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What is "Open Peer Review" process?

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- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

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Jundishapur Journal of Natural Pharmaceutical Products

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Peer Review Report for "Protective Role of Vanillic Acid Against Sodium Arsenite-Induced Cardiotoxicity in Male Mice: A Focus on Antioxidant and Anti-inflammatory Properties"

Author(s): Mohammad Javad Khodayar, Maryam Shirani, Shokooh Mohtadi, Mohammad Amin Behmanesh, Reza Azadnasab, Saeedeh Shariati

Review Timeline:

Submit Date:	22 Sep 2025
Revised Date:	15 Nov 2025
Accepted Date:	16 Nov 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

22 Sep 2025

Dear Editor,

The manuscript titled "Unveiling the Power of Vanillic Acid Against Sodium arsenite - Induced Cardiotoxicity" was reviewed as follows:

- 1- The Introduction should specify the type and tolerable level of human exposure to sodium arsenite.
- 2- Please clarify the rationale for the chosen dosages of compounds in the treatment groups.
- 3- The Discussion mentions a dose-dependent response of VA. Could a VA dosage be

determined that effectively eliminates SA's negative effects without causing side effects?

4- For improved clarity, please consolidate multi-part images (e.g., Image 2). Image 4's description should include A, B, C labels. Figures should use symbols that highlight key results; for instance, Figure 3 should indicate the significant difference between both VA and SA dose groups and the control group.

5- In Figure 5, the difference in damage between the two VA and SA groups is not readily apparent.

REFEREE : Reviewer

5 Oct 2025

This manuscript [Unveiling the Power of Vanillic Acid Against Sodium arsenite - Induced Cardiotoxicity;] has been reviewed for its methodological and statistical aspects.

The study is described as an experimental animal study involving five distinct treatment groups, with an allocation ratio resulting in (n=6) animals per group. While this fits the definition of a controlled laboratory experiment, the manuscript frequently employs terminology traditionally associated with higher-level clinical research (e.g., using phrases suggesting high efficacy or clinical relevance) without the corresponding methodological rigor or justification required for such claims.

- Absence of Blinding Documentation: There is no explicit statement confirming that investigators were blinded during any critical stage of the study, including:
 - o Administration of treatments (if applicable to the blinding schedule).
 - o Data collection (e.g., behavioral observations, if any).
 - o Biochemical assay interpretation (e.g., spectrophotometer readings or ELISA plate analyses).
 - o Histopathological scoring (where subjective interpretation is highly susceptible to experimenter expectation).
- No sample size calculation or a priori power analysis is reported. Ethical guidelines mandate that researchers justify the number of animals used, ensuring that neither an excessive number is used (wasting resources and animal lives) nor too few are used (rendering the study statistically inconclusive, which is also unethical).
- The manuscript provides no evidence or reporting of tests conducted to verify these assumptions for any of the dependent variables analyzed (e.g., serum enzyme levels, protein expression ratios). Standard checks, such as the Shapiro–Wilk test (for normality) and Levene's test or Bartlett's test (for homogeneity), must be performed and reported.
- No adjustment for multiple comparisons (e.g., using the Bonferroni correction, or controlling the False Discovery Rate (FDR) via Benjamini-Hochberg procedure) has been applied or documented. This methodological oversight means that reported significant findings might simply be artifacts of repeated testing.
- Effect sizes (e.g., Cohen's (d) for pairwise comparisons, or (η^2) (eta-squared) for ANOVA results) are mandatory. Effect sizes quantify the magnitude of the difference or relationship, independent of sample size. A small (p)-value with a trivially small effect size is less informative than a moderate (p)-value with a large effect size.
- Figures should not simply display data points or means with error bars. They must

clearly indicate the results of the statistical comparisons performed (e.g., using asterisks or letters above the bars corresponding to significance levels determined by the post-hoc tests). Exact (p)-values or CI bounds should ideally be presented alongside the figure or in accompanying tables.

- A common error is the inconsistent use of error bars. All error bars must be explicitly defined in the figure legend as representing either Standard Deviation (SD) or Standard Error of the Mean (SEM). For describing the data variability of the sample, SD is generally preferred; for inferential statistics presentation, SEM is often used, but consistency is vital.

REFeree : Reviewer

6 Oct 2025

Dear AE,

Thank you very much for giving me the opportunity to review this manuscript.

Here are my comments:

1. The abstract omits critical information required to assess the study's validity. The full manuscript must specify the animal model (e.g., strain of mice), the doses of both SA and VA used, the group design and sample size (n) per group.
2. The results are described qualitatively ("significantly elevated," "notably reduced"). The full manuscript must provide the actual numerical data (mean $\pm$ SD), report the results of statistical tests (e.g., p-values), and specify the number of replicates.
3. The conclusion that VA is a "therapeutic agent" is premature without discussing the dose used relative to a feasible human equivalent intake and without commenting on the pharmacokinetics or potential toxicity of VA itself.
4. As part of the review process, we would like to draw the authors' attention to several recent publications that may be relevant to their study. These references are suggested to help strengthen the manuscript's background, contextualize its findings within the broader literature, and ensure that it reflects the most up-to-date research in the field. These references are provided solely as suggestions, and citing them is not mandatory. The aim is to enhance the academic rigor and comprehensiveness of the manuscript.

These article(s) are as below:

A. High-intensity Interval Training Protectively Modulates Doxorubicin-Induced Changes in Cardiac Mitochondrial Dynamics and Autophagy Process

* Reason: This study is highly relevant as it provides direct mechanistic evidence that a non-pharmacological intervention (HIIT) protects against doxorubicin cardiotoxicity by modulating key cellular processes, offering a strong comparative strategy to your pharmacological approach with vanillic acid. Citing it would powerfully position your research within the broader field of cardioprotection, highlighting that both exercise and antioxidant compounds target similar pathways (oxidative stress, mitochondrial function) to counteract drug-induced cardiac injury.

** Link: <https://brieflands.com/articles/jcrps-159586>

B. Impact of Low Sodium Concentