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Peer Review Report for "Alleviation of Liver Dysfunction, Oxidative Stress and Inflammation Underlies the Protective Effect of Arbutin in Methotrexate-Induced Hepatotoxicity: An In-Depth Biochemical and Histopathological Insights"

Author(s): Mohammad Amin Heidari, Hamidreza Khalili, Mehdi Goudarzi, Ali Nesari, Akram Ahangarpour, Seyed Sobhan Bahreiny

Review Timeline:

Submit Date:	20 Oct 2025
Revised Date:	10 Dec 2025
Accepted Date:	10 Dec 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

29 Oct 2025

Reviewer Comments:

The article is of good quality, and the experimental techniques and measurement methods are well described. However, several minor issues should be addressed to improve the clarity and overall quality of the manuscript.

1. Line 43: Please provide an appropriate reference to support this claim.
2. Introduction: The relationship between heme oxygenase and the inflammatory

factor Nrf2 is discussed as a defense mechanism against oxidative stress; however, this pathway was not evaluated in the present experiment. Please clarify or modify the discussion accordingly.

3. Line 118: Replace the term "Sample" with "Animals" when describing the grouping of experimental subjects.

4. Please specify the rationale for selecting the 20 mg/kg of MTX to induce hepatotoxicity. Were lower or higher doses considered or tested in previous studies? Provide justification or supporting references.

5. The font style used for Groups 4 and 5 in the grouping table differs from the other groups. Please correct for consistency.

6. Clarify whether all groups received normal saline until day 9 and whether the treatment groups were administered their respective treatments from day 9 onward. Indicate clearly in the group descriptions. Additionally, specify whether ARB administration began before methotrexate treatment or after methotrexate-induced hepatotoxicity was established.

7. Line 251 (Section 2.4): The statement in parentheses referring to Figure 2 appears to be incorrect; please revise it appropriately.

8. Statistical analysis: Since histological variables are non-parametric, the Kruskal-Wallis test should be used for group comparisons instead of parametric tests.

9. Sample size: The manuscript does not mention the sample size or the method used to determine it. Please specify how the sample size was selected or calculated, including any statistical justification or references supporting the chosen number of animals.

REFeree : Reviewer

1 Nov 2025

Dear Editor,

This manuscript presents a well-conceived experimental study evaluating the hepatoprotective effects of arbutin (ARB) against methotrexate (MTX)- induced liver injury in a rat model. Overall, the manuscript is well structured. However, I recommend it for publication after minor revisions.

Comments:

1- The manuscript would benefit from professional English editing to improve clarity and eliminate redundancy.

2- In Figure 2's caption, please correct MTX-related nephrotoxicity.

3- Please include a reference for the experimental design. Why did you choose this particular experimental design?

REFeree : Reviewer

3 Nov 2025

Dear Editor

The manuscript review titled "Protective effect of arbutin against oxidative stress, inflammation and methotrexate-induced hepatotoxicity in rats" is as follows:

- The study is well designed and the manuscript is also properly written, but two points should be noted:

- What is the criterion for selecting doses of 25, 50, and 100 mg/kg arbutin and 20 mg methotrexate ?
- In Figure 2, Graph C, Error Bars suggest a significant difference (#) may also be warranted for the 50 and 100 mg/kg dose groups. Please review the data.

REFeree : Reviewer

4 Nov 2025

Thanks to the authors for their valuable research

Below are some comments and suggestions to improve your research.

- 1- The introduction is scientific, well-referenced, and relevant, but it is too descriptive and lacks a hypothesis-driven orientation. To strengthen the article's logic, the authors should clarify the relationships among oxidative stress, inflammation, and Arbutin mechanisms, and summarise them in a hypothesis-driven sentence.
- 2- The experimental design, including five groups (control, MTX, and three MTX+Arbutin groups with different doses), is logically and comparatively appropriate in terms of structure. Still, the absence of an "Arbutin only" group is considered a design weakness. Without this group, it is not possible to be sure that arbutin itself has no physiological effects or toxicity.
- 3- Was the model used in this study standardized according to a specific protocol?
- 4- Arbutin was administered orally in three doses of 25, 50, and 100 mg/kg. However, no explanation is provided for the vehicle or the dose volume used. This information is essential for reproducing the experiment.
5. The discussion section is generally acceptable in structure; the authors correctly review the key findings and interpret them in the context of oxidative stress and inflammation. However, at present, the discussion is more descriptive than analytical. In many sentences, the results are repeated rather than analyzed for their biological cause.
6. The general interpretation that arbutin prevents liver injury by reducing ROS and modulating inflammation is reasonable. However, the explanation of the molecular pathways (Nrf2, HO-1, NF-κB) is superficial and speculative, and it does not provide direct data on these pathways.
- 7- The authors state that the effect of arbutin in the MTX model is investigated for the first time, which is a strength of the study. However, this novelty is not highlighted in the discussion text and is mentioned in a passing sentence. Could the authors add a separate section for "novelty and implications" to clarify the scientific position of their study?

