Hepatitis Monthly
Volume 25 · Issue 1 · e164840

OPEN PEER REVIEW

# Astragaloside IV Improves Cyclophosphamide-Induced Liver Injury in Mice by Mediating Immunosuppression and Oxidative Stress Through the PI3K/Akt Signaling Pathway: A Randomized Trial

Yue e Sun, Jiahui Cao, Mengtao Li, Jingwen Ma, Weidong Wang

| SUBMITTED | REVISED | ACCEPTED | DOI |
| --- | --- | --- | --- |
| 26 Jul 2025 | 21 Sep 2025 | 6 Oct 2025 | 10.5812/hepatmon-164840 |

## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

## Contents

1 **Original submission** Referee 1, Referee 2, Referee 3, Associate Editor, Editor-in-Chief ..... 2
2 **Revision 1** Referee 3, Referee 1, Referee 2, Associate Editor, Editor-in-Chief ..... 5
Authors' reply to reviewers ..... 6
Reviewer and editor comments ..... 13
3 **Revision 2** Referee 2, Associate Editor, Editor-in-Chief ..... 15
Authors' reply to reviewers ..... 16
Reviewer and editor comments ..... 18

## 1 Original submission

5 comments · 30 Jul 2025 – 6 Aug 2025

### Referee 1 Reviewer

MAJOR REVISION 30 Jul 2025

Reviewer Comments

The manuscript presents an important investigation into the hepatoprotective potential of Astragaloside IV (AS-IV) against cyclophosphamide (CTX)-induced liver injury in mice, primarily through modulation of the PI3K/Akt signaling pathway. The study offers valuable insight into the immunomodulatory and antioxidative roles of AS-IV and uses a variety of established techniques including ELISA, HE staining, Western blotting, and oxidative stress assays to support its findings.

The authors can find the comments, concerns, and questions below.

The introduction, although informative, lacks a coherent transition from the problem statement to the study rationale. Key terms such as PI3K/Akt axis, CTX-induced immunosuppression, and oxidative stress are mentioned but insufficiently contextualized. The authors should clearly state the gap in current knowledge and explain why AS-IV is a promising candidate. The introduction would benefit from a concise mechanistic overview of the PI3K/Akt signaling pathway in relation to liver damage and immunity.

The authors are strongly advised to incorporate recent studies (from 2024 onward) to support novelty, particularly in the introduction, discussion, and conclusion sections. You should include more references to recent studies and explain their contributions in greater detail. The citation of these articles provides valuable insights that closely align with the themes and research objectives of the work. Read and cite the following articles: (a) VEGF-FGF Signaling Activates Quiescent CD63+ Liver Stem Cells to Proliferate and Differentiate. doi: https://doi.org/10.1002/advs.202308711 (b) Noncoding RNAs in sepsis-associated acute liver injury: doi: https://doi.org/10.1016/j.phrs.2025.107596 (c) doi: 10.1080/03602532.2024.2405163 (d) doi: 10.3389/fendo.2023.1321576 (e) doi: 10.1248/bpb.b17-00882 (f) doi: https://doi.org/10.1016/j.biopha.2024.117163 (g) doi: 10.1080/14740338.2025.2494685 (h) doi: 10.3923/ijp.2024.1331.1338 (i) doi: 10.24976/Discov.Med.202436184.99 (j) doi: https://doi.org/10.1016/j.jep.2025.120099.

Concerns:
1. Lines 143–148:
 The use of multiple ELISA kits from Abcam is appropriate; however, the rationale for selecting these specific cytokines and immunoglobulins should be justified.
 How was the specificity validated for mouse serum? Are there cross-reactivity concerns?
 How was the double dilution method standardized across kits?
2. Lines 256–265:
 The dose-dependency of AS-IV is mentioned, but the concentration choice (100 mg/kg) needs better justification supported by previous studies.
 The link between observed biochemical markers and inhibition of PI3K/Akt signaling by LY294002 requires deeper interpretation. Were compensatory mechanisms or feedback loops assessed?
3. Lines 382–388:
 The conclusion overstates the therapeutic potential of AS-IV without long-term toxicity or dose-tolerance data.
 The sentence structure is redundant and could be streamlined. Suggest emphasizing “AS-IV may represent a potential adjunct treatment strategy, pending further mechanistic and clinical validation.”
4. Figures should be rechecked for clarity and appropriate statistical labeling.
5. Grammatical errors and sentence fragmentation appear in several places. A professional English editing

service is recommended.
6. Abbreviations (e.g., CTX, AS-IV, ROS) should be defined at first mention.
7. Include a limitations section acknowledging species-specific effects and translation to human systems.

