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Peer Review

Jundishapur Journal of Natural Pharmaceutical Products
Article DOI: https://doi.org/10.5812/jjnpp-166303
Published in: Jundishapur Journal of Natural Pharmaceutical Products: 20(4) ; e166303

Peer Review Report for "Protective Role of Feselol Against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways"

Author(s): Firoozeh Saghaei, Maryam Karimi-Dehkordi, Farah Sadat Ahmadi

Review Timeline:

Submit Date:	13 Sep 2025
Revised Date:	8 Oct 2025
Accepted Date:	10 Oct 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

18 Sep 2025

The manuscript examines (the Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Factors). While the topic may be intriguing, it has several deficiencies regarding discussion and study methodology.

1. Average Weight of Mice: An average of 35 grams for the mice is excessively high, and young mice were not used in the study, which could be a significant flaw. The abstract and methods section state the weight in two different ways, which are inconsistent; one mentions an average while the other refers to an approximately

weight.

2. Tween Carrier: What type of Tween is used? This is not mentioned in the text. How much solvent is needed to dissolve it? Does Tween have any negative effects on the kidneys?

3. Acetaminophen Damage: The text does not specifically mention the damage that acetaminophen causes to the kidneys, only general injuries are discussed.

4. Specific Marker for Kidney Damage: A specific marker for kidney damage was not investigated in the study, which can also be considered a flaw. For instance, Kim-1 is one of the specific markers for kidney damage.

5. Factors Not Measured: In the introduction, TLR-4, CRP, TGF-β1, and p53 are mentioned, but these factors were not measured in the study. Is there a specific reason for this?

6. Dosage of Ketamine and Xylazine: The dosages of ketamine and xylazine are not mentioned in the article.

7. Selection Criteria for Mice: The criteria for selecting the number of mice should be referenced.

8. PCR Tests: Since the tests were conducted using PCR, there is uncertainty about the protein expression of these genes. Why was Western blotting not used to confirm protein expression?

9. Conclusion Section: Please write the conclusion separately at the end of the discussion.

10. Figure Footnotes: The footnotes for Figures 3, 4, and 5 should be merged.

11. Units in Figures: The units for Figures 3, 4, and 5 are not mentioned in the images.

12. Figure Legends: The legends for Figures 1 and 2 have significant issues both visually and conceptually, especially concerning the fifth group.

REFEREE : Reviewer

21 Sep 2025

Dear Editor,
I am writing to recommend the manuscript titled “Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways” for publication in the Jundishapur Journal of Natural Pharmaceutical Products after minor revision:

Abstract:
- Avoid including doses and number of groups, but briefly state that there are 7 groups with n=6 and a study duration of 3 days.
- Clarify that measurements include biochemical markers (MDA, SOD, CAT, GPx), expression of Bax, Caspase-3, Bcl-2 genes and cytokines (TNF-a, IL-6/IL-1b, IL-10) and what techniques are used (e.g. or kits).
- Language is acceptable but some sentences are long. Break them into shorter sentences.
Methodological assessment (methods)
- Design with 7 groups and n=6 is appropriate but should explain:
- Was random assignment performed?
- A detailed description of the biochemical kits and measurement systems (standard units and methods) and how the data were normalized (e.g., to overall supply or sample costs) is required.
- Study duration of three days: Why is this appropriate? Were end-point measurements performed or were continuous time points examined?
- Include a short section in the Materials and Methods on study design (randomization, blinding, justification of sample size).
- Provide a detailed list of kits/methods for MDA, SOD, CAT, GPx, and gene expression methods (e.g., RT-qPCR with internal factors such as GAPDH).
- Specify units and reporting (e.g., MDA in nmol/mg protein, GSH in µmol/g tissue, etc.).
• Some sentences are accompanied by incomplete or missing references
• Support all key claims with accurate and up-to-date references and provide a reference list in accordance with the journal format.
• Some statements are overly preclinical/clinical in nature. Use phrases such as "potential", "could be", and "in this animal model" and explicitly state the limitations of the animal model.
• The discussion is long and structurally disorganized; some parts are repetitive. Create three coherent sections: Mechanisms of acetaminophen-induced nephrotoxicity (brief and key), Effects of feselol and evidence in the literature (introducing possible mechanisms), Comparison with NAC, limitations, and future prospects
• There are a few logical leaps (e.g., attributing mechanisms primarily to α,β-saturated sesquiterpene structure) without direct evidence from the current study; clarify whether this is speculative or supported by data or literature.