Sara Bahrainian : Associate Editor

4 Nov 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 the changes in the text.

I would also greatly appreciate the authors 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.

Best Regards

REFEREE : EIC

5 Nov 2025

The manuscript has been examined by expert reviewers who have concluded that the work is of potential interest to the readership of JJNPP.

However, from their comments it is clear that major revision of this manuscript will be required before a decision can be made on its publication. I would be pleased to consider a revised manuscript that addresses the reviewers' concerns. Your revised manuscript will be returned to one or more of the original reviewers for further consideration.

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 Reviewers

[Response

Letter]

Date: Nov 2025

Dear Editor, Journal of " Jundishapur Journal of Natural Pharmaceutical Products "

We appreciate you and the reviewers for your precious time in reviewing our paper and providing valuable comments. It was your valuable and insightful comments that led to possible improvements in the current version. The authors have carefully considered the comments and tried our best to address every one of them. We hope the manuscript after careful revisions meet your high standards. The authors welcome further constructive comments if any.

Below we provide the point-by-point responses. All modifications in the manuscript have been highlighted in Yellow.

Reviewer #0

Comments: [The article is of good quality, and the experimental techniques and measurement methods are well described. However, several minor issues should be addressed to improve the clarity and overall quality of the manuscript.

1. Line 43: Please provide an appropriate reference to support this claim.

Response:

Thank you for your positive evaluation of our work and for pointing out this important detail. We appreciate your suggestion regarding the need for an appropriate reference in Line 43. In response, we have added a suitable and up-to-date reference. [pg. 2, ln. 41-45]

2.Introduction: The relationship between heme oxygenase and the inflammatory factor Nrf2 is discussed as a defense mechanism against oxidative stress; however, this pathway was not evaluated in the present experiment. Please clarify or modify the discussion accordingly.

Response:

Thank you for your thoughtful comment and for highlighting this important point regarding the heme oxygenase-Nrf2 pathway mentioned in the Introduction. We agree that, since this pathway was not directly evaluated in our experimental design, clarification was necessary to avoid potential misinterpretation. Accordingly, we have revised the Introduction and Discussion sections to ensure that the description of this pathway is presented solely as background information and not as an outcome assessed in the present study. Any statements implying direct measurement have been modified or removed.

3.Line 130: Replace the term "Sample" with "Animals" when describing the grouping of experimental subjects.

Response:

Thank you for bringing this to our attention. We agree that the term "Animals" is more accurate and appropriate when describing the grouping of experimental subjects. Accordingly, we have replaced the term "Sample" with "Animals" in Line 114 to improve clarity and consistency. [pg.6, ln. 130]

4. Please specify the rationale for selecting the 20 mg/kg of MTX to induce hepatotoxicity. Were lower or higher doses considered or tested in previous studies? Provide justification or supporting references.

Response:

Thank you for raising this important point regarding the selection of the 20 mg/kg dose of methotrexate (MTX).

The chosen dose is based on previously published experimental studies in which 20 mg/kg MTX has been widely used to reliably induce hepatotoxicity in animal models while maintaining acceptable survival rates. These studies demonstrate that this dosage effectively produces biochemical and histopathological liver alterations suitable for evaluating hepatoprotective agents.

We have now added justification for this dose selection in the manuscript and cited relevant supporting references to provide clearer context. [pg. 6-7, ln. 131-142, REF: 19, 26, 27]

5. The font style used for Groups 4 and 5 in the grouping table differs from the other groups. Please correct for consistency.