It requires major revisions for clarity, scientific soundness, and structural refinement.

**Referee 2** Reviewer — MINOR REVISION — 30 Jul 2025

*Subject
- yes

*Title
- yes

*Abstract
- yes

*Introduction
- yes

*Materials And Methods
They are somewhat appropriate.
It would be better to mention in the histopathology section what type of tissue lesions were observed.
For example, in this study, in the histopathology section, we examined cases of hemorrhage, necrosis, atrophy, inflammatory cell infiltration, fibrosis, etc.

*Results
- It seems that in the histopathology results section, changes and improvements in liver, spleen, and thymus tissue are shown with arrows and stars, and the lesions are also graded.

*Discussion
It is somewhat appropriate.

*References
yes

**Referee 3** Reviewer — MAJOR REVISION — 3 Aug 2025

Dear AE,
This study investigates the protective effects of Astragaloside IV (AS-IV) against cyclophosphamide (CTX)-induced liver injury, immunosuppression, and oxidative stress in mice, with a focus on the PI3K/Akt signaling pathway. The topic is timely and relevant to the fields of pharmacology and hepatotoxicity. The experimental design is logical, and the use of the PI3K inhibitor (LY294002) adds mechanistic insight. However, several major and minor issues need to be addressed before the manuscript is suitable for publication:
1- The abstract is inadequate in all respects.
2- The sample size was not specified in the abstract.
3- Some sentences in the introduction lack references. All sentences quoted from other articles must have references at the end of each sentence.
4- No explanation or power calculation is provided for choosing 6 mice per group. Given the number of groups (8), the power of the study is questionable.
5- The inclusion of lung tissue in protein analysis (Section 2.9) is unexplained and inconsistent with the

liver/spleen focus.
6- The manuscript is generally understandable but would benefit from professional English editing. Examples of awkward or incorrect phrasing include:

“RISE SOD and CAT contents” → “increase SOD and CAT levels”

“Liver cell structure’ integrity and clarity” → “the integrity and clarity of liver cell structure”

“AS-IV had no hepatotoxicity” → “AS-IV showed no hepatotoxicity”
7- Several sections, especially the Results and Discussion, repeat findings without adding insight. Condense redundant explanations to improve readability.

**Associate Editor** MAJOR REVISION 3 Aug 2025

Dear EIC,
I thank the authors and express my appreciation to the researchers. The manuscript is a valuable piece of research. But some revisions are needed. The authors should revise it based on the comments. Also, a minor change should be made in the abstract:
*The study design should be mentioned in the first line of the method section of the ABSTRACT.

**Editor-in-Chief** MAJOR REVISION 6 Aug 2025

Major Revision

2

# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear editors and reviewers,

Thank you very much for your comments and professional suggestions. These comments are very important to our articles and help to improve the academic rigor of our articles. According to the suggestions and requirements of you and the reviewer, we have done our best to make a comprehensive and detailed revision of the paper, hoping that the revised paper can meet the requirements of Hepatitis Monthly. Next, we reply to the reviewers' comments point by point.

**Reviewers' Comments:**

**Reviewer 0:**

Dear AE,

This study investigates the protective effects of Astragaloside IV (AS-IV) against cyclophosphamide (CTX)-induced liver injury, immunosuppression, and oxidative stress in mice, with a focus on the PI3K/Akt signaling pathway. The topic is timely and relevant to the fields of pharmacology and hepatotoxicity. The experimental design is logical, and the use of the PI3K inhibitor (LY294002) adds mechanistic insight. However, several major and minor issues need to be addressed before the manuscript is suitable for publication:

1-Abstract part is too poor in all parts.

**Response:** Thanks for your kind suggestion. The abstract has been rewritten according to the journal 's author 's guide.

2-Sample size was not denoted in abstract.

**Response:** Thanks for your kind suggestion. The sample size has been supplemented in the abstract.

3-Some sentences in introduction lack references. All sentences quoted from other articles must have references at the end of themselves.