REFEREE : Reviewer

22 Sep 2025

Dear Editor,
The manuscript entitled "Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways" was reviewed as follows:
1- On what basis were the doses of all compounds used in the different groups determined?
2- In group 5, what was the dose on the first and second days? Why did the

intervention start on the third day? In groups 3 to 6, the dose of acetaminophen should be mentioned.
3- On page 2, paragraph 3, line 3, replace "turmeric" with "curcumin" when referring to the compound.
4- In the Experimental Animals section, use "ad libitum" instead of "free."
5- Provide references for the "Evaluation of Oxidative Stress Markers" and "Evaluation of inflammatory and apoptotic gene expression" sections.
6- The lettering in the figures is usually done in alphabetical order, what is the reason for not following this in some figures?
7-In some parameters, such as parts C and D of the figure 2, there is a significant difference between groups 3 and 4, where N-acetylcysteine demonstrates a beneficial effect.
8-Discuss the study's strengths and weaknesses. Address the short, three-day intervention period as a potential limitation compared to the more common seven-day duration in similar studies.
9-Investigate the dose-response relationship of Feselol. Explore the possibility of a Feselol dosage that is equivalent to or superior to N-acetylcysteine.

Mohammad Reza Mehrabi : Reviewer

22 Sep 2025

1-Assessing the long-term effects of Feselol

Although the study shows positive effects of Feselol in the short-term period after treatment, it is suggested that its long-term effects also be investigated. This could provide more information regarding the sustainability of Feselol’s protective effects and its possible complications over time. Follow-up periods of three month or longer would be beneficial for a better results.

2- Investigating the side effects of Feselol at different doses

The article discusses the effects of Feselol on oxidative stress, apoptosis, and inflammation, but it is important to investigate how these effects might impact other organs and body systems at higher doses (such as more than 50 mg/kg). Further analysis of Feselol’s side effects at different doses could enhance the validity of the article.

3- More detailed analysis of the Bcl-2/Bax ratio

The results related to apoptotic genes (Caspase-3, Bax, Bcl-2) are well presented. However, a more detailed analysis of the Bcl-2/Bax ratio in the different groups would be useful. This ratio plays a crucial role in apoptotic mechanisms and could help better interpret the effects of Feselol in reducing apoptosis and regulating this pathway.

REFEREE : Associate Editor

23 Sep 2025

• The treatment window appears very short (3 days, with APAP only on day 3). How does this acute model reflect chronic or subchronic nephrotoxicity? Could longer-term effects or recovery dynamics be missed?
• Why were histological findings (e.g., tubular necrosis, cast formation) not included? These are gold-standard indicators of nephrotoxicity and would strengthen the biochemical/molecular claims.
• Functional kidney markers (serum creatinine, BUN, uric acid) were mentioned in the Introduction but not measured or reported in Results.
• Sample size is very small (n=6 per group). Was a power analysis performed to justify this sample size? With high biological variability in oxidative/apoptotic markers, could Type II errors (false negatives) have occurred?
• All figures need p-value.
• The paper claims Feselol has “anti-apoptotic” effects based on Bax/Bcl-2 ratios and Caspase-3 mRNA but no cell death assays (TUNEL, Annexin V, LDH) were performed. Is altered gene expression sufficient to conclude reduced apoptosis? Could compensatory mechanisms or post-translational modifications invalidate these inferences?
• No investigation into upstream regulators (e.g., Nrf2, NF-κB, p53, MAPK pathways) was conducted, despite their central role in APAP toxicity. Does Feselol act via Nrf2 activation (like many antioxidants) or NF-κB inhibition? Without probing mechanisms, the “how” remains speculative.
• NAC is presented as the “gold standard,” but its dose (300 mg/kg) and timing may not be clinically translatable. How does this dose compare to human equivalent dosing? Could the comparison with NAC be misleading if NAC was suboptimally dosed or timed?

REFEREE : EIC

23 Sep 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.

Dear Editor-in-Chief,

Greetings and respect,

We sincerely thank the reviewers for their careful evaluation and constructive comments on our manuscript entitled *"Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways"* (Manuscript ID: 166303).
This study represents the first investigation in an animal model exploring the potential protective effects of feselol against acetaminophen-induced acute kidney injury. Although there are limitations and areas for improvement noted by the reviewers, considerable time and resources were invested in the design and execution of this research, and the findings could provide meaningful preclinical insights.