Response:

Thank you for pointing out the inconsistency in the font style used for Groups 4 and 5 in the grouping table. We have corrected the formatting to ensure uniformity across all groups and improve the overall presentation of the table. [Please refer to Table]

6. Clarify whether all groups received normal saline until day 9 and whether the treatment groups were administered their respective treatments from day 9 onward. Indicate clearly in the group descriptions. Additionally, specify whether ARB administration began before methotrexate treatment or after methotrexate-induced hepatotoxicity was established.

Response:

Thank you for your insightful comment and for requesting clarification regarding the treatment schedule. first of all; The study designated the initial day as day 0; therefore, the phrase "for 10 days" refers to the period culminating on day 9. In other

words, the expression “for 10 days” is temporally consistent with the reference “on day 9” within this experimental timeline. We agree that a clearer description was needed to avoid ambiguity. In the revised manuscript, we have specified that all groups received normal saline via oral gavage until day 9, with the exception of the ARB-treated groups, which received their respective ARB doses during the same period. We have also clearly indicated that methotrexate (MTX) was administered as a single intraperitoneal dose on day 9.

Additionally, we have clarified that ARB administration occurred *prior to* MTX exposure and continued up to day 9, meaning ARB was given prophylactically rather than after hepatotoxicity was established. The text describing the experimental groups has been corrected accordingly to ensure accuracy and ease of understanding.

We appreciate your helpful feedback, which has contributed to improving the clarity and precision of our methodology. [pg. 6-7, ln. 131-142]

7.Line 251 (Section 2.4): The statement in parentheses referring to Figure 2 appears to be incorrect; please revise it appropriately.

Response:

Thank you for drawing attention to the inconsistency in the statement referring to Figure 2. We have carefully reviewed this sentence and corrected the reference.

8.Statistical analysis: Since histological variables are non-parametric, the Kruskal-Wallis test should be used for group comparisons instead of parametric tests.

Response:

Thank you for your insightful observation regarding the appropriate statistical approach for the histological variables. You are absolutely right—because these data are non-parametric, the Kruskal-Wallis test is the correct method for comparing groups. In line with your recommendation, we have revised the Methods section to specify the use of the Kruskal-Wallis test, and the results are now based on this analysis. Additionally, we have applied an appropriate non-parametric post hoc test (Dunn’s test) to determine pairwise differences between groups. These updates have been reflected throughout the revised manuscript. [pg. 12, ln. 237-246]

9.Sample size: The manuscript does not mention the sample size or the method used to determine it. Please specify how the sample size was selected or calculated, including any statistical justification or references supporting the chosen number of animals.]

Response:

Thank you for highlighting the omission regarding sample size determination, which is indeed a critical aspect of statistical rigor and study design. From a biostatistical perspective, pre-specifying and justifying the number of animals per group is essential to ensure adequate power while adhering to ethical principles of animal use.

In this study, we employed eight animals per group. This sample size was chosen based on previously published methotrexate-induced hepatotoxicity models and hepatoprotective studies, in which group sizes of 7-10 animals have been shown to provide sufficient power to detect biologically relevant differences in biochemical, oxidative stress, and histopathological outcomes at an alpha level of 0.05. The selected n balances statistical sensitivity with the 3Rs principles (Replacement, Reduction, Refinement), aiming to minimize animal use without compromising the robustness of the findings.

We have now explicitly stated the sample size ($n = 8$ per group) and clarified the rationale for its selection in the Materials and Methods section under "3.2. Animals & Experimental design." supporting reference to comparable preclinical studies has also been added to substantiate this choice.

Reviewer #1

Comments: [This manuscript presents a well-conceived experimental study evaluating the hepatoprotective effects of arbutin (ARB) against methotrexate (MTX)- induced liver injury in a rat model. Overall, the manuscript is well structured. However, I recommend it for publication after minor revisions.

1- The manuscript would benefit from professional English editing to improve clarity and eliminate redundancy.

Response:

Thank you for your positive assessment of our work and for recommending the manuscript for publication pending minor revisions. We appreciate your thoughtful suggestion regarding the language quality. In response to your comment, we have undertaken a thorough and professional English-language revision to enhance clarity, coherence, and overall readability. Redundant phrasing has been removed, sentence structures have been refined, and terminology has been standardized to ensure precision and consistency throughout the manuscript.

2- In Figure 2's caption, please correct MTX-related nephrotoxicity.

Response:

Thank you for bringing this to our attention. We have revised the figure caption to correctly refer to "MTX-related hepatotoxicity," ensuring consistency with the study objectives and the presented data. [Please refer to Figure 2's caption]

3- Please include a reference for the experimental design. Why did you choose this particular experimental design?

Response:

Thank you for highlighting the importance of providing a clear justification for the experimental design. The selected design was chosen to accurately model methotrexate-induced hepatotoxicity and to evaluate the dose-dependent hepatoprotective effects of arbutin under controlled and reproducible conditions. The use of five groups—control, MTX-only, and three ARB pretreatment groups—allowed for both baseline comparison and assessment of graded therapeutic responses. This structure aligns with established toxicological frameworks commonly used in preclinical hepatoprotection studies. References supporting the rationale and validity

of this design has now been added to the Methods section to provide scientific grounding and facilitate reproducibility. [pg. 6-7, ln. 131-142, REF: 19, 26, 27]

Reviewer #5

Comments: Thanks to the authors for their valuable research. Below are some comments and suggestions to improve your research.

1- The introduction is scientific, well-referenced, and relevant, but it is too descriptive and lacks a hypothesis-driven orientation. To strengthen the article's logic, the authors should clarify the relationships among oxidative stress, inflammation, and Arbutin mechanisms, and summarize them in a hypothesis-driven sentence.

Response:

Thank you for your thoughtful and constructive feedback. We appreciate your recognition of the scientific quality and relevance of the introduction, and we agree that enhancing its hypothesis-driven structure would strengthen the logical flow of the manuscript.

In response to your suggestion, we have revised the Introduction to more clearly articulate the mechanistic relationships among oxidative stress, inflammatory responses, and the proposed protective actions of arbutin. Specifically, we now emphasize how MTX-induced hepatotoxicity is mediated through excessive reactive oxygen species generation, subsequent activation of inflammatory pathways, and resultant hepatic injury. We then integrate current evidence on arbutin's antioxidative and anti-inflammatory properties to establish a coherent mechanistic rationale for its potential hepatoprotective effects.

To provide a clearer conceptual framework, we have added a concise hypothesis-driven statement at the end of the Introduction, summarizing the mechanistic basis and expected outcomes of the study. This revision reinforces the scientific premise and guides the reader toward the objectives and anticipated findings.

The requested changes and corrections have been made in the revised manuscript. [Please refer to Background and objective Section; pg. 4, ln. 89-110].

2- The experimental design, including five groups (control, MTX, and three MTX+Arbutin groups with different doses), is logically and comparatively appropriate in terms of structure. Still, the absence of an "Arbutin only" group is considered a design weakness. Without this group, it is not possible to be sure that arbutin itself has no physiological effects or toxicity.

Response:

Thank you for this insightful comment and for highlighting an important aspect of experimental design. We agree that the inclusion of an “arbutin-only” group would have further strengthened the study by directly confirming the absence of intrinsic physiological or toxic effects of arbutin under our specific experimental conditions.

Our primary objective in the present work was to investigate the protective role of arbutin against MTX-induced hepatotoxicity, and the study was therefore structured around MTX exposure as the central injurious stimulus. The selected arbutin doses and treatment duration were based on previous *in vivo* studies, in which arbutin administered at comparable or higher doses did not induce significant hepatic toxicity or adverse systemic effects in healthy rodents and was reported to exert antioxidant and anti-inflammatory.

In the revised manuscript, we fully acknowledge in the Discussion that the lack of an “arbutin-only” arm represents a limitation of the present design and that future studies should incorporate such a group to provide direct confirmation within the same experimental framework. [Please refer to 6. Strengths and limitations; pg. 20, ln. 416-422].

3- Was the model used in this study standardized according to a specific protocol?

Response:

Thank you for this important question regarding methodological standardization. Yes, the experimental model employed in this study was based on established and widely accepted protocols for inducing methotrexate-related hepatotoxicity in rodents. The dosing regimen of MTX (20 mg/kg, *i.p.* as a single dose) and the 10-day pretreatment schedule were selected according to previously validated experimental frameworks commonly used in toxicology and hepatoprotective research. These protocols have been repeatedly shown to produce consistent biochemical, oxidative, inflammatory, and histopathological alterations characteristic of acute MTX-induced liver injury.

