected doses of *Rosa damascena* essential oil determined? Were they based on previous studies, or was a preliminary dose-response experiment conducted?

Has the toxicity of the essential oil been evaluated at higher doses to determine its safety threshold?

Could the molecular mechanisms underlying the essential oil's effects be further explored, such as its influence on specific signaling pathways?

3. Results

Why was gene expression analysis not conducted at earlier time points (e.g., 12 hours)? Some rapid cellular responses may not be captured in the 24-hour data. Were sex differences (male vs. female) in neural cell responses to treatment considered? If not, would this be an area for future investigation?

4. Discussion and Conclusion

The study does not discuss its limitations. Have the long-term effects of the essential oil on neural cells been examined?

There is no clear proposal for future research. Would it be feasible to extend the study to in vivo animal models for further validation?

Some of the references cited are relatively outdated. Incorporating more recent studies, particularly on the neuroprotective properties of *Rosa damascena* and similar plant extracts, would strengthen the manuscript.

Zahra Mansouri : Reviewer

24 Mar 2025

Thank you for the opportunity to review this manuscript titled "Neuroprotective Effects of *Rosa damascena* Essential Oil Against Ethanol-Induced Toxicity in Neural Progenitor Cells." The study offers intriguing findings on the potential therapeutic benefits of *Rosa damascena*, particularly in mitigating damage to neural cells. While the manuscript presents interesting results, several areas require clarification and further discussion. I have listed my comments below:

1. The Introduction lacks references. Please provide appropriate citations
2. Once an abbreviation is introduced in the main text, there is no need to repeat the full term. It would be better to use the abbreviation instead, as in line 107. Revise this throughout the entire manuscript.
3. The control group should be written in the same format as the others.
4. Big mistake! Immunohistochemistry (IHC) is used for tissue studies, while immunocytochemistry (ICC) is the appropriate method for cell-based studies.

5. In line 172, the first mention of SEM should include its full form: Standard Error of the Mean
6. There is inconsistency in referring to figures ('Fig.' in some parts and 'Figure' in others). Please use a consistent format throughout the manuscript.
7. The asterisk annotations on the graphs should be corrected. The correct approach is to compare the disease group with the control group and the treatment group with the disease group.
8. There is an inconsistency between the figure annotations and the text. The graph uses two asterisks (indicating $p < 0.01$), while the Results section states $p < 0.001$. Please ensure consistency between the figure and the text.
9. In the figure legend (Figure 1), it is stated that asterisks indicate p-values of less than 0.05 and less than 0.001, but the graph shows two asterisks, which represent $p < 0.01$. There are no single or three asterisks to indicate $p < 0.05$ or $p < 0.001$, as described in the legend. When mentioning p-values of less than 0.05 or 0.001 in the caption, it should be specified how many asterisks correspond to these p-values. Please revise the figure to ensure consistency between the asterisk annotations and the p-value descriptions. These issues should be reviewed and corrected in all figures, their captions, and also in the corresponding sections of the Results in the main text to ensure consistency and clarity.
10. In line 227, ' $p < 0.005$ ' should be corrected
11. The figures should be revised, and the asterisks should be placed above each graph.
12. Could you please justify the observed increase in gene expression or cell viability compared to the control group? What are the potential mechanisms or effects of the treatment that might explain these changes? Given that cell viability typically should not exceed the control group, could you please clarify the rationale for the observed increase in viability in the treated group compared to the control
13. The discussion section highlights oxidative stress and ROS, which are presented as the starting point for the study's results. However, oxidative stress and ROS were not measured in the study. Given the emphasis on these factors, it would have been more thorough to assess ROS levels in the experiment to strengthen the connection between the discussed mechanisms and the observed results.

REFeree : Reviewer

25 Mar 2025

Comments to the authors

This study investigates the neuroprotective effects of *Rosa damascena* essential oil against ethanol-induced toxicity in neural progenitor cells. Several issues necessitate careful consideration by the authors to enhance the clarity and quality of the manuscript.

1) In the Results section of the Abstract:

- "Ethanol exposure significantly reduced cell viability in a dose- and time-dependent manner (24, 48, 72 hours), while treatment with 200 μ M ESO significantly improved

survival". It should be mentioned "in NPCs and NCs".

- The results related to protein expression of TAU should be mentioned.

- P-values should be checked for consistency with the results section of the manuscript.

2) References are not cited within the text and require comprehensive correction throughout the manuscript.

3) The authors are advised to carefully revise the entire manuscript for grammatical errors and syntax.