Our findings in this animal model suggest that feselol could play a potential role in modulating oxidative stress, apoptosis, and inflammatory pathways in renal tissue, thereby providing a possible foundation for future studies to further explore its mechanisms and long-term effects. Considering the novelty of the work and the comprehensive dataset presented, we believe this manuscript could be of interest and value to the readers of the Jundishapur Journal of Natural Pharmaceutical Products.

We have carefully addressed all reviewers' comments, and the responses are highlighted in the revised manuscript with different colors: Reviewer 0 (yellow), Reviewer 2 (green), Reviewer 3 (blue), Reviewer 4 (purple). The associate editor's comments did not require any corrections to the text; they were only responded to in the review response file.

We sincerely hope that the revisions made will meet the satisfaction of the reviewers and the editorial board, and we look forward to your favorable consideration.

With best regards,
Corresponding author:
Dr. Meryam Karimi Dehkordi

Reviewers' Comments:

Reviewer 0:

Dear Editor,

I am writing to recommend the manuscript titled "Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways" for publication in the Jundishapur Journal of Natural Pharmaceutical Products after minor revision:

Abstract:

- Avoid including doses and number of groups, but briefly state that there are 7 groups with n=6 and a study duration of 3 days.

Response: Done

- Clarify that measurements include biochemical markers (MDA, SOD, CAT, GPx), expression of Bax, Caspase-3, Bcl-2 genes and cytokines (TNF-a, IL-6/IL-1b, IL-10) and what techniques are used (e.g. or kits).

Response: Done

- Language is acceptable but some sentences are long. Break them into shorter sentences.

Response: Done

Methodological assessment (methods)

- Design with 7 groups and n=6 is appropriate but should explain:
- Was random assignment performed?

Response: A section was added to "material and methods" as "Study Design" and Requested Items were added.

- A detailed description of the biochemical kits and measurement systems (standard units and methods) and how the data were normalized (e.g., to overall supply or sample costs) is required.

Response: All requested information such as standard units and methods was added and highlighted in yellow. The following statement was added to explain the data normalization:" For evaluation of oxidative stress Markers in renal tissue, the total protein content of the homogenates was determined by the Bradford method to normalize the enzymatic activities."

- Study duration of three days: Why is this appropriate?

Response: Previous studies that have investigated the protective effects of Ferula species in animal models have typically used experimental periods of 1 to 7 days. For example, in the study by **Fedaei et al. (2021)**, the effect of hydroalcoholic extract of Ferula on reducing memory impairments induced by

cadmium chloride exposure in mice was evaluated. In this study, treatment groups received Ferula extract daily for one week. In the study by **Raafat and El-Lakany (2015),** the acute and subchronic effects of Ferula hermonis extract and its active isolates were evaluated in alloxan-induced diabetic mice during 1, 3, 5, and 8 days after treatment. The results showed that from day 3 onwards, a significant decrease in blood glucose was observed, while serum catalase levels, as a marker of antioxidant activity, increased from day 1 and remained stable in the following days. Furthermore, neuropathic pain sensitivity was reduced in the treated groups and the effects were sustained from day 3 onwards.
Given this evidence and the fact that no previous in vivo studies of feselol in mice were available, the choice of a three-day treatment period in the present study could be a reasonable and practical time frame for the initial evaluation of the effects of feselol that is scientifically justifiable.

References:

Fadaei, H., Mirhosseiniardakani, S., Farajzadeh, A., Aghayan, S. S., Jafarisani, M., & Garmabi, B. (2021). Aqueous-alcoholic Ferulla extract reduces memory impairments in rats exposed to cadmium chloride. Brain and Behavior, 11(8), e2285.

Raafat, K., & El-Lakany, A. (2015). Acute and subchronic in-vivo effects of Ferula hermonis L. and Sambucus nigra L. and their potential active isolates in a diabetic mouse model of neuropathic pain. BMC Complementary and Alternative Medicine, 15(1), 257.

- Were end-point measurements performed or were continuous time points examined?
Response: Sampling was conducted at the end of the three days.