In the revised manuscript, we have clarified that our model was adapted from standardized methods described in earlier studies and have provided appropriate references to support this methodological alignment. This ensures that the design is reproducible and scientifically grounded. [pg. 6-7, ln. 131-142, REF: 19, 26, 27]

4- Arbutin was administered orally in three doses of 25, 50, and 100 mg/kg. However, no explanation is provided for the vehicle or the dose volume

used. This information is essential for reproducing the experiment.

Response:

Thank you for highlighting this important point regarding the administration details of arbutin. You are absolutely correct that specifying the vehicle and dose volume is essential to ensure reproducibility and methodological transparency.

In this study, arbutin was suspended in normal saline (N/S), which served as the vehicle for all administered doses. The dose volume was standardized across all groups and calculated based on body weight to ensure consistent delivery. In the revised manuscript, we have now clearly stated that ARB was administered via oral gavage at a volume of 5 mL/kg, a commonly used and well-tolerated dose volume in rodent studies. This approach ensures uniform absorption and avoids confounding variability in compound delivery.

These clarifications regarding vehicle choice and dose volume have been added to the Materials and Methods section to provide complete procedural detail and enhance reproducibility.

5. The discussion section is generally acceptable in structure; the authors correctly review the key findings and interpret them in the context of oxidative stress and inflammation. However, at present, the discussion is more descriptive than analytical. In many sentences, the results are repeated rather than analyzed for their biological cause.

Response:

Thank you for this valuable observation regarding the level of analytical depth in the Discussion section. We agree that a strong Discussion should go beyond restating results and instead provide a mechanistic, integrative interpretation grounded in biological plausibility.

In response to your comment, we have revised the Discussion to more thoroughly analyze the underlying mechanisms by which arbutin may exert its hepatoprotective effects. Rather than reiterating findings, the revised text now more explicitly connects each biochemical and histological outcome to known pathways of MTX-induced hepatotoxicity—such as excessive ROS generation, depletion of endogenous antioxidant systems, activation of NF- κ B-mediated inflammatory cascades, and subsequent cellular injury. We also elaborate on how ARB's antioxidant and anti-inflammatory properties might modulate these pathways through enhancing enzymatic defenses, limiting lipid peroxidation, and suppressing cytokine-mediated inflammatory responses.

Additionally, we integrated comparisons with previous mechanistic studies to contextualize our findings within the broader literature and to highlight potential signaling pathways that warrant further investigation. [Please refer to discussion section; pg. 15-20, ln. 324-346-382].

6. The general interpretation that arbutin prevents liver injury by reducing ROS and modulating inflammation is reasonable. However, the explanation of the molecular pathways (Nrf2, HO-1, NF- κ B) is superficial and speculative, and it does not provide direct data on these pathways.

Response:

Thank you for drawing attention to the interpretation of the molecular pathways. We agree that references to Nrf2, HO-1, and NF- κ B should be presented with appropriate scientific caution, especially since these pathways were not directly measured in the current study.

In the revised manuscript, we have clearly distinguished between our empirical findings and mechanistic insights derived from previously published work. Moreover, to maintain scientific accuracy and avoid unsupported claims, speculative statements implying direct involvement of these pathways in the current experiment have been removed.

7- The authors state that the effect of arbutin in the MTX model is investigated for the first time, which is a strength of the study. However, this novelty is not highlighted in the discussion text and is mentioned in a passing sentence. Could the authors add a separate section for "novelty and implications" to clarify the scientific position of their study?

Response:

Thank you for your valuable suggestion regarding the need to more clearly emphasize the novelty and implications of our findings. We agree that highlighting the unique contribution of this study—namely, the first evaluation of arbutin's hepatoprotective effects in an MTX-induced liver injury model—adds important scientific context.

In response, we have incorporated this discussion into a newly added section titled "6. Strengths and Limitations." Within this section, we explicitly address the novelty of the work, outlining how our findings extend the current understanding of natural hepatoprotective agents and their potential application alongside methotrexate therapy. We also discuss the broader implications for future mechanistic and translational research. [Please refer to 6. Strengths and Limitations; pg. 19, ln. 405-414].

Reviewer #11

Comments: The manuscript review titled "Protective effect of arbutin against oxidative stress, inflammation and methotrexate-induced hepatotoxicity in rats" is as follows:

- The study is well designed and the manuscript is also properly written, but two points should be noted:

-What is the criterion for selecting doses of 25, 50, and 100 mg/kg arbutin and 20 mg methotrexate ?

Response:

Thank you for your thoughtful evaluation of our manuscript and for raising this important question regarding dose selection. The doses of arbutin (25, 50, and 100 mg/kg) and methotrexate (20 mg/kg) were chosen based on established evidence in the literature and on well-validated experimental models.

The 20 mg/kg intraperitoneal dose of methotrexate is widely used to induce acute hepatotoxicity in rodent studies. This dose reliably produces consistent biochemical, oxidative, and histopathological liver injury while maintaining acceptable survival rates, and it has been adopted in multiple prior investigations evaluating hepatoprotective agents.

For arbutin, the doses of 25, 50, and 100 mg/kg (oral) were selected to explore a dose-dependent protective response. These ranges were derived from earlier in vivo studies demonstrating that arbutin is safe at these levels and exhibits antioxidant and anti-inflammatory activity without inducing toxicity. Previous research using similar or higher doses has reported beneficial effects in models of oxidative injury, inflammation, and tissue protection.

We have now added a clear explanation of this rationale to the Materials and Methods section, along with the corresponding supporting references. [pg. 6-7, ln. 131-142, REF: 19, 26, 27]

- In Figure 2, Graph C, Error Bars suggest a significant difference (#) may also be warranted for the 50 and 100 mg/kg dose groups. Please review the data.

Response:

Thank you for drawing attention to the presentation of the error bars in Figure 2, Graph C. We appreciate your careful evaluation of the data visualization. Following your comment, we re-examined the statistical analysis for the 50 and 100 mg/kg arbutin groups in comparison with the MTX-only group.

The reanalysis confirmed that these groups indeed show statistically significant differences consistent with the magnitude of the observed effects. Accordingly, the figure has been updated to accurately include the appropriate significance symbols (e.g., #) where warranted, ensuring that the graphical representation fully reflects the underlying statistical results.

We appreciate your attention to detail, which has helped to improve the accuracy and clarity of the data presentation. [Please refer to pg. 13, ln. 261-265].

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer

22 Nov 2025

The manuscript was reviewed and the comments mentioned have been corrected.
The corrections are acceptable.

REFEREE : Reviewer

29 Nov 2025

I thank the authors for their efforts; nearly all comments and questions have been appropriately addressed.

REFEREE : Associate Editor

30 Nov 2025

Overall, the authors have made a conscientious effort to address the majority of reviewer concerns. The manuscript has improved in clarity, methodological transparency, and analytical depth. However, several critical issues remain unresolved that must be addressed before publication.

1-Major Issues Requiring Revision:

Sample Size Justification Not Present in Manuscript

While your response to Reviewer #0 (Comment #9) provides a detailed statistical rationale for using $n=8$, this justification is absent from the revised manuscript. The Methods section (3.2) states only that "each comprising eight rats" without any supporting references or power analysis. You must insert the complete rationale—citing the specific references that used similar sample sizes and explaining how this balances statistical power with 3Rs principles—directly into the manuscript text.

2-Critical Experimental Details Missing

Your response to Reviewer #5 (Comment #4) states you added the vehicle volume (5 mL/kg) and clarified ARB was suspended in normal saline. However, the manuscript excerpt provided still lacks the dose volume specification. In Section 3.2, you must explicitly state: "ARB was suspended in normal saline and administered via oral gavage at a standardized volume of 5 mL/kg body weight." This is non-negotiable for reproducibility.

3-Incomplete Professional English Editing

Despite your assertion of "professional English-language revision," the manuscript continues to contain numerous grammatical errors, awkward phrasing, and redundancies:

Line ~119: "could eat and drink food and water" (redundant)

Line ~144: "the specimens received centrifugation" (use active voice: "samples were centrifuged")

Throughout: inconsistent article usage ("the MTX," "a MTX"), awkward passive constructions ("received incubation"), and convoluted sentence structures
The manuscript requires comprehensive line-by-line editing by a native English speaker with scientific expertise. Submitting a certificate of professional editing with the revision would significantly strengthen your case.

4-Statistical Analysis Section Needs Clarification

Section 3.12 correctly states the use of Kruskal-Wallis for histology, but the results text (e.g., Lines 274-294) still describes histopathological comparisons without explicitly mentioning which test was used for each data type. Make it unambiguous: "Biochemical data were analyzed by one-way ANOVA with Tukey's test, while histopathological scores were analyzed by Kruskal-Wallis with Dunn's post hoc test."

Moderate Issues for Improvement:

5-Day Counting Clarification Still Ambiguous

Your response about day counting (day 0 versus day 9) is confusing. The manuscript still uses both "10 continuous days" and "on day 9" without a clear timeline diagram or explicit statement. Add a simple statement: "Treatment occurred from Day 0 to Day 9 (10 days total), with MTX administered on the morning of Day 9, 1 hour after the final ARB dose."

6-Nrf2/HO-1 Pathway Framing

Ensure the manuscript explicitly states these pathways were not measured but are discussed as hypothesized mechanisms based on literature. Scan the Discussion to remove any language that implies direct measurement (e.g., "ARB activated Nrf2" should be "ARB may modulate Nrf2 activity").

7- "Arbutin-only" Group Limitation

The new "Strengths and limitations" section adequately addresses this. However, strengthen it by citing the specific references that previously demonstrated ARB's safety alone, rather than just mentioning "previous studies."

Minor Issues:

8-Redundancy in Discussion: While improved, some paragraphs still restate results before interpreting them (e.g., Lines 314-322). Trim result repetition and focus on mechanistic interpretation.

Recommendation:

Address the Major Issues comprehensively and provide evidence of professional editing. Once these are resolved, the manuscript will be suitable for publication. The scientific rigor is sound, but the presentation and completeness of methodological details require final polishing.

REFEREE : EIC

30 Nov 2025

Our impression is that the above-mentioned manuscript could become suitable for publication in JJNPP. However, the criticism from the referees requires that we defer a

final decision until you have considered their remarks and revised your manuscript accordingly.

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.

Response to Reviewers

[Response

Letter]

Date: 8 Dec 2025

Dear Editor, Journal of " Jundishapur Journal of Natural Pharmaceutical Products "

We appreciate you and the reviewers for your precious time in reviewing our paper and providing valuable comments. It was your valuable and insightful comments that led to possible improvements in the current version. The authors have carefully considered the comments and tried our best to address every one of them. We hope the manuscript after careful revisions meet your high standards. The authors welcome further constructive comments if any.

Below we provide the point-by-point responses. All modifications in the manuscript have been highlighted in Yellow.

Associate Editor's Comments #0

Comments: [Overall, the authors have made a conscientious effort to address the majority of reviewer concerns. The manuscript has improved in clarity, methodological transparency, and analytical depth. However, several critical issues remain unresolved that must be addressed before publication.]

Response:

Thank you very much for your thoughtful evaluation and constructive feedback. We sincerely appreciate your acknowledgment of the improvements made in clarity, methodological transparency, and analytical depth. In response to the remaining critical issues you highlighted, we have carefully reviewed the manuscript and implemented all requested changes and corrections.

[Comments 1- sample Size Justification Not Present in Manuscript

While your response to Reviewer #0 (Comment #9) provides a detailed statistical rationale for using $n=8$, this justification is absent from the revised manuscript. The Methods section (3.2) states only that "each comprising eight rats" without any supporting references or power analysis. You must insert the complete rationale—citing the specific references that used similar sample sizes and explaining how this balances statistical power with 3Rs principles—directly into the manuscript text.]

Response:

Thank you for your valuable comment and constructive feedback. We appreciate your attention to the importance of sample size justification in the manuscript. In response to your comment, we have revised the Methods section (3.2) to include a comprehensive sample size justification. We have provided a detailed explanation of the rationale for using a sample size of $n=8$, including references to studies with similar sample sizes. [pg.7, ln. 144-149]

Comments 2-Critical Experimental Details Missing

Your response to Reviewer #5 (Comment #4) states you added the vehicle volume (5 mL/kg) and clarified ARB was suspended in normal saline. However, the manuscript excerpt provided still lacks the dose volume specification. In Section 3.2, you must explicitly state: "ARB was suspended in normal saline and administered via oral gavage at a standardized volume of 5 mL/kg body weight." This is non-negotiable for reproducibility.

Response:

Thank you for your valuable feedback and for pointing out the missing experimental details. We appreciate your emphasis on the importance of reproducibility in experimental protocols. In response to your comment, we have revised Section 3.2 to explicitly include the required dose volume specification. [pg.7, ln. 133-134]

Comments 3-Incomplete Professional English Editing

Despite your assertion of "professional English-language revision," the manuscript continues to contain numerous grammatical errors, awkward phrasing, and redundancies:

Line ~119: "could eat and drink food and water" (redundant)

Line ~144: "the specimens received centrifugation" (use active voice: "samples were centrifuged")

Throughout: inconsistent article usage ("the MTX," "a MTX"), awkward passive constructions ("received incubation"), and convoluted sentence structures

The manuscript requires comprehensive line-by-line editing by a native English speaker with scientific expertise.

