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OPEN PEER REVIEW

The Role of GRP78/ATF6/IRE1 and Caspase-12 Signaling Pathways in the Protective Effects of n-Hexane Oil Extract of Black Soldier Flies' Larvae (*Hermetia illucens*) Against Aflatoxin-Induced Hepatotoxicity in Rats

Fateme Heidari, Tahereh Zarei Taher, Yalda Arast

SUBMITTED	REVISED	ACCEPTED	DOI
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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.

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Contents

1 **Original submission** Referee 1, Referee 2, Associate Editor, Editor-in-Chief 2
2 **Revision 1** Referee 1, Associate Editor, Editor-in-Chief 4
Authors' reply to reviewers 5
Reviewer and editor comments 11

1 Original submission

4 comments · 19 Nov 2025 – 27 Nov 2025

Referee 1 Reviewer

MINOR REVISION 19 Nov 2025

The novelty of the study should be added at the end of the introduction.
Why was only the n-hexane extract used?
The number of animals should be listed uniformly throughout the article.
The Discussion largely repeats results and cites previous studies but does not critically analyze discrepancies or limitations.
The abstract should include statistical significance levels for key findings.

Referee 2 Reviewer

MINOR REVISION 25 Nov 2025

The use of Black Soldier Fly Larvae (BSFL) n-hexane extract as a bioactive substance with antioxidant properties against AFB1-induced hepatotoxicity in rats is innovative. The inclusion of ER stress pathways (GRP78/ATF6/IRE1) adds mechanistic depth that is relatively less explored in mycotoxin studies. The novelty could be highlighted more clearly through comparison with previous antioxidant or BSFL studies. If BSFL has not been used in hepatotoxicity models before, this should be emphasized.

The use of five groups (Control, AFB1, BSFL alone, BSFL+AFB1 at two doses) provides a well-structured design and allows dose-response evaluation. The 28-day treatment duration is adequate to observe sub-chronic hepatotoxicity and protective effects. The sample size (n=7 per group) may be limited; a power calculation should be mentioned. Details on randomization, blinding, and extract preparation are missing; including them would increase methodological rigor. The rationale for the BSFL doses should be supported by references or pilot studies.

The article structure is clear. The integration of biochemical, inflammatory, and histopathological outcomes is logical. Some sentences are long and complex; for example, “At the end of the treatment…” could be split for clarity. Minor punctuation and grammatical issues exist (commas, colons, etc.). Abbreviations should be consistent (e.g., IL-6b → IL-6). “Administration of BSFLextract (at a more potent dose…)” is vague; “higher dose” or “dose-dependent effect” is clearer.

Multiple parameters are assessed: liver enzymes, oxidative stress, inflammation, ER stress, apoptosis markers, and histopathology. Consistency between biochemical and histopathological data strengthens validity. Statistical significance (p-values) is not mentioned; even in an abstract, reporting key p-values is recommended. Statistical methods (ANOVA, post hoc tests) should be stated briefly.

BSFL extract mitigates AFB1-induced hepatotoxicity. The mechanistic insight into biochemical indices, oxidative stress, and inflammation aligns with the results. The wording may be slightly strong; “can have beneficial effects” could be replaced with “may mitigate” for a more cautious tone.

Associate Editor

MINOR REVISION 26 Nov 2025

Dear Dr. Arast,
As seen in their comments, both reviewers requested some minor revisions to your initial submission before it can be considered acceptable for publication in IJPR. I therefore ask you to please carefully read all

comments and provide thoughtful answers to each one, point by point. Finally, revise your manuscript according to the reviewers' comments.
With kind regards,
Jalal Pourahmad
Associate Editor