**Response:** Sorry for our negligence. The missing references in the introduction have been supplemented.

4-No explanation or power calculation is provided for choosing 6 mice per group. Given the number of groups (8), the power of the study is questionable.

**Response:** Thanks for your suggestion. In this study, the sample size of 6 mice in each group was based on the following basis: (1) According to the standard of $\alpha = 0.05$ and Power = 0.8 and the minimum effect size, the sample size was calculated by ANOVA in GPOWER software. This may have some limitations, so we will expand the sample size in future research. (2) Reference to the conventional practice of similar studies (DOI: 10.3892/mmr.2021.12076, DOI: 10.1016/j.fshw.2021.07.009, DOI: 10.1515/chem-2024-0056, DOI: 10.1556/2060.2023.00190).

5-The inclusion of lung tissue in protein analysis (Section 2.9) is unexplained and inconsistent with the liver/spleen focus.

**Response:** Sorry for the inappropriate expression. The protein analysis was conducted on liver and spleen tissues, which have been revised in the manuscript.

6-The manuscript is generally understandable but would benefit from professional English editing. Examples of awkward or incorrect phrasing include:

**Response:** Thanks for your comment. The manuscript has been revised by professional editors to enhance the language and grammatical quality of the article.

“RISE SOD and CAT contents” → “increase SOD and CAT levels”

**Response:** Thanks for your comment. "rise" has been changed to "increase".

“Liver cell structure’ integrity and clarity” → “the integrity and clarity of liver cell structure”

**Response:** Thanks for your comment. the phrase "liver cell structure' integrity and clarity" has been modified to "the integrity and clarity of liver cell structure".

“AS-IV had no hepatotoxicity” → “AS-IV showed no hepatotoxicity”

**Response:** Thanks for your comment. "had" has been changed to "showed".

7-Several sections, especially the Results and Discussion, repeat findings without adding insight. Condense redundant explanations to improve readability.

**Response:** Thank you for pointing it out. We have removed repetitive expressions and condensed the content of the results and discussion sections to enhance readability.

**Reviewer 5:**

**Reviewer Comments**

The manuscript presents an important investigation into the hepatoprotective potential of Astragaloside IV (AS-IV) against cyclophosphamide (CTX)-induced liver injury in mice, primarily through modulation of the PI3K/Akt signaling pathway. The study offers valuable insight into immunomodulatory and antioxidative roles of AS-IV and uses a variety of established techniques including ELISA, HE staining, Western blotting, and oxidative stress assays to support its findings.

Authors can find the comments, concerns, and questions below.

The introduction, although informative, lacks a coherent transition from the problem statement to the study rationale. Key terms such as PI3K/Akt axis, CTX-induced immunosuppression, and oxidative stress are mentioned but insufficiently contextualized. The authors should clearly state the gap in current knowledge and explain why AS-IV is a promising candidate. The introduction would benefit from a concise mechanistic overview of the PI3K/Akt signaling pathway in relation to liver damage and immunity.

**Response:** Thank you for your thoughtful suggestion. The connection between the problem statement and the research basis has been supplemented. Some explanations have been added to the background of the PI3K/Akt signaling pathway, CTX-induced immunosuppression, and oxidative stress. Astragaloside IV (AS-IV), AS the main active component of Astragalus membranaceus, can regulate the PI3K/Akt pathway in other disease models, significantly improving inflammation, immunosuppression and oxidative stress. Relevant studies have also confirmed that AS-IV can significantly alleviate drug-induced liver injury (PMID: 38798265, PMID: 38689349, PMID: 38521426, PMID: 36368505). However, it remains unknown whether AS-IV can improve liver injury and immunosuppression caused by CTX through the PI3K/Akt pathway. Therefore, AS-IV is an ideal research object. The relationship between the PI3K/Akt signaling pathway and liver injury as well as immune function has been expounded in the introduction part, which will make the content more academically valuable.

Authors are strongly advised to incorporate recent studies (from 2024 onward) to support novelty, particularly in the introduction, discussion, and conclusion sections. You should include more references to recent studies and explain their contributions in greater detail. The citation of these articles provides valuable insights that closely align with the themes and research objectives of the

work. Read and cite the following article; (a) VEGF-FGF Signaling Activates Quiescent CD63+ Liver Stem Cells to Proliferate and Differentiate. doi: https://doi.org/10.1002/advs.202308711 (b) Noncoding RNAs in sepsis-associated acute liver injury: doi:https://doi.org/10.1016/j.phrs.2025.107596 (c) doi: 10.1080/03602532.2024.2405163 (d) doi: 10.3389/fendo.2023.1321576 (e) doi: 10.1248/bpb.b17-00882 (f) doi: https://doi.org/10.1016/j.biopha.2024.117163 (g) doi: 10.1080/14740338.2025.2494685 (h) doi: 10.3923/ijp.2024.1331.1338 (i) doi: 10.24976/Discov.Med.202436184.99 (j) doi: https://doi.org/10.1016/j.jep.2025.120099.

**Response:** Thanks for your kind suggestion. The latest research findings from 2024 and beyond have been cited in the introduction, discussion and conclusion sections to enhance innovation. And the recommended literature has been cited.

Concerns:

1. Lines 143–148:

The use of multiple ELISA kits from Abcam is appropriate; however, the rationale for selecting these specific cytokines and immunoglobulins should be justified.

How was the specificity validated for mouse serum? Are there cross-reactivity concerns?

How was the double dilution method standardized across kits?

**Response:** Thanks for your suggestion. (1) The basic principles of cytokines and immunoglobulins have been explained in "3.6 Enzyme-linked immunosorbent assay (ELISA)". (2) The instructions for the Abcam ELISA kit clearly state that there is no cross-reaction between rat and human proteins. (3) All reference standards are standardized using the same initial concentration and the same dilution solution, with a fixed dilution factor.

2. Lines 256–265:

The dose-dependency of AS-IV is mentioned, but the concentration choice (100 mg/kg) needs better justification supported by previous studies.

The link between observed biochemical markers and inhibition of PI3K/Akt signaling by LY294002 requires deeper interpretation. Were compensatory mechanisms or feedback loops assessed?

**Response:** Thanks for your comment. The selection of AS-IV concentration was based on the reference PMID: 37100262, and this reference was introduced into the manuscript. Regarding whether the compensation mechanism was evaluated, this study indeed did not systematically assess the compensation process. This will be our limitation and also a key direction for future research.

3. Lines 382–388:

The conclusion overstates the therapeutic potential of AS-IV without long-term toxicity or dose-tolerance data.

The sentence structure is redundant and could be streamlined. Suggest emphasizing “AS-IV may represent a potential adjunct treatment strategy, pending further mechanistic and clinical validation.”

**Response:** Thank you for pointing it out. We have simplified this passage, emphasizing that "AS-IV may represent a potential adjuvant therapeutic strategy, awaiting further mechanism and clinical validation."

4. Figures should be rechecked for clarity and appropriate statistical labeling.

**Response:** Thank you to the reviewers for their comments. We have checked the clarity of the

result graphs and found that they meet the journal's requirements for image clarity. At the same time, we have marked the results without significant differences with ns.

5. Grammatical errors and sentence fragmentation appear in several places. A professional English editing service is recommended.

**Response:** Thanks for your comment. The manuscript has been revised by professional English editor to enhance the language and grammatical quality of the article.

6. Abbreviations (e.g., CTX, AS-IV, ROS) should be defined at first mention.

**Response:** Thanks for your suggestion. The full names of CTX, AS-IV, etc. in the abstract and the main text are all added when they are first mentioned. In addition, the full names of abbreviations such as ROS, MDA, and SOD have been supplemented in the abstract.

7. Include a limitations section acknowledging species-specific effects and translation to human systems.

**Response:** Thanks for your kind suggestion. The limitations of the manuscript have been supplemented in the discussion section, and species-specific effects and transformations on human systems have been acknowledged.

It requires major revisions for clarity, scientific sounds, and structural refinement.

**Reviewer 6:**

*Subject

- yes

*Title

- yes

*Abstract

- yes

*Introduction

- yes

*Materials And Methods

They are somewhat appropriate.

It would be better to mention in the histopathology section what type of tissue lesions were observed.

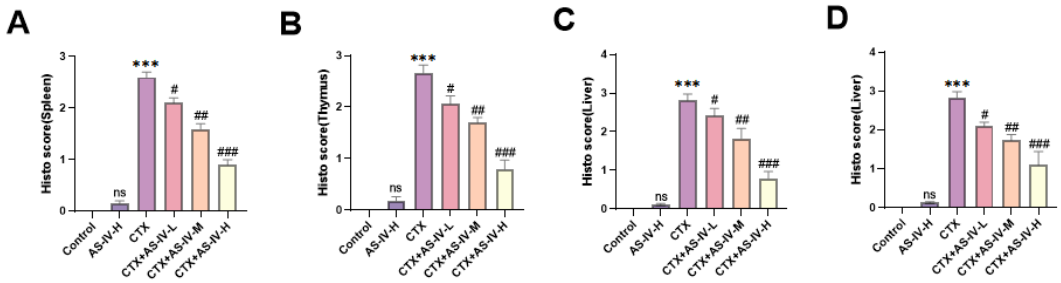
For example, in this study, in the histopathology section, we examined cases of hemorrhage, necrosis, atrophy, inflammatory cell infiltration, fibrosis, etc.

*Results

- It seems that in the histopathology results section, changes and improvements in liver, spleen, and thymus tissue are shown with arrows and stars, and the lesions are also graded.

**Response:** Thanks for your kind suggestion. The types of tissue lesions observed (swelling, necrosis, atrophy, inflammatory cell infiltration, etc.) have been mentioned in the 3.4 HE staining chapter. In the section on histopathological results, we have marked the changes and improvements in liver, spleen and thymus tissues with arrows, classified the lesions and provided

relevant explanations. The statistical chart of related lesion scores can be found in the supplementary data.




*Discussion

They are somewhat appropriate.

*References

yes

---

Associate Editor's Comments:

Associate Editor 0:

Dear EIC,

Thanks to the authors and appreciation to the researchers. The manuscript is a valuable research. But some revisions are needed. Authors should revise it based on comments. Also minor change should be done in abstract:

*Study design should be mentioned in first line of method section of ABSTRACT

**Response:** Thanks for your comment. The research design has been mentioned in the first line of the methods section of the abstract.

Reviewer Assistant Comments:

These comments were generated by AI based on your manuscript. Please review them and consider incorporating the suggestions to enhance your article.

I will now perform a peer review of the abstract using the CONSORT checklist for Randomized Trial Studies. Here are the questions from the checklist along with the corresponding answers from the abstract:

1. Identification as a randomized trial in the title.

Peer Review: The abstract does not explicitly identify the study as a randomized trial in the title.

**Response:** Thanks for your comment. It has been noted in the title that this is a randomized trial.

2. Structured summary of trial design, methods, results, and conclusions.

Peer Review: The abstract provides a structured summary including background, methods, results, and conclusion. However, it does not explicitly mention the trial design as randomized.

**Response:** Thanks for your comment. The format of the abstract has been modified in accordance with the author's guidelines, and it has been stated that the trial design is randomized.

3. Scientific background and explanation of rationale.

Peer Review: The abstract provides a background explaining the hepatotoxicity caused by Cyclophosphamide (CTX) and the potential immune-enhancing effects of Astragaloside IV (AS-IV).

**Response:** Thanks for your comment. The hepatotoxicity caused by cyclophosphamide (CTX) and the potential immune-enhancing effect of astragaloside IV (AS-IV) have been explained previously.

4. Specific objectives or hypothesis.

Peer Review: The objective to evaluate the effects of AS-IV on CTX-induced hepatotoxicity and immunosuppression is clear.

**Response:** Thanks for your comment. It has been stated that the purpose of this study is to evaluate the effect of AS-IV on CTX-induced hepatotoxicity and immunosuppression.

5. Description of trial design (such as parallel, factorial), including allocation ratio.

Peer Review: The abstract does not describe the trial design or allocation ratio.

**Response:** Thanks for your comment. The experimental design has been described in the abstract.

6. Eligibility criteria for participants.

Peer Review: The abstract does not specify eligibility criteria for the mice used in the study.

**Response:** Thanks for your comment. The qualified criteria for the mice used have been described in the abstract: BALB/C mice aged 6-8 weeks.

7. Settings and locations where the data were collected.

Peer Review: The abstract does not provide information on the settings and locations of data collection.

**Response:** Thanks for your comment. The environmental and location information for data collection is too long and has been mentioned in the method, so it is not provided in the abstract.

8. The experimental and control interventions for each group with sufficient details to allow replication, including how and when they were actually administered.

Peer Review: The abstract details the administration of AS-IV and PI3K inhibitor LY294002, but does not mention a control group.

**Response:** Thanks for your comment. The details of the experimental intervention and control intervention for each group have been supplemented and explained.

9. Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed.

Peer Review: The abstract describes several outcome measures such as blood composition, cytokines, and liver injury indexes, but does not specify which are primary or secondary.

**Response:** Thanks for your comment. This study mainly evaluated the regulation of astragaloside IV on immune function and its improvement of liver injury in mice. There was no way to distinguish between primary and secondary indicators.

10. Statistical methods used to compare groups for primary and secondary outcomes.

Peer Review: The abstract does not mention any statistical methods used.

**Response:** Thanks for your comment. Too many statistical methods were used in this study and have been uniformly described in the methods.

11. For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome.

Peer Review: The abstract does not provide information on the number of mice used or their assignment to groups.

**Response:** Thanks for your comment. The information on the number of mice used or their groups has been supplemented in the abstract.

12. Dates defining the periods of recruitment and follow-up.

Peer Review: The abstract does not mention the dates of recruitment or follow-up.

**Response:** Thanks for your comment. This study was conducted on mice, so clinical terms such as "recruitment" and "follow-up" are not applicable. The abstract method and the method involve the experimental timeline.

13. A table showing baseline demographic and clinical characteristics for each group.

Peer Review: The abstract does not include a table or description of baseline characteristics.

**Response:** Thanks for your comment. This study was a mouse experiment, so there are no tables of baseline demographic and clinical characteristics for each group.

14. For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval).

Peer Review: The abstract provides results but does not include effect sizes or confidence intervals.

**Response:** Thanks for your comment. The results of each group and the estimated effect size have been supplemented in the abstract.

15. All important harms or unintended effects in each group.

Peer Review: The abstract does not mention any harms or unintended effects.

**Response:** Thanks for your comment. No harm or unexpected effects were found in this study.

Major Comments:

The abstract lacks explicit identification as a randomized trial.

There is no mention of trial design, allocation ratio, or control group.

Statistical methods and effect sizes are not described.

**Response:** Thanks for your comment. It has been clearly indicated that this experiment is a randomized trial. The experimental design, allocation ratio, control group and effect size have all been supplemented and explained.

Minor Comments:

The abstract does not specify eligibility criteria or settings for data collection.

There is no information on the number of participants or baseline characteristics.

Overall, the abstract provides a general overview of the study but lacks specific details required for a comprehensive understanding of the trial design and outcomes as per the CONSORT checklist【5:2†source】.

**Response:** Thanks for your comment. We fully agree with the significance of transparency in research design for reproducibility. It should be noted that this study belongs to preclinical basic research (focusing on molecular mechanisms and animal models), rather than a clinical randomized controlled trial. Therefore, the requirements for clinical trials in the CONSORT statement do not fully apply to this study. However, we have already marked the P-values for each outcome indicator described in the abstract.

## 2 Revision 1

5 comments · 30 Aug 2025 – 1 Sep 2025

### Referee 3 Reviewer

ACCEPT 30 Aug 2025

Dear AE,
I reviewed the manuscript again. The great effort in improving the manuscript is highly appreciated.
The authors have addressed all comments.
Thank you

### Referee 1 Reviewer

ACCEPT 31 Aug 2025

Review Report R2

Thank you for submitting the revised manuscript titled “Astragaloside IV improves cyclophosphamide-induced liver injury in mice by mediating immunosuppression and oxidative stress through the PI3K/Akt signaling pathway: a randomized trial”. I have carefully reviewed your responses and the updated version, and I am pleased to confirm that all previous concerns have been satisfactorily addressed. The revisions, particularly the clarification of the introduction, refinement of the figures, and enhancement of the discussion, have notably improved the manuscript's overall quality. I recommend acceptance in its current form.

### Referee 2 Reviewer

MINOR REVISION 31 Aug 2025

According to the histopathological changes, the lesions observed in the histopathology slides should be marked with arrows, asterisks, etc.