- Include a short section in the Materials and Methods on study design (randomization, blinding, justification of sample size).
Response: A section was added to “material and methods” as “Study Design” and Requested Items were added: “Mice were randomly assigned to seven groups (n = 6) to reduce selection bias, and investigators were blinded during data collection. The sample size was chosen based on previous studies in similar animal models and expected effect sizes, which ensures sufficient statistical power for detecting differences between groups.”

- Provide a detailed list of kits/methods for MDA, SOD, CAT, GPx, and gene expression methods (e.g., RT-qPCR with internal factors such as GAPDH).
Response: We thank the reviewer for the comment. A detailed list of kits and methods used for MDA, SOD, CAT, GPx, and gene expression analysis (e.g.,

RT-qPCR) has been added to the Methods section. These additions are highlighted in the revised manuscript. GAPDH was used as the internal reference (housekeeping) gene to normalize gene expression data.

- Specify units and reporting (e.g., MDA in nmol/mg protein, GSH in μmol/g tissue, etc.).
Response: Done

• Some sentences are accompanied by incomplete or missing references
Response: The text has been reviewed and edited.

• Support all key claims with accurate and up-to-date references and provide a reference list in accordance with the journal format.
Response: Done

• Some statements are overly preclinical/clinical in nature. Use phrases such as "potential", "could be", and "in this animal model"
Response: All sentences that lead the reader to the definitive clinical effects of the drug have been removed or modified.
- and explicitly state the limitations of the animal model.
Response: Thank you for your valuable comment. As suggested, we have now explicitly stated the limitations of the animal model in the conclusion section. Specifically, we acknowledged that the present findings are based on a short-term animal model of acute toxicity and therefore should not be directly extrapolated to humans without further studies.

• The discussion is long and structurally disorganized; some parts are repetitive. Create three coherent sections: Mechanisms of acetaminophen-induced nephrotoxicity (brief and key), Effects of feselol and evidence in the literature (introducing possible mechanisms), comparison with NAC, limitations, and future prospects
Response: Done

• There are a few logical leaps (e.g., attributing mechanisms primarily to α,β-saturated sesquiterpene structure) without direct evidence from the current study; clarify whether this is speculative or supported by data or literature.
Response: Thank you for your insightful comment. We agree that attributing feselol’s activity mainly to the α,β-saturated sesquiterpene structure is speculative. Accordingly, we have revised the text to clarify that this interpretation is based on previous literature and not on direct evidence from our current data.

Reviewer 2:

1-Assessing the long-term effects of Feselol

Although the study shows positive effects of Feselol in the short-term period after treatment, it is suggested that its long-term effects also be investigated. This could provide more information regarding the sustainability of Feselol's protective effects and its possible complications over time. Follow-up periods of three month or longer would be beneficial for a better results.

Response: Thanks to the esteemed referee's suggestion, this study was only designed to investigate the short-term effects of feselol, and unfortunately, it is not possible to continue the study for three months in the current work. Hopefully, in future work, we will investigate the long-term effects of feselol, as suggested by the esteemed referee.

2- Investigating the side effects of Feselol at different dosesThe article discusses the effects of Feselol on oxidative stress, apoptosis, and inflammation, but it is important to investigate how these effects might impact other organs and body systems at higher doses (such as more than 50 mg/kg). Further analysis of Feselol's side effects at different doses could enhance the validity of the article.

Response: This article examined the effects of feselol at two doses of 25 and 50 mg. Due to the high cost of feselol, which was a limitation of this study, it was unfortunately not possible to simultaneously examine higher doses (such as more than 50 mg/kg). In agreement with the reviewer, different and higher doses will be used in future studies to further analyze the side effects of feselol.

3- More detailed analysis of the Bcl-2/Bax ratio

The results related to apoptotic genes (Caspase-3, Bax, Bcl-2) are well presented. However, a more detailed analysis of the Bcl-2/Bax ratio in the different groups would be useful. This ratio plays a crucial role in apoptotic mechanisms and could help better interpret the effects of Feselol in reducing apoptosis and regulating this pathway.

Response: Added in Result, **4.2.** Gene expression of apoptosis-related markers in renal tissue

Reviewer 3:

The manuscript examines (the Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Factors). While the topic may be intriguing, it has several deficiencies regarding discussion and study methodology.

1. Average Weight of Mice: An average of 35 grams for the mice is excessively high, and young mice were not used in the study, which could be a significant flaw. The abstract and methods section state the weight in two different ways,

which are inconsistent; one mentions an average while the other refers to an approximately weight.
Response: The text in the abstract and methods was reread and revised.

2. Tween Carrier: What type of Tween is used?
Response: Tween 80 (Sigma-Aldrich, Germany), a non-ionic surfactant, was used in this study. Tween 80 is widely compatible with various chemical, pharmaceutical, and biological substances, minimizing the risk of adverse reactions. The specific type and supplier of Tween are detailed in the Materials section of the manuscript.

This is not mentioned in the text. How much solvent is needed to dissolve it?
Response: To prepare the feselol solution, 100 mg of feselol was dissolved in 16 mL of Tween 80. This solvent volume was sufficient to fully dissolve the compound for use in the study.

Does Tween have any negative effects on the kidneys?
Response: Tween is widely used as a solvent or vehicle in numerous chemical, pharmaceutical, and biomedical studies, and it is generally considered non-toxic under the conditions commonly employed in such experiments. In the present study, one experimental group was specifically designed to evaluate the potential combined effects of Tween and acetaminophen. The outcomes in this group were comparable to those observed in the acetaminophen-only group, indicating that Tween did not influence the results. Therefore, any observed renal changes can be attributed to acetaminophen rather than Tween.
3. Acetaminophen Damage: The text does not specifically mention the damage that acetaminophen causes to the kidneys, only general injuries are discussed.
Response: The nephrotoxic mechanism of acetaminophen has been highlighted and clearly described in the revised manuscript to ensure that its specific effects on the kidneys are fully conveyed.

4. Specific Marker for Kidney Damage: A specific marker for kidney damage was not investigated in the study, which can also be considered a flaw. For instance, Kim-1 is one of the specific markers for kidney damage.
Response: We thank the reviewer for this valuable comment. The aim of the present study was to investigate the potential protective effects of Feselol against acetaminophen-induced kidney injury through oxidative stress, apoptotic, and inflammatory markers in a short-term animal model. Although the use of a specific marker such as KIM-1 could have provided higher sensitivity, time and financial constraints prevented its assessment. Nevertheless, significant changes in MDA, SOD, CAT, GPx, Caspase-3, Bax, Bcl-2, TNF-α, IL-1β, and IL-10 clearly indicate kidney injury and the potential protective effect of Feselol in this model. Therefore, the absence of KIM-1

assessment does not compromise the overall validity of the findings within the context of this animal model, and the results still provide valuable preclinical insights.

5. Factors Not Measured: In the introduction, TLR-4, CRP, TGF-β1, and p53 are mentioned, but these factors were not measured in the study. Is there a specific reason for this?

Response: As noted in our response to the previous comment, the present study aimed to investigate the potential protective effects of Feselol against acetaminophen-induced kidney injury through oxidative stress, apoptotic, and inflammatory markers. Due to time and financial constraints, assessment of additional factors was not feasible. Therefore, markers such as TLR-4, CRP, TGF-β1, and p53 were not measured in this study. Nevertheless, the results obtained from the measured parameters clearly indicate kidney injury and the potential protective effect of Feselol. We recommend that future studies include these additional markers to further elucidate the underlying molecular mechanisms.

6. Dosage of Ketamine and Xylazine: The dosages of ketamine and xylazine are not mentioned in the article.

Response: The dosages of the anesthetic agents used in this study were Ketamine 90 mg/kg and Xylazine 4 mg/kg, as described by David et al. (2022). These details have now been added to the Methods section of the manuscript.

References:

David EM, Pacharinsak C, Jampachaisri K, Hagan L, Marx JO. Use of ketamine or xylazine to provide balanced anesthesia with isoflurane in C57BL/6J mice. Journal of the American Association for Laboratory Animal Science. 2022 Sep 1;61(5):457-67.

7. Selection Criteria for Mice: The criteria for selecting the number of mice should be referenced.

Response: The number of animals per group (n = 6) was selected based on the standards of similar animal studies (Sedghi et al., 2025) and Festing's guidelines (2006). With this sample size, the study's statistical power to detect the expected effects between groups is estimated to be approximately 80% at a significance level of 0.05, which is considered adequate for evaluating the protective effects of feselol.

References:

Festing, M. F. (2006). Design and statistical methods in studies using animal models of development. Ilar Journal, 47(1), 5-14.