Reviewer 1:
The novelty of the study should be added at the end of the introduction.
Why was only the n-hexane extract used?
The number of animals should be listed uniformly throughout the article.
The Discussion largely repeats results and cites previous studies but does not critically analyze discrepancies or limitations.
The abstract should include statistical significance levels for key findings.
Decision:
Minor revision
Reviewer 2:
The use of Black Soldier Fly Larvae (BSFL) n-hexane extract as a bioactive substance with antioxidant properties against AFB1-induced hepatotoxicity in rats is innovative. The inclusion of ER stress pathways (GRP78/ATF6/IRE1) adds mechanistic depth that is relatively less explored in mycotoxin studies. Novelty could be highlighted more clearly through comparison with previous antioxidant or BSFL studies. If BSFL has not been used in hepatotoxicity models before, this should be emphasized.
The use of five groups (Control, AFB1, BSFL alone, BSFL+AFB1 at two doses) is well-structured and allows dose-response evaluation. The 28-day treatment duration is adequate to observe sub-chronic hepatotoxicity and protective effects. The sample size (n=7 per group) may be limited; a power calculation should be mentioned. Details on randomization, blinding, and extract preparation are missing, and including them would increase methodological rigor. The rationale for the BSFL doses should be supported by references or pilot studies.
The article structure is clear. The integration of biochemical, inflammatory, and histopathological outcomes is logical. Some sentences are long and complex; e.g., “At the end of the treatment…” could be split for clarity. Minor punctuation and grammatical issues exist (commas, colons, etc.). Abbreviations should be consistent (e.g., IL-6b → IL-6). “Administration of BSFLextract (at a more potent dose…)” is vague; “higher dose” or “dose-dependent effect” is clearer.
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BSFL extract mitigates AFB1-induced hepatotoxicity. Mechanistic insight into biochemical indices, oxidative stress, and inflammation aligns with the results. The wording may be slightly strong; “can have beneficial effects” could be replaced with “may mitigate” for a more cautious tone.
Decision:
Minor revision

Editor-in-Chief

MINOR REVISION 27 Nov 2025

Minor 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.

Manuscript ID: ID 167846
Title: The role of GRP78/ATF6/IRE1 and caspase-12 signaling pathways in the protective effects of n-hexane oil extract of Black soldier flies' larvae (Hermetia illucens) against Aflatoxin-Induced Hepatotoxicity in Rats

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[d] Department of Tissue Engineering and Applied Cell Sciences, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran.

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We sincerely thank the reviewers for their time, insightful comments, and constructive suggestions. Their valuable feedback has significantly improved the quality and clarity of our manuscript. We have carefully considered all points raised and have made the necessary revisions to the manuscript. Our point-by-point responses to the comments are detailed below.

Responses to Reviewer 1

Comment 1.1: The novelty of the study should be added at the end of the introduction.

Response: We thank the reviewer for this excellent suggestion. The following paragraph has been added to the end of the Introduction section to explicitly state the novelty of our work. Please see the revised version. All the corrections appear in yellow bands.

"Therefore, to the best of our knowledge, this is the first study to investigate the protective effects of the n-hexane extract of Black Soldier Fly Larvae against AFB1-induced hepatotoxicity, with a specific focus on elucidating its underlying mechanisms through the modulation of the GRP78/ATF6/IRE1 and caspase-12 signaling pathways, oxidative stress, and inflammation simultaneously."

Comment 1.2: Why was only the n-hexane extract used?

Response: We appreciate the reviewer's question regarding the extraction solvent. The following justification has been added to the 'Materials' subsection (Section 3). Please see the revised version. All the corrections appear in yellow bands.

"The n-hexane solvent was selected for extraction due to its high efficiency in extracting non-polar bioactive compounds, particularly lipids and fat-soluble antioxidants such as tocopherols (Vitamin E), which are abundant in BSFL oil and are known to contribute significantly to its antioxidant properties [5, 6]. This method ensures a high yield of the oil-rich fraction responsible for the purported bioactivity."

Comment 1.3: The number of animals should be listed uniformly throughout the article.