4) Throughout the manuscript, ensure that after the initial explanation of the abbreviations NPCs and NCs, the respective abbreviations are used in the text.

5) Line 83: The age of the animals should be mentioned.

6) Line 107, 156, 179, and 186: Replace "NSCs" with "NPCs".

7) Line 139: "During the 7-day treatment period". Was the duration of treatment for the groups 7 days or 24, 48, and 72 hours? It should be revised.

8) The method of differentiating NPCs to NCs should be mentioned in the "Methods" section.

9) Some abbreviations, such as Nestin, Sox2, Pax6, Tuj1, and Map2, should be fully explained the first time they are used.

10) Line 178: "Ethanol toxicity on NPCs and NCs".

11) Line 191: Graphs 1B, C, D, and E have no control groups. How was the statistical comparison of different concentrations at different times conducted?

12) Statistical comparisons presented in the results section, along with the symbols used, may be confusing to the readers. It is recommended to use the symbol * to denote comparisons between the control group and the ethanol group, while the symbol # should be used to indicate comparisons between the ethanol group and the ethanol + essential oil group. Furthermore, p-values are defined as follows: *P < 0.05, **P < 0.01, and ***P < 0.001.

13) Line 202: ... was evaluated at 24, 48, and 72 hours.

14) Line 204: Insert "after 48 and 72 hours" after "(Fig. 1G)". Furthermore, replace "control group" with "ETH group". Co-treatment of cells with ethanol and essential oil should be compared to the ethanol group for statistical analysis.

15) In the "Gene expression analysis" section:

- first explain what each graph is about and then present the results.
- Significant differences are not shown in graphs 2B and 2D.
- In Graphs 2B and 2D, why is the effect of the ethanol + essential oil treatment is compared to the control group rather than the ethanol group?
- In the caption of Figure 2, provide a description of the graph. For example: A) The effect of ethanol on the expression levels of Nestin, Sox2, and Pax6 genes in NPCs after 24, 48, and 72 hours. Same for B, C, and D graphs.

16) Line 281: "significantly enhanced cell viability, particularly at higher concentrations".

Rosa Damascena essential oil was used to investigate its effect on the reduction of cell viability induced by ethanol at a concentration of 200 μ M. So how did it increase cell viability at higher concentrations?

17) Line 316: "Rosa Damascena essential oil, either alone or in combination with ethanol".

The graphs related to the expression of these genes do not show the effect of essential oil alone in regulating these genes. It should be revised.

18) The results related to the expression of TAU protein are not discussed and should be addressed in the Discussion section.

19) The authors state in the last paragraph of the discussion that "Rosa Damascena essential oil may offer a promising natural remedy for protecting neural stem cells and promoting neurogenesis, particularly in the context of oxidative stress induced by ethanol". However, this study did not measure any factors related to oxidative stress. Therefore, how can the observed effects be attributed to the modulation of ethanol-induced oxidative stress?

20) Lines 328-330: How does essential oil prevent spontaneous pathological cell death in neural stem cells and which graph supports this?

Sara Bahrainian : Associate Editor

25 Mar 2025

Thank you for submitting your manuscript to JJNPP.

Please revise the manuscript and submit a point-by-point response to the reviewers' comments and concerns and highlight any changes in the text.

I would also greatly appreciate the author's contribution and time for reviewing manuscripts for this journal. Without the dedication of reviewers like you, it would be impossible to manage an efficient peer review process and maintain the high standards necessary for a successful journal.

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.

Dear editor:

We revised the manuscript entitled “**Neuroprotective Effects of Rosa damascena Essential Oil Against Ethanol-Induced Toxicity in Neural Progenitor Cells**” based on the comments of the reviewers. We thank the reviewers for their comments, as addressing these issues has substantially improved the manuscript's clarity and rigor. We have edited the text based on reviewers' comments. Point-by-point responses to each of the reviewer's concerns are included below.

Reviewers' Comments:

- **Reviewer 1:**
Thank you for your valuable contribution to the field of neuroprotection. Below, I have outlined several questions and suggestions to further improve the manuscript.

1. Introduction

- what was the rationale for selecting Rosa damascena essential oil for this study?
Have comparisons been made with other plant-derived antioxidant compounds?

Thanks for reviewer's #1 comments to improve the manuscript. We chose Rosa damascena essential oil due to its rich flavonoid content and well-documented traditional use for antioxidant and anti-inflammatory properties (Akram et al., 2020). While direct comparisons with other plant-derived antioxidants were not performed in this study, we note that compounds like quercetin have shown neuroprotective effects (Chan et al., 2014). Our focus was to explore Rosa damascena's specific potential in neural progenitor cells (NPCs), a novel application. This rationale has been added to the Introduction.

- Previous studies on Rosa damascena have primarily focused on its antioxidant properties. Are there any direct prior findings supporting its neuroprotective effects in neural progenitor cells?

To our knowledge, this is the first study examining Rosa damascena essential oil's neuroprotective effects specifically in NPCs. Prior research, such as Homayoun et al. (2015), demonstrated its ability to reduce dark neuron formation in the hippocampus, suggesting broader neuroprotective potential. We've clarified this in the Introduction, positioning our study as an extension to NPCs.

2. Methodology

- how were the selected doses of Rosa damascena essential oil determined? Were they based on previous studies, or was a preliminary dose-response experiment conducted?

The doses were determined via a preliminary dose-response experiment testing concentrations from 25 to 500 μ M, identifying 200 μ M as the optimal non-toxic dose. This has been explicitly detailed in the Methods section.

- Has the toxicity of the essential oil been evaluated at higher doses to determine its safety threshold?

Yes, we tested concentrations up to 500 μ M, observing reduced cell viability above 200 μ M. This safety threshold has been added to the Results section.

- Could the molecular mechanisms underlying the essential oil's effects be further explored, such as its influence on specific signaling pathways?

We agree that exploring pathways like PI3K/Akt or MAPK could elucidate the mechanisms. While beyond this study's scope, we've added this as a future research direction in the Discussion.

3. Results

- Why was gene expression analysis not conducted at earlier time points (e.g., 12 hours)? Some rapid cellular responses may not be captured in the 24-hour data.

We selected 24, 48, and 72 hours to capture both short- and long-term effects. Earlier time points (e.g., 12 hours) could indeed reveal rapid responses, and we've noted this limitation in the Discussion, suggesting it for future studies.

- Were sex differences (male vs. female) in neural cell responses to treatment considered? If not, would this be an area for future investigation?

Sex differences were not assessed, as cells were pooled from both male and female rats. We've acknowledged this limitation in the Discussion and proposed it as a future research area.

4. Discussion and Conclusion

- The study does not discuss its limitations. Have the long-term effects of the essential oil on neural cells been examined?

No long-term effects were examined (limited to 72 hours). We've added a limitations paragraph in the Discussion, noting the need for extended in vivo studies.

- There is no clear proposal for future research. Would it be feasible to extend the study to in vivo animal models for further validation?

We've added a future research proposal in the Discussion, suggesting in vivo validation in models of ethanol-induced neurotoxicity.

- Some of the references cited are relatively outdated. Incorporating more recent studies, particularly on the neuroprotective properties of *Rosa damascena* and similar plant extracts, would strengthen the manuscript.

We've updated the reference list with recent studies (more than a dozen other articles from the last couple of years), such as, Chroho et al. (2022) on *Rosa damascena*'s phenolic composition and Beigom et al. (2023) on Effect of *Rosa damascena* extract on rat model Alzheimer's disease, etc.

Reviewer 2:

Comments to the authors

This study investigates the neuroprotective effects of *Rosa damascena* essential oil against ethanol-induced toxicity in neural progenitor cells. Several issues necessitate careful consideration by the authors to enhance the clarity and quality of the manuscript.

1) In the Results section of the Abstract:

- "Ethanol exposure significantly reduced cell viability in a dose- and time-dependent manner (24, 48, 72 hours), while treatment with 200 μ M ESO significantly improved survival". It should be mentioned "in NPCs and NCs". The results related to protein expression of TAU should be mentioned. P-values should be checked for consistency with the results section of the manuscript.

Thanks for reviewer's #2 comments to improve the manuscript. The Abstract now specifies effects in NPCs and NCs, includes TAU protein expression results, and ensures p-value consistency with the Results section.

2) References are not cited within the text and require comprehensive correction throughout the manuscript.

All statements requiring citations have been referenced in-text, and the reference list has been corrected.

3) The authors are advised to carefully revise the entire manuscript for grammatical errors and syntax.

The manuscript has been thoroughly proofread for grammar and syntax.

4) Throughout the manuscript, ensure that after the initial explanation of the abbreviations NPCs and NCs, the respective abbreviations are used in the text.

Abbreviations are now consistently used after initial definitions.

5) Line 83: The age of the animals should be mentioned.

The Methods section now specifies that baby Wistar rats were 1-2 weeks old.

6) Line 107, 156, 179, and 186: Replace "NSCs" with "NPCs".

All instances of "NSCs" have been corrected to "NPCs".

7) Line 139: "During the 7-day treatment period". Was the duration of treatment for the groups 7 days or 24, 48, and 72 hours? It should be revised.

Corrected to reflect the actual treatment durations of 24, 48, and 72 hours.

8) The method of differentiating NPCs to NCs should be mentioned in the "Methods" section.

Details on differentiation by withdrawing growth factors have been added to the Methods.

9) Some abbreviations, such as Nestin, Sox2, Pax6, Tuj1, and Map2, should be fully explained the first time they are used.

Full gene names are now provided at first mention.

10) Line 178: “Ethanol toxicity on NPCs and NCs”.

Revised to a complete heading: “Ethanol Toxicity on NPCs and NCs”.

11) Line 191: Graphs 1B, C, D, and E have no control groups. How was the statistical comparison of different concentrations at different times conducted?

Control groups (0% ethanol or 0 μ M ESO) were considered to have 100% survival and the results are measured relative to the control group with 100% survival.

12) Statistical comparisons presented in the results section, along with the symbols used, may be confusing to the readers. It is recommended to use the symbol * to denote comparisons between the control group and the ethanol group, while the symbol # should be used to indicate comparisons between the ethanol group and the ethanol + essential oil group. Furthermore, p-values are defined as follows: *P < 0.05, **P < 0.01, and ***P < 0.001.

Adopted this notation for clarity in figures and Results.

13) Line 202: ... was evaluated at 24, 48, and 72 hours.

Sentence completed for clarity.

14) Line 204: Insert “after 48 and 72 hours” after “(Fig. 1G)”. Furthermore, replace “control group” with “ETH group”. Co-treatment of cells with ethanol and essential oil should be compared to the ethanol group for statistical analysis.

Text revised to compare ETH+ESO to the ETH group, with time points specified.

15) In the “Gene expression analysis” section:

- first explain what each graph is about and then present the results.
- Significant differences are not shown in graphs 2B and 2D.
- In Graphs 2B and 2D, why is the effect of the ethanol + essential oil treatment is compared to the control group rather than the ethanol group?
- In the caption of Figure 2, provide a description of the graph. For example: A) The effect of ethanol on the expression levels of Nestin, Sox2, and Pax6 genes in NPCs after 24, 48, and 72 hours. Same for B, C, and D graphs.

Section restructured with graph descriptions first, significance added to 2B and 2D, comparisons adjusted to ETH group, and Figure 2 captions enhanced.

16) Line 281: “significantly enhanced cell viability, particularly at higher concentrations”. Rosa Damascena essential oil was used to investigate its effect on the reduction of cell viability induced by ethanol at a concentration of 200 μ M. So how did it increase cell viability at higher concentrations?

Clarified that higher ESO concentrations alone reduced viability, but 200 μ M with ethanol improved it compared to ethanol alone.

17) Line 316: “Rosa Damascena essential oil, either alone or in combination with ethanol”.

The graphs related to the expression of these genes do not show the effect of essential oil alone in regulating these genes. It should be revised.

Revised to reflect only tested groups (ETH and ETH+ESO).

18) The results related to the expression of TAU protein are not discussed and should be addressed in the Discussion section.

Added a paragraph discussing TAU protein results in the Discussion.

19) The authors state in the last paragraph of the discussion that “Rosa Damascena essential oil may offer a promising natural remedy for protecting neural stem cells and promoting neurogenesis, particularly in the context of oxidative stress induced by ethanol”. However, this study did not measure any factors related to oxidative stress. Therefore, how can the observed effects be attributed to the modulation of ethanol-induced oxidative stress?

Tempered the claim, noting that while ethanol induces oxidative stress and ESO has antioxidant properties, we didn’t measure oxidative stress markers, suggesting this for future studies.

20) Lines 328-330: How does essential oil prevent spontaneous pathological cell death in neural stem cells and which graph supports this?

Corrected to “ethanol-induced cell death,” supported by Figure 1F and 1G.

Reviewer 3:

Thank you for the opportunity to review this manuscript titled” Neuroprotective Effects of Rosa damascena Essential Oil Against Ethanol-Induced Toxicity in Neural Progenitor Cells.” The study offers intriguing findings on the potential therapeutic benefits of Rosa damascena, particularly in mitigating damage to neural cells. While the manuscript presents interesting results, several areas require clarification and further discussion. I have listed my comments below:

1. The Introduction lacks references. Please provide appropriate citations.

Thanks for reviewer’s #3 comments to improve the manuscript. Citations added throughout the Introduction.

2. Once an abbreviation is introduced in the main text, there is no need to repeat the full term. It would be better to use the abbreviation instead, as in line 107. Revise this throughout the entire manuscript.

Ensured consistent abbreviation use after initial definitions.

3. The control group should be written in the same format as the others.

Control group labeling standardized in text and figures.

4. Big mistake! Immunohistochemistry (IHC) is used for tissue studies, while immunocytochemistry (ICC) is the appropriate method for cell-based studies.

Corrected to “immunocytochemistry” throughout.

5. In line 172, the first mention of SEM should include its full form: Standard Error of the Mean.

Defined as “Standard Error of the Mean” at first use.

6. There is inconsistency in referring to figures ('Fig.' in some parts and 'Figure' in others). Please use a consistent format throughout the manuscript.

Standardized to “Figure” in full sentences and “Fig.” in parentheses.

7. The asterisk annotations on the graphs should be corrected. The correct approach is to compare the disease group with the control group and the treatment group with the disease group.

Adjusted comparisons in figures as per Reviewer 2's notation.

8. There is an inconsistency between the figure annotations and the text. The graph uses two asterisks (indicating $p < 0.01$), while the Results section states $p < 0.001$. Please ensure consistency between the figure and the text.

P-values aligned between text and figures.

9. In the figure legend (Figure 1), it is stated that asterisks indicate p-values of less than 0.05 and less than 0.001, but the graph shows two asterisks, which represent $p < 0.01$. There are no single or three asterisks to indicate $p < 0.05$ or $p < 0.001$, as described in the legend. When mentioning p-values of less than 0.05 or 0.001 in the caption, it should be specified how many asterisks correspond to these p-values. Please revise the figure to ensure consistency between the asterisk annotations and the p-value descriptions. These issues should be reviewed and corrected in all figures, their captions, and also in the corresponding sections of the Results in the main text to ensure consistency and clarity.

Revised figures and legends to use * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) consistently.

10. In line 227, ' $p < 0.005$ ' should be corrected.

Corrected to $p < 0.05$ based on standard thresholds.

11. The figures should be revised, and the asterisks should be placed above each graph.

Asterisks placed above bars in all figures.

12. Could you please justify the observed increase in gene expression or cell viability compared to the control group? What are the potential mechanisms or effects of the treatment that might explain these changes? Given that cell viability typically should not exceed the control group, could you please clarify the rationale for the observed increase in viability in the treated group compared to the control.

None of the treatments had greater survival or gene expression than the control group, although there was a minor mistake in explaining the results that was corrected in result and discussion sections.

13. The discussion section highlights oxidative stress and ROS, which are presented as the starting point for the study's results. However, oxidative stress and ROS were not measured in the study. Given the emphasis on these factors, it would have been more thorough to assess ROS levels in the experiment to strengthen the connection between the discussed mechanisms and the observed results.

Revised Discussion to clarify that oxidative stress is a known ethanol effect, but not measured here, suggesting future ROS assessments.

OPEN PEER REVIEW

Revision (1)

REFeree : Reviewer

7 Jul 2025

The authors' response to the reviewers demonstrates a robust and systematic approach to addressing critiques, significantly enhancing the manuscript's scientific rigor and clarity. Key revisions include methodological clarifications, such as detailing dose determination via preliminary experiments and correcting immunocytochemistry terminology, which strengthen experimental transparency. Statistical rigor was improved by standardizing p-value annotations (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$) and aligning comparisons between ethanol-treated and control groups. The addition of a limitations section—acknowledging the absence of long-term data, sex-specific analyses, and oxidative stress marker measurements—reflects scholarly humility. Novelty is underscored by positioning the study as the first to explore *Rosa damascena*'s neuroprotective effects in NPCs, supported by updated references to recent literature. However, mechanistic gaps persist: while oxidative stress is theorized as a pathway, the lack of direct ROS measurement weakens causal claims, necessitating future validation. Reviewer 1's request for comparative analysis with other antioxidants (e.g., quercetin) remains partially addressed, suggesting a benchmark study could further contextualize *Rosa damascena*'s efficacy. Minor inconsistencies in figure annotations (e.g., asterisk placement) and the rationale for selecting 200 μM ESO—despite higher concentrations reducing viability—warrant explicit clarification. Final recommendations include proofreading for residual grammatical errors, adding supplemental data (dose-response curves), and expanding the future work section to prioritize in vivo models and pathway-specific investigations. Overall, the revisions position the manuscript for acceptance, pending minor editorial refinements to ensure figure-text consistency and mechanistic coherence.