

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.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:

<https://brieflands.com/briefland/knowledgebase/category/tree.html#opr.html>



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Peer Review Report for "Chrysin and Gallic Acid Protect the Hippocampal Neurons and Mitigate Blood-Brain Barrier Disruption in a Mouse Model of Global Cerebral Ischemia"

Author(s): Ali Vakili, Shahein Momenabadi, Ahmad Reza Bandegi, Abbas Ali Vafaei, Abedin Vakili

Review Timeline:

Submit Date:	25 Jan 2025
Revised Date:	15 Feb 2025
Accepted Date:	19 Feb 2025

Revision (0)

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

Soheil Abbaspour-Ravasjani : Reviewer

31 Jan 2025

Abstract

- * Specify how the combination of Chrysin and Gallic Acid (GA) performed better than individual treatments in quantitative terms (e.g., percentage improvement in BBB integrity or memory retention).
- * Highlight the potential clinical implications of this combined therapy.
- * Replace "Chrysin (30 mg/kg, IP), G.A (50 mg/kg, IP), and their combination were given at the start of reperfusion, as well as 30 minutes and 1 hour afterward" with "Chrysin (30 mg/kg, IP), G.A (50 mg/kg, IP), and their combination were administered at the start of reperfusion and subsequently at 30 minutes and 1 hour."

- * Briefly mention the molecular pathway through which the treatments might exert their effects (e.g., claudin-5 regulation).

Introduction

- * Provide additional background on the limitations of existing treatments for cerebral ischemia.
- * Justify the choice of targeting claudin-5 as a marker for BBB integrity.
- * Simplify "Chrysin is a natural flavonoid found in plants, honey, and propolis (6), exhibits, antioxidant, anti-inflammatory, and anti-apoptotic properties, making it a compound of significant interest in therapeutic applications" to "Chrysin, a natural flavonoid found in plants, honey, and propolis, exhibits antioxidant, anti-inflammatory, and anti-apoptotic properties, making it therapeutically significant."
- * Include comparisons with other flavonoids or polyphenols that have been studied for similar neuroprotective effects.

Materials and Methods

- * Provide more justification for the selected doses of Chrysin and GA.
- * Explain how randomization and blinding were implemented to avoid bias.
- * Replace "Brains were carefully extracted and preserved in a 10% formalin solution for the evaluation of neuronal damage" with "Brains were carefully extracted and fixed in a 10% formalin solution for neuronal damage evaluation."
- * Justify the use of the Evans Blue (EB) assay for assessing BBB permeability compared to other potential methods.

Results

Hippocampal Neuron Protection

- * Provide quantitative data comparing the neuronal survival rates between the combination and individual treatment groups.
- * Clarify whether the results were dose-dependent.
- * Replace "The combined Chrysin and G.A treatment further enhanced neuronal survival in all regions (Figure 1A-D; $P<0.001$)" with "Combined Chrysin and G.A treatment significantly enhanced neuronal survival across all regions (Figure 1A-D; $P<0.001$)."
- * Discuss the potential mechanisms contributing to the observed synergy between Chrysin and GA.

Behavioral and Cognitive Improvements

- * Specify whether escape latency and target zone time improvements correlated with neuronal survival data.
- * Discuss potential variability in behavioral assessments.
- * Simplify "Neurological evaluations revealed a notable reduction in the neurological deficit score in animals treated with Chrysin + G.A" to "Chrysin + G.A treatment significantly reduced neurological deficit scores."
- * Mention whether stress or anxiety levels were controlled during the Radial Arm Water Maze (RAWM) test.

Claudin-5 Expression and BBB Integrity

- * Highlight the magnitude of improvement in claudin-5 expression compared to controls.
- * Discuss the clinical relevance of reducing EB leakage.
- * Replace "The combination of Chrysin + G.A more effectively increased the expression of claudin-5 compared to the G.A group" with "The Chrysin + G.A combination significantly increased claudin-5 expression compared to the G.A group."
- * Explain why claudin-5 was specifically chosen as a marker over other tight junction proteins.

Discussion

- * Provide a more detailed analysis of the synergy observed between Chrysin and GA.
- * Discuss the translational potential of these findings for human clinical applications.
- * Replace "This study revealed that temporary BCCAO, led to significant hippocampal damage, spatial memory and neurological dysfunction" with "Temporary BCCAO resulted in significant hippocampal damage, as well as spatial memory and neurological dysfunction."
- * Elaborate on potential molecular pathways (e.g., PI3K/Akt, MAPK/ERK) involved in neuroprotection.

Conclusion

- * Summarize key findings and their implications for clinical research.
- * Suggest future studies to validate the findings in chronic ischemia models.
- * Replace "The treatment also preserved BBB integrity by upregulating claudin-5 expression" with "The treatment preserved BBB integrity through upregulation of claudin-5 expression."
- * Address potential limitations, such as sample size and lack of long-term follow-up.

Figures and Tables

- * Ensure all figures include error bars and statistical significance markers.
- * Improve figure legends for clarity, specifying the experimental conditions.
- * Maintain consistent language in figure legends, avoiding redundancy.
- * Provide more detailed explanations for observed trends in the graphical data.

Here is a useful reference for authors observation:

<https://www.sciencedirect.com/science/article/pii/S0378517323002351>

Esmail Mohammadian : Reviewer

1 Feb 2025

Dear Editor

Have a nice day.

The author investigated "Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia".

In my opinion, this manuscript cannot be considered for publication. The main reasons for this outcome are as follows:

1. The manuscript is not well written in all parts.

mahmoud omidi : Reviewer

4 Feb 2025

Dear Editor- in - Chief,

After carefully evaluating the above article entitled: " Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia", I found that this research is a valuable scientific work, and in my opinion, it has all the criterias of acceptance in your journal. Thank you for allowing me the honor of being a reviewer for your magazine.

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 Dr. Moghimipour,

We sincerely appreciate the rapid evaluation and the insightful comments and feedback from the reviewers on our manuscript (JJNPP, Manuscript ID: 159908) titled “Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia.” We have carefully reviewed the feedback, incorporated the necessary revisions, and updated our manuscript accordingly. The modifications in the text are highlighted in blue. A detailed, point-by-point response to the reviewers' comments is provided below.

Reviewer #1: The author investigated “Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia”.

In my opinion, this manuscript cannot be considered for publication. The main reasons for this outcome are as follows:

1. The manuscript is not well written in all parts.

Response: Thank you for the comment. We revised comprehensively the manuscript to improve the language and clarity. To achieve this, we used professional language editing tools and services, including AI-based platforms like ChatGPT and Grammarly software.

Reviewer 2: The manuscript "Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia" was reviewed, and the following objections were raised:

1. It is better to check again written language with a native editor or relevant software.

Response: Thank you for your comment. We have carefully revised the manuscript to enhance both the language and clarity. To do so, we employed professional language editing tools and services, including AI-powered platforms like ChatGPT and Grammarly software.

2. When mentioning dosages, include units consistently and instead of “Chrysin (30 mg/kg, IP)” consider writing “Chrysin (30 mg/kg, intraperitoneally)”.

Response: Thank you for the suggestion. We have revised the manuscript to include consistent units when mentioning dosages. Additionally, we have updated the phrase 'Chrysin (30 mg/kg, IP)' to 'Chrysin (30 mg/kg, intraperitoneally)' for greater clarity

3. "evans blue (E.B)" should be "Evans blue (EB)" for consistency in capitalization.

Response: Thank you for pointing this out. We have corrected the capitalization to maintain consistency, changing 'evans blue (E.B.)' to 'Evans blue (EB)' throughout the manuscript.

4. Inconsistent abbreviation use (e.g., "G.A" vs. "GA").

Response: Thank you for bringing this to our attention. We have reviewed the manuscript and standardized the abbreviation usage, ensuring consistency by using 'G.A' throughout instead of GA

5. Remove the duplicate "in": "Transient global cerebral ischemia (24) was induced in in mice under anesthesia with ketamine.

Response: Thank you for the observation. This error has been corrected.

6. Ensure consistency in capitalization for “p” in “post ischemic treatment with Chrysin and G.A significantly increased neuronal survival in this regions”.

Response: Thank you for the observation. This error has been corrected.

Reviewer 3:

After carefully evaluating the above article entitled: " Chrysin and Gallic acid protect the hippocampal neurons and mitigate blood-brain barrier disruption in a mouse model of global cerebral ischemia", I found that this research is a valuable scientific work, and in my opinion, it has all the criterias of acceptance in your journal. Thank you for allowing me the honor of being a reviewer for your magazine.

Response: We greatly appreciate your positive evaluation of our work. It is an honor to have had our research reviewed by you, and we are grateful for the time and effort you put into the process

Reviewer 4:

1. Abstract

* Specify how the combination of Chrysin and Gallic Acid (GA) performed better than individual treatments in quantitative terms (e.g., percentage improvement in BBB integrity or memory retention).

* Highlight the potential clinical implications of this combined therapy.

* Replace "Chrysin (30 mg/kg, IP), G.A (50 mg/kg, IP), and their combination were given at the start of reperfusion, as well as 30 minutes and 1 hour afterward" with "Chrysin (30 mg/kg, IP), G.A (50 mg/kg, IP), and their combination were administered at the start of reperfusion and subsequently at 30 minutes and 1 hour."

* Briefly mention the molecular pathway through which the treatments might exert their effects (e.g., claudin-5 regulation).

Response: Thank you for your valuable feedback. The abstract of the manuscript has been completely revised as requested (Page 1).

Word Count Policy for Brieflands Journals (including tables and references for original articles):

Maximum Word Count = 3500. Therefore, we are unable to include additional data in the abstract, which has a 250-word limit. However, we have incorporated the key points to revise and improve the abstract.

The point regarding the molecular pathway through which the treatments may exert their effects (e.g., claudin-5 regulation) has been addressed and briefly mentioned in the discussion section of the manuscript.

2. Introduction

- * Provide additional background on the limitations of existing treatments for cerebral ischemia.
- * Justify the choice of targeting claudin-5 as a marker for BBB integrity.
- * Simplify "Chrysin is a natural flavonoid found in plants, honey, and propolis (6), exhibits, antioxidant, anti-inflammatory, and anti-apoptotic properties, making it a compound of significant interest in therapeutic applications" to "Chrysin, a natural flavonoid found in plants, honey, and propolis, exhibits antioxidant, anti-inflammatory, and anti-apoptotic properties, making it therapeutically significant."
- * Include comparisons with other flavonoids or polyphenols that have been studied for similar neuroprotective effects.

Response: Thank you for your insightful comments. The introduction part of the manuscript has been revised as requested. Due to the word count limitation (Maximum Word Count = 3500), the key points suggested by the reviewer have been incorporated into the introduction section of the manuscript as follows (Page2, Paragraph 1):

1. We have added background information on the limitations of existing treatments for cerebral ischemia in the manuscript (Page 8 and Paragraph 2).
2. Due to the word count limitation, this point was briefly included, along with a key reference on claudin-5 as a marker for BBB integrity to the discussion section (Page 8 and Paragraph 1).
3. This point has been corrected and replaced with a new sentence as suggested.
4. Thank you for your suggestion. However, due to the word count limitation, we are unable to include 'comparisons with other flavonoids or polyphenols that have been studied for similar neuroprotective effects' in the introduction. The current word count of the manuscript exceeds 4000 words after revision.

3. Materials and Methods

1. Provide more justification for the selected doses of Chrysin and GA.

Response: Thank you for your valuable feedback. The dose of Chrysin and GA was selected, based on previous research on the experimental model of cerebral ischemia, this study selected therapeutic dosages of 30 mg/kg for Chrysin [14,29,30] and 50 mg/kg for G.A [20,31,32] to balance efficacy with minimal toxicity. Xiao-Li and colleagues demonstrated Chrysin at the dosage of 30 mg/kg significantly reduced neuronal damage and improved cognitive function, whereas the dosage of 100 mg/kg did not provide beneficial effects in cerebral ischemia induced by bilateral common carotid arteries in rats [29]. These results are consistent with the findings of the present study. They further suggested that higher doses of Chrysin might have toxic effects, potentially diminishing its therapeutic efficacy [29].

However, due to the word count limitation, we are unable to include these points in the manuscript. Instead, the references have been added to the Materials and Methods section (Page 4, Paragraph 1).

2. Explain how randomization and blinding were implemented to avoid bias.

Response: In this study, although drug administration was not blinded, the overall experimental procedure was conducted in a blinded manner to minimize bias. Tissue sample evaluations, including hematoxylin and eosin (H&E) staining and immunohistochemistry analyses, were coded according to group assignment. This coding ensured that the experimenters conducting the evaluations remained unaware of the experimental conditions. Furthermore, spatial memory and neurological evaluations were performed by students who were blinded to the experimental groupings.

This point shortly has been included in the data analysis section (Page 6, Paragraph 1).

3. Replace "Brains were carefully extracted and preserved in a 10% formalin solution for the evaluation of neuronal damage" with "Brains were carefully extracted and fixed in a 10% formalin solution for neuronal damage evaluation."

Response: This point has been corrected

4. Justify the use of the Evans Blue (EB) assay for assessing BBB permeability compared to other potential methods.

Response: Thank you for your suggestion. Due to the word count limitation policy, we are unable to include this topic in detail. However, we have added the reference to the Materials and Methods section. It is worth noting that EB is a widely used as a reliable method for assessing BBB permeability in many studies (Belayev et. al., 1996; Yao et.al., 2022, Mehr MH et.al., 2024, Xiao et.al., 2024).

3. Results

Thank you for your valuable feedback, Due to the word count limitation policy, the key points suggested have been incorporated into the results section of the manuscript as follows:

3.1. Provide quantitative data comparing the neuronal survival rates between the combination and individual treatment groups.

Response: The quantitative data for neuronal survival rates has been included in the result section (Page 6, Paragraph 1).

3.2. Clarify whether the results were dose-dependent.

Response: In this study, a single dose of Chrysin and/or G.A, as approved by previous research, was used, without focusing on dose-response relationships.

3.3. Replace "The combined Chrysin and G.A treatment further enhanced neuronal survival in all regions (Figure 1A-D; $P < 0.001$)" with "Combined Chrysin and G.A treatment significantly enhanced neuronal survival across all regions (Figure 1A-D; $P < 0.001$)."

Response: This point has been corrected

3.4. Discuss the potential mechanisms contributing to the observed synergy between Chrysin and GA.

Response: This point has been briefly included in the discussion section due to the word count limitation (Page7, Paragraph 3).

3.5. Specify whether escape latency and target zone time improvements correlated with neuronal survival data.

Response: This point has been briefly included in the discussion section due to the word count limitation (Page7, Paragraph 4).

The most significant finding of this study demonstrated that combined intervention notably reduced sensory-motor and spatial memory dysfunction. These behavioral improvements were further supported by histological analysis, which revealed a significant increase in neuronal survival in the combined treatment group. These results suggest a positive correlation between the improvements in behavioral outcomes and enhanced neuronal survival rates.

3.6. Discuss potential variability in behavioral assessments.

Response: Potential variability in behavioral assessments may arise due to factors such as individual differences in baseline performance, environmental influences, and experimental conditions. Additionally, the complexity of behavioral tasks and subject stress levels can contribute to inconsistencies in the results.

In this study, behavioral assessments were performed under minimal stress conditions by an evaluator who was blinded to the group assignments. Due to the word count limitation policy, we are unable to include this topic in detail in the manuscript.

3.7, Simplify "Neurological evaluations revealed a notable reduction in the neurological deficit score in animals treated with Chrysin + G.A" to "Chrysin + G.A treatment significantly reduced neurological deficit scores."

Response: This point has been corrected

3.8.Mention whether stress or anxiety levels were controlled during the Radial Arm Water Maze (RAWM) test.

Response: This point has been briefly addressed in the RAWM section of the Materials and Methods. The protocol comprised the following phases: Habituation (1 day): Mice were introduced to the water and maze environment to minimize stress and acclimate them to swimming. Training Phase (4 days): Mice were trained to find a submerged platform at the end of one arm of the maze. Each mouse underwent five trials per day, using spatial cues for navigation. Mice that failed to locate the platform within 30 seconds were gently guided to it. Probe trial (1 day): A probe trial assessed memory retention following 4 days of training (Page4, Paragraph 6).

3.10. Highlight the magnitude of improvement in claudin-5 expression compared to controls.

Response: This point has been corrected and quantitative data has been added to the result (Page 7, Paragraph 1).

3.11. Discuss the clinical relevance of reducing EB leakage.

Response: This point has been briefly addressed in the discussion section of the manuscript due to the word count limitation.

Reducing EB leakage is clinically relevant as it indicates improved blood-brain barrier (BBB) integrity. A more intact BBB can help protect the brain from harmful substances and support better therapeutic delivery. This could have significant implications for treating neurological conditions, such as stroke, traumatic brain injury, or neurodegenerative diseases, where BBB dysfunction is often a key factor in disease progression. By preventing EB leakage, treatments could potentially reduce neuroinflammation, promote neuronal survival, and improve overall brain health.

3.12. Replace "The combination of Chrysin + G.A more effectively increased the expression of claudin-5 compared to the G.A group" with "The Chrysin + G.A combination significantly increased claudin-5 expression compared to the G.A group."

Response: This point has been corrected.

3.13. Explain why claudin-5 was specifically chosen as a marker over other tight junction proteins.

Response: This point has been briefly addressed in the discussion section of the manuscript due to the word count limitation (Page 8, Paragraph 2).

Claudin-5 was specifically chosen as a marker over other tight junction proteins because it is highly enriched in endothelial cells of the blood-brain barrier (BBB) and plays a crucial role in maintaining barrier integrity. Unlike other claudins that are expressed in various epithelial and endothelial tissues, claudin-5 is predominantly found in brain microvascular endothelial cells, making it a reliable marker for studying BBB function and permeability (Greene et. al., 2019, Nitta et al., 2003; Ma et. al.2017; Jia et al., 2013) Additionally, its regulation is closely associated with pathological conditions such as neuroinflammation, stroke, and neurodegenerative diseases, further emphasizing its relevance in BBB-related research (Greene et. al., 2019, Nitta et al., 2003; Ma et. al.2017; Jia et al., 2013)

4. Discussion

Thank you for your valuable comments, Due to the word count limitation policy, briefly the key points have been incorporated into the discussion section of the manuscript as follows:

4.1. Provide a more detailed analysis of the synergy observed between Chrysin and GA.

Response: This point has been briefly addressed in the discussion section of the manuscript due to the word count limitation

4.2. Discuss the translational potential of these findings for human clinical applications.

Response: This point has been briefly addressed in the conclusion section of the manuscript due to the word count limitation

4.3. Replace "This study revealed that temporary BCCAO, led to significant hippocampal damage, spatial memory and neurological dysfunction" with "Temporary BCCAO resulted in significant hippocampal damage, as well as spatial memory and neurological dysfunction."

Response: This point was removed in the revised form of the manuscript.

4.4. Elaborate on potential molecular pathways (e.g., PI3K/Akt, MAPK/ERK) involved in neuroprotection.

Response: This point has been briefly addressed in the discussion section of the manuscript due to the word count limitation(Page 8, Paragraph 2).

5. Conclusion

- * Summarize key findings and their implications for clinical research.
- * Suggest future studies to validate the findings in chronic ischemia models.
- * Replace "The treatment also preserved BBB integrity by upregulating claudin-5 expression" with "The treatment preserved BBB integrity through upregulation of claudin-5 expression."
- * Address potential limitations, such as sample size and lack of long-term follow-up.

Response: The conclusion section of the manuscript has been fully and concisely revised as requested due to the word count limitation (Page 8).

6. Figures and Tables

- * Ensure all figures include error bars and statistical significance markers.

- * Improve figure legends for clarity, specifying the experimental conditions.
- * Maintain consistent language in figure legends, avoiding redundancy.
- * Provide more detailed explanations for observed trends in the graphical data.

Response: All figures were rechecked to ensure the inclusion of error bars and statistical significance markers. The figure legends were updated for clarity, and the language was standardized to eliminate redundancy.

We hope that these revisions sufficiently address your comments. Please let us know if any clarifications or additional modifications are needed. We look forward to your decision on our revised manuscript.

Sincerely,

A. V.

A handwritten signature in black ink, appearing to be 'A. V.', with a stylized flourish extending to the right.

OPEN PEER REVIEW

Revision (1)

Soheil Abbaspour-Ravasjani : Reviewer

16 Feb 2025

accept