Regarding the grading of lesions, the meaning of no obvious lesions (what do you mean? How many cells are infiltrating? What is the amount of necrotic tissue? What is the extent of the atrophy?) and the other lesion grades should be specified. Use both qualitative and quantitative scoring to grade the lesions.
For example, grade zero (inflammatory cell infiltration 0, necrosis 0, atrophy 0), without obvious lesions (inflammatory cell infiltration ..., necrosis ..., atrophy ...), and other grades should be specified as such.
On the other hand, you wrote that you used a microscope (NIKON ECLIPSE E100, Nikon, Tokyo, Japan) to observe histopathological changes and examine swelling, necrosis, atrophy, inflammatory cell infiltration, etc. What damage is meant by "other"?
In this regard, you can get help from pathology colleagues.

Good luck and victory

### Associate Editor

MINOR REVISION 31 Aug 2025

Dear Editor-in-Chief,

I would like to extend my gratitude to the authors for their contribution and commend the researchers for their valuable work presented in the manuscript. However, I believe that certain revisions are necessary, and I

kindly request the authors to consider incorporating these suggested changes based on the provided comments.

**Editor-in-Chief** MINOR REVISION 1 Sep 2025

Minor Revision

3

# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear editors and reviewers,
Thank you very much for your comments and professional suggestions. These comments are very important to our articles and help to improve the academic rigor of our articles. According to the suggestions and requirements of you and the reviewer, we have done our best to make a comprehensive and detailed revision of the paper, hoping that the revised paper can meet the requirements of Hepatitis Monthly. Next, we reply to the reviewers' comments point by point.

**Reviewers' Comments:**

**Reviewer 0:**
Dear AE,
I reviewed the manuscript again. The great effort in improving the manuscript is highly appreciated.
The authors had addressed all comments.
Thank you

**Reviewer 1:**
Review Report R2

Thank you for submitting the revised manuscript titled “Astragaloside IV improves cyclophosphamide-induced liver injury in mice by mediating immunosuppression and oxidative stress through the PI3K/Akt signaling pathway: a randomized trial”. I have carefully reviewed your responses and the updated version, and I am pleased to confirm that all previous concerns have been satisfactorily addressed. The revisions, particularly in introduction clarification, figure refinement, and discussion enhancement, have notably improved the manuscript's overall quality. I recommend acceptance in its current form.

**Reviewer 2:**
According to the histopathological changes, the lesions observed in the histopathology slides should be marked with arrows, asterisks, etc.
And also regarding the grading of lesions, no obvious lesions (what do you mean? How many cells are infiltrating? How much is the amount of necrotic tissue? How much is the atrophy?) and other lesion grading should be specified. Use both qualitative and quantitative scoring to grade the lesions.
For example, grade zero (inflammatory cell infiltration 0, necrosis 0, atrophy 0) without obvious lesions (inflammatory cell infiltration ..., necrosis ..., atrophy ...) and other grades should be specified as such.
On the other hand, you wrote that using a microscope (NIKON ECLIPSE E100, Nikon, Tokyo, Japan) to observe histopathological changes, examining swelling, necrosis, atrophy, inflammatory cell infiltration, etc. What damage is meant by "other"?
In this regard, you can get help from pathology colleagues
**Response:** Thanks for your kind suggestion. Based on your reference suggestions and after discussing with colleagues in pathology, we have found that the descriptions in the traditional 0-3 scoring system are often rather vague (such as "mild", "moderate", "severe") and highly dependent

on the personal experience of pathologists, which can easily lead to significant inter-observer differences. Therefore, we made changes to the scoring system and re-conducted statistical analysis, enabling us to provide a more precise semi-quantitative definition for each grade. This makes the scoring results more consistent and repeatable, and is also in line with the current trend of pathological scoring standardization. And all the types of pathological changes we observed were clearly listed. In addition to the classic lesions (swelling, congestion, inflammatory cell infiltration), we also observed and evaluated vacuolization, which has been incorporated into the above-mentioned scoring system. Furthermore, in the legend of the result graph, we illustrate that different markers represent different pathological lesions.

## 3 Revision 2

3 comments · 28 Sep 2025 – 6 Oct 2025

### Referee 2 Reviewer

ACCEPT 28 Sep 2025

Hello
Good day
Wishing you success.

### Associate Editor

ACCEPT 29 Sep 2025

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

### Editor-in-Chief

ACCEPT 6 Oct 2025

Accept