Response: We thank the reviewer for pointing out this inconsistency. The number of animals has been standardized throughout the manuscript. In the abstract, "35" has been changed to "thirty-five" to match the narrative style. In the 'Materials and Methods' section, the number "35" is retained, which is standard for this section. Reference to the total number in the results has been avoided, consistently referring to the group size (n=7) where appropriate. Please see the revised version. All the corrections appear in yellow bands.

Comment 1.4: The Discussion largely repeats results and cites previous studies but does not critically analyze discrepancies or limitations.

Response: We have thoroughly revised the Discussion section to reduce repetition of results and incorporate a critical analysis of our findings. A dedicated paragraph discussing the limitations and a critical appraisal of the results, including analysis of potential discrepancies and future research directions, has been added before the conclusion (Line 428 and 510). A new paragraph about Limitations of the study has been added before the conclusion. Please see the revised version. All the corrections appear in yellow bands.

Line 428: The significant reduction in serum hepatic enzymes by BSFL extract not only confirms its hepatoprotective property but also suggests a potential mechanism shared with lauric acid, its predominant component. While our findings align with the study by Namachivayam et al. on lauric acid [10], it is noteworthy that the whole BSFL extract exhibited effects at doses that deliver a lower equivalent amount of lauric acid. This discrepancy may indicate a synergistic contribution from other bioactive lipids or compounds in the extract, amplifying the protective effect beyond that of lauric acid alone. Future studies isolating individual components are warranted to test this hypothesis.

Line 510: While this study provides compelling evidence for the hepatoprotective effects of BSFL extract, it is important to critically appraise its findings within their context. A key limitation is the use of a whole extract, which, while ecologically relevant, makes it challenging to attribute the observed effects solely to lauric acid despite its predominance. The efficacy of our extract at doses delivering a lower equivalent of lauric acid compared to some pure compound studies [10] suggests possible synergism with other medium-chain fatty acids or minor constituents, a hypothesis requiring validation through compound isolation studies. Furthermore, the focus on a single extraction solvent (n-hexane) may have selected for a specific lipid profile; testing extracts from other solvents could reveal additional bioactive spectra. Finally, while the acute AFB1 model is well-established, the therapeutic potential of BSFL extract needs evaluation in chronic, low-dose exposure scenarios, which more accurately mimic human dietary exposure, and in other species to assess translatability.

Comment 1.5: Abstract should include statistical significance levels for key findings.

Response: We agree with the reviewer. Statistical significance levels ($p<0.05$, $p<0.01$, $p<0.001$) for key findings have been added throughout the abstract. Please see the revised version. All the corrections appear in yellow bands.

Responses to Reviewer 2

Comment 2.1: Novelty could be highlighted more clearly by comparing to previous antioxidant or BSFL studies. If BSFL has not been used in hepatotoxicity models before, this should be emphasized.

Response: We thank the reviewer for reinforcing this point. As detailed in our response to Comment 1.1, the novel aspect of our work—being the first study to use BSFL extract against AFB1-induced hepatotoxicity and to investigate the mentioned mechanisms—is now explicitly stated in the final paragraph of the Introduction. Please see the revised version. All the corrections appear in yellow bands.

Comment 2.2: Sample size (n=7 per group) may be limited; power calculation should be mentioned. Details on randomization, blinding, and extract preparation are missing and would increase methodological rigor. Rationale for BSFL doses should be justified with references or pilot studies

Response: We thank the reviewer for these crucial methodological suggestions. The following changes have been made to the 'Materials and Methods' section. Please see the revised version. All the corrections appear in yellow bands.

- **Rationale for BSFL Doses:** Added to the 'Experimental Design' subsection (Section 4):

  "The selected doses of BSFL extract (180 and 360 mg/kg) were based on previous studies investigating the bioactive effects of BSFL oil and its fatty acid components in rodent models, which demonstrated efficacy and absence of toxicity within this range [10, Namachivayam et al., Heliyon, 2023]."