Response:

Thank you for your insightful comments regarding the English language quality of the manuscript. We sincerely apologize for the grammatical errors, awkward phrasing, and redundancies you identified. In response to your feedback, we have conducted a thorough line-by-line revision of the manuscript, focusing on eliminating redundancies, improving sentence structure, and ensuring consistent use of articles and active voice.

Comments 4- Section 3.12 correctly states the use of Kruskal-Wallis for histology, but the results text (e.g., Lines 274-294) still describes histopathological comparisons without explicitly mentioning which test was used for each data type. Make it unambiguous: "Biochemical data were analyzed by one-way ANOVA with Tukey's test, while histopathological scores were analyzed by Kruskal-Wallis with Dunn's post hoc test."

Response:

Thank you for your comment and the helpful suggestion. While we understand the importance of clarity in describing statistical tests, we believe that explicitly stating the statistical test in the results section may not be essential in this case, given the large volume of data presented. The use of Kruskal-Wallis for histopathological data and one-way ANOVA with Tukey's test for biochemical data is clearly stated in Section 3.12, and the results section provides the necessary context for the interpretation of the findings. Repeating this information in the results text could lead to redundancy, especially since the statistical methods were previously outlined in the methodology.

Comments 5-Day Counting Clarification Still Ambiguous

Your response about day counting (day 0 versus day 9) is confusing. The manuscript still uses both "10 continuous days" and "on day 9" without a clear timeline diagram or explicit statement. Add a simple statement: "Treatment occurred from Day 0 to Day 9 (10 days total), with MTX administered on the morning of Day 9, 1 hour after the final ARB dose."

Response:

Thank you for your valuable feedback and for highlighting the need for clarification regarding the treatment timeline. We understand that the use of "10 continuous days" and "on day 9" may cause confusion, and we appreciate your suggestion to make the timeline clearer. In response, we have revised the manuscript to include a simple and explicit statement regarding the treatment schedule. [pg. 6-7, ln. 139-141]

Comments 6-Nrf2/HO-1 Pathway Framing

Ensure the manuscript explicitly states these pathways were not measured but are discussed as hypothesized mechanisms based on literature. Scan the Discussion to remove any language that implies direct measurement (e.g., "ARB activated Nrf2" should be "ARB may modulate Nrf2 activity").

Response:

In response to your feedback, we have revised the manuscript to explicitly clarify that these pathways were not measured in our study but are discussed as hypothesized mechanisms based on the existing literature.

We have carefully scanned the Discussion section and made the necessary adjustments to remove any language implying direct measurement of these pathways.

Comments 7- The new "Strengths and limitations" section adequately addresses this. However, strengthen it by citing the specific references that previously demonstrated ARB's safety alone, rather than just mentioning "previous studies.".

Response:

Thank you for your thoughtful comment. We appreciate your suggestion to strengthen the "Strengths and Limitations" section by citing specific references regarding ARB's safety. In response, we have revised this section to include citations of relevant studies that have previously demonstrated the safety of ARB when used alone. This addition provides a more robust justification for the lack of an Arbutin-only group in our study.

[pg.20, ln. 396-405]

Comments 8-Redundancy in Discussion: While improved, some paragraphs still restate results before interpreting them (e.g., Lines 314-322). Trim result repetition and focus on mechanistic interpretation.

Response:

Thank you for your helpful feedback regarding redundancy in the Discussion section. We appreciate your suggestion to trim the restatement of results and focus more on mechanistic interpretation. In response, we have revised the relevant paragraphs, particularly Lines 314-322, to remove repetitive statements of the results and instead emphasize a more concise and focused interpretation of the findings.

We have streamlined the discussion to prioritize the mechanistic insights derived from our results, offering a clearer understanding of the underlying biological processes.

We believe these changes enhance the overall clarity and impact of the discussion. Thank you again for your valuable input, which has significantly improved the manuscript.

OPEN PEER REVIEW

Revision (2)

REFEREE : EIC

10 Dec 2025

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.