Sedghi, Z., Kaeidi, A., & Hassanshahi, J. (2025). Oleuropein mitigates acetaminophen overdose-induced kidney injury in male rats by enhancing

antioxidant defense and suppressing inflammatory and apoptotic pathways. Drug and Chemical Toxicology, 1-10.

8. PCR Tests: Since the tests were conducted using PCR, there is uncertainty about the protein expression of these genes. Why was Western blotting not used to confirm protein expression?
Response: We thank the reviewer for this comment. The primary aim of this study was to assess gene expression changes related to apoptosis and inflammation at the mRNA level. Functional or protein-level analyses, such as Western blotting, were not performed in this study and could be addressed in future investigations.

9. Conclusion Section: Please write the conclusion separately at the end of the discussion.
Response: Done

10. Figure Footnotes: The footnotes for Figures 3, 4, and 5 should be merged.
Response: Done

11. Units in Figures: The units for Figures 3, 4, and 5 are not mentioned in the images.
Response: Done

12. Figure Legends: The legends for Figures 1 and 2 have significant issues both visually and conceptually, especially concerning the fifth group.
Response: Done

Reviewer 4:
Dear Editor,
The manuscript entitled "Protective Role of Feselol against Acetaminophen-Induced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic, and Inflammatory Pathways" was reviewed as follows:

1- On what basis were the doses of all compounds used in the different groups determined?
Feselol: Since no previous animal studies have been conducted on this compound, the doses of 25 and 50 mg/kg were selected based on our prior experience with bioactive plant compounds, including the protective effects of chlorogenic acid in diclofenac-induced toxicity (Yazdani et al., 2024)
Acetaminophen and N-acetylcysteine (NAC): The doses of acetaminophen (Karthivashan et al., 2016) and NAC (Shen et al., 2016) were chosen based on previously published studies in similar animal models.

References:

Yazdani, M. H., Saghaei, F., & Karimi-Dehkordi, M. (2024). Protective effect of chlorogenic acid on diclofenac-induced oxidative stress and inflammation in the liver of BALB/c mice. Feyz Medical Sciences Journal, 28(4), 375-382.
Shen, Y., Miao, N. J., Xu, J. L., Gan, X. X., Xu, D., Zhou, L., ... & Lu, L. M. (2016). N-acetylcysteine alleviates angiotensin II-mediated renal fibrosis in mouse obstructed kidneys. Acta pharmacologica sinica, 37(5), 637-644.
Karthivashan, G., Kura, A. U., Arulselvan, P., Isa, N. M., & Fakurazi, S. (2016). The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. PeerJ, 4, e2127.

2- In group 5, what was the dose on the first and second days? Why did the intervention start on the third day? In groups 3 to 6, the dose of acetaminophen should be mentioned.

Response: group 5, correct in the study design, as:

Group 5 (NAC + Acetaminophen): Administered N-acetylcysteine (300 mg/kg, IP) for three days; two hours after the last dose, acetaminophen was injected.

The experimental period in this study was three days. All drugs, including feselol 25 and 50 (groups 3 and 4), N-acetylcysteine (group 5) and Tween 80 (group 6), were administered for three days. Only acetaminophen was administered as a single dose on the third day, two hours after the last dose of the drug, to induce acute toxicity.

The grouping in the method was reviewed and revised, and ambiguities were resolved.

3- On page 2, paragraph 3, line 3, and replace "turmeric" with "curcumin" when referring to the compound.

Response: Done

4- In the Experimental Animals section, use "ad libitum" instead of "free."

Response: Done

5- Provide references for the "Evaluation of Oxidative Stress Markers" and "Evaluation of inflammatory and apoptotic gene expression" sections.

Response: Done

6- The lettering in the figures is usually done in alphabetical order, what is the reason for not following this in some figures?

Response: Done

7-In some parameters, such as parts C and D of the figure 2, there is a significant

difference between groups 3 and 4, where N-acetylcysteine demonstrates a beneficial effect.
Response: Done

8-Discuss the study's strengths and weaknesses. Address the short, three-day intervention period as a potential limitation compared to the more common seven-day duration in similar studies.

Response:
Strengths: Despite the short, three-day intervention period, significant changes were observed in oxidative stress, apoptotic, and inflammatory markers, clearly indicating the potential protective effect of feselol against acetaminophen-induced kidney injury. This highlights the potency of feselol even within a limited timeframe.
Limitations: The three-day intervention period is shorter than the more commonly used seven-day duration in similar studies, which may be considered a limitation. Future studies with longer intervention periods could provide a more comprehensive understanding of the long-term therapeutic effects of feselol.
The conclusion section has been revised to incorporate both the strengths and limitations of the study.

9-Investigate the dose-response relationship of Feselol. Explore the possibility of a Feselol dosage that is equivalent to or superior to N-acetylcysteine.
Response: Results were carefully re-read and revised to place greater focus on the dose-response relationship of feselol and on comparing the effects of feselol doses with those of N-acetylcysteine

Associate Editor's Comments:

Associate Editor 1:
• The treatment window appears very short (3 days, with APAP only on day 3). How does this acute model reflect chronic or subchronic nephrotoxicity? Could longer-term effects or recovery dynamics be missed?
Response: The present study aimed to evaluate the protective effects of feselol against acute kidney injury induced by a toxic dose of acetaminophen. The original study design was intended to assess acetaminophen-induced hepatotoxicity and was conducted as part of a thesis. In this thesis, a three-day model of acetaminophen-induced hepatotoxicity was designed based on previous studies (Zhang et al., 2017). During the study, it was decided to also examine kidney samples to determine whether the acute acetaminophen toxicity model and the potential protective effects of feselol observed in the liver would similarly manifest in the kidney. Interestingly, the results demonstrated that this dosing and timeframe were sufficient to induce acute renal injury by day three, allowing assessment of protective effects of feselol.

As this model focuses solely on acute effects, chronic or subchronic outcomes and long-term recovery were not assessed. Investigation of these aspects is recommended for future studies to gain a more comprehensive understanding of therapeutic potential of feselol.

References:

Zhang, J., Song, Q., Han, X., Zhang, Y., Zhang, Y., Zhang, X., ... & Chu, L. (2017). Multi-targeted protection of acetaminophen-induced hepatotoxicity in mice by tannic acid. International immunopharmacology, 47, 95-105.

• Why were histological findings (e.g., tubular necrosis, cast formation) not included? These are gold-standard indicators of nephrotoxicity and would strengthen the biochemical/molecular claims.

Response: We thank the reviewer for highlighting the importance of histological indicators. The primary aim of the present study was to evaluate the protective effects of feselol against acetaminophen-induced kidney injury through biochemical and molecular markers. Unfortunately, histological samples were not assessed in this study. Nevertheless, the biochemical and molecular data—including significant changes in MDA, SOD, CAT, GPx, Caspase-3, Bax, Bcl-2, TNF-α, IL-1β, and IL-10—clearly indicate renal injury and the protective effects of feselol. Histological assessments are currently being conducted as part of a separate thesis by another student, and inclusion of these results in the present study is not feasible at this time.

• Functional kidney markers (serum creatinine, BUN, uric acid) were mentioned in the Introduction but not measured or reported in Results.

Response: We thank the reviewer for their careful observation. As noted in the Introduction, some studies have evaluated kidney functional markers such as serum urea and creatinine. In the present study, we focused primarily on molecular markers and cellular pathways underlying acetaminophen-induced kidney injury to gain deeper mechanistic insights. Nevertheless, significant changes in MDA, SOD, CAT, GPx, Caspase-3, Bax, Bcl-2, TNF-α, IL-1β, and IL-10 clearly demonstrate the strong protective effects of feselol on the kidney. Serum markers such as urea and creatinine, together with histopathological assessments, are currently being investigated as part of a separate thesis project by another student; inclusion of these results in the present study is therefore not feasible at this time.

• Sample size is very small (n=6 per group). Was a power analysis performed to justify this sample size? With high biological variability in oxidative/apoptotic markers, could Type II errors (false negatives) have occurred?

Response: The number of animals per group (n = 6) was selected based on Festing's recommendations (2006) and the standards of similar animal studies (Sedghi et al., 2025), which are considered sufficient for preclinical studies with comparable biochemical and genetic markers and allow detection of significant differences. With this sample size, the statistical power of the study to detect the expected effects between groups is estimated to be approximately 80% at a significance level of 0.05, which is considered adequate for evaluating the protective effects of feselol.

Furthermore, our results showed clear and significant changes in oxidative, apoptotic, and inflammatory markers, indicating adequate study power and a low likelihood of

Type II errors (false negatives). Nevertheless, assessing the effects with a larger number of animals could be recommended in future studies to improve precision and generalizability of the results.

References:

Festing, M. F. (2006). Design and statistical methods in studies using animal models of development. Ilar Journal, 47(1), 5-14.

Sedghi, Z., Kaeidi, A., & Hassanshahi, J. (2025). Oleuropein mitigates acetaminophen overdose-induced kidney injury in male rats by enhancing antioxidant defense and suppressing inflammatory and apoptotic pathways. Drug and Chemical Toxicology, 1-10.

• All figures need p-value.

Response: All figures were subjected to statistical analysis, and it is indicated below each figure that shared letters (a, b, etc.) denote no significant differences ($p > 0.05$). Therefore, the presented results are supported by p-values and are statistically valid.

• The paper claims Feselol has “anti-apoptotic” effects based on Bax/Bcl-2 ratios and Caspase-3 mRNA but no cell death assays (TUNEL, Annexin V, LDH) were performed. Is altered gene expression sufficient to conclude reduced apoptosis? Could compensatory mechanisms or post-translational modifications invalidate these inferences?

Response: This study focused on the protective effects of feselol on acetaminophen-induced renal apoptosis based on biochemical markers and gene expression. No direct functional assays of cell death were performed, and the conclusions rely on these molecular indicators. Future studies could include functional assays to further validate these findings.

• No investigation into upstream regulators (e.g., Nrf2, NF-κB, p53, MAPK pathways) was conducted, despite their central role in APAP toxicity. Does Feselol act via Nrf2 activation (like many antioxidants) or NF-κB inhibition? Without probing mechanisms, the “how” remains speculative.

Response: The aim of this study was to evaluate the protective effects of feselol against acetaminophen-induced kidney injury via oxidative stress, apoptosis, and inflammation markers. Upstream pathways such as Nrf2, NF-κB, p53, and MAPK were beyond the scope of this study, and the precise mechanisms of feselol require further investigation. Nevertheless, the results demonstrate reduced oxidative stress, modulation of apoptotic gene expression, and attenuation of inflammation.»

• NAC is presented as the “gold standard,” but its dose (300 mg/kg) and timing may not be clinically translatable. How does this dose compare to human equivalent dosing? Could the comparison with NAC be misleading if NAC was suboptimally dosed or timed?

Response: The dose and timing of NAC in this study were selected based on previous animal studies in similar mouse models to reliably assess the known protective effects

of NAC (Shen et al., 2016). The use of NAC as a positive control provided a comparative standard within the same animal model. Investigation of the human equivalent dose of NAC was beyond the scope of this study and could be addressed in future research.

References:

Shen, Y., Miao, N. J., Xu, J. L., Gan, X. X., Xu, D., Zhou, L., ... & Lu, L. M. (2016). N-acetylcysteine alleviates angiotensin II-mediated renal fibrosis in mouse obstructed kidneys. Acta pharmacologica sinica, 37(5), 637-644.

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer — 8 Oct 2025

All identified concerns have been addressed, and the manuscript now meets the journal's quality standards. The study on the protective role of Feselol against acetaminophen-induced nephrotoxicity in mice, including the modulation of oxidative stress, apoptosis, and inflammatory markers, is now fully prepared for consideration and review for publication.

REFEREE : Reviewer — 8 Oct 2025

Dear Editor, Based on the referee's responses, the manuscript is acceptable for publication.

REFEREE : Reviewer — 8 Oct 2025

Dear Editor,
The manuscript entitled "Protective Role of Feselol against AcetaminophenInduced Nephrotoxicity in Mice: Modulation of Oxidative Stress, Apoptotic,and Inflammatory Pathways" was reviewed as follows:
Satisfied with the authors' corrections, I approve the manuscript for acceptance.

REFEREE : Associate Editor — 10 Oct 2025

The Ms is now suitable for publication in JJNPP

REFEREE : EIC — 10 Oct 2025

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

Your work underwent a rigorous peer-review process, and the reviewers and editorial board were impressed by the originality, scientific rigor, and relevance of your findings. We believe your contribution will be of significant interest to our readership and will advance the field of natural pharmaceutical products.

Before publication, our production team will contact you regarding final formatting, proofs, and any necessary administrative steps. Please ensure that all authors have

approved the final version of the manuscript and that any required ethical or copyright documentation is in order.

On behalf of the editorial board, I congratulate you and your co-authors on this excellent work and thank you for choosing to publish in JJNPP.