- **Randomization:** Added to the 'Animals and Housing' subsection (Section 4):

  "Rats were randomly assigned to experimental groups using a random number table to minimize bias."

- **Blinding:** Emphasized in the 'Histopathological examination' subsection (Section 8):

  "Histological evaluation and imaging were performed by an experienced pathologist who was blinded to the experimental groups." Blinding during densitometry analysis of Western blot data was also explicitly mentioned.

- **Extract Preparation:** Details added to the 'Materials' subsection (Section 3):

"The n-hexane extract was prepared using the Soxhlet extraction method. The resulting oil was concentrated under reduced pressure using a rotary evaporator, and the solvent was completely removed. The final extract was stored at -20°C until use."

- **Power Calculation/Sample Size:** Acknowledged in the 'Statistical Analysis' subsection (Section 9) and in the 'Limitations' paragraph of the Discussion:

"A power calculation was not performed a priori; the sample size was determined based on common practices in similar toxicological studies. This is acknowledged as a limitation in the Discussion section."

Comment 2.3: Some sentences are long and complex... Minor punctuation and grammatical issues exist... Abbreviations should be consistent (e.g., IL-6b → IL-6). “Administration of BSFLextract (at a more potent dose…)” is vague.

Response: We appreciate the reviewer's careful attention to language and clarity. The manuscript has been thoroughly edited to address these points:

- Long, complex sentences have been split or rephrased for better readability.
- The entire text has been checked and corrected for punctuation, grammar, and spacing (e.g., "BSFLextract" is now "BSFL extract").
- Abbreviations have been standardized (e.g., "IL-6b" has been corrected to "IL-6" throughout the text).
- Vague phrases like "at a more potent dose" have been replaced with clearer terms such as "at the higher dose of 360 mg/kg" or "in a dose-dependent manner".

Please see the revised version. All the corrections appear in yellow bands.

Comment 2.4: Statistical significance (p-values) is not mentioned; even in an abstract, reporting key p-values is recommended. Statistical methods (ANOVA, post-hoc tests) should be stated briefly.

Response: We thank the reviewer for this suggestion. The following changes have been made. Please see the revised version. All the corrections appear in yellow bands.

- **Abstract:** Key p-values ($p<0.001$) have been added to the results described in the abstract.
- **Abstract:** The following sentence has been added to the 'Methods' part of the abstract:

"Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test, with p<0.05 considered statistically significant."

Comment 2.5: Wording may be slightly strong; “can have beneficial effects” could be replaced with “may mitigate” for a more cautious tone.

Response: We thank the reviewer for this precise suggestion. We fully agree and have adjusted the tone throughout the manuscript to be more cautious and scientific, as recommended.

- In the **Abstract**, the concluding phrase has been changed from *"can have beneficial effects"* to *"***may attenuate biochemical indices, oxidative stress, and inflammation...**"
- In the final **Conclusion** section, the phrasing has been moderated. The statement *"exerts a robust protective effect"* has been revised to **"may confer a protective effect"**, and *"highlight the therapeutic potential"* has been softened to **"point to the therapeutic potential"**.

We believe that the revisions have significantly strengthened the manuscript, and we hope that the changes made are now satisfactory. Thank you again for the opportunity to improve our work.

Sincerely,

The Corresponding Author,
Yalda Arast

2 Revision 1

3 comments · 17 Dec 2025 – 19 Dec 2025

Referee 1 Reviewer

ACCEPT 17 Dec 2025

Accept

Associate Editor

ACCEPT 18 Dec 2025

Dear Authors,
I am glad to inform you that your revised manuscript ID 167846 R1 was accepted by the final reviewer. Please see his positive comment below, indicating that your answers to the reviewers' concerns and your final revision are satisfactory.
Congratulations and kind regards,
Jalal Pourahmad
Associate Editor

Review for 167846 R1
Reviewer Comment:
Accept
Decision:
Accept

Editor-in-Chief

ACCEPT 19 Dec 2025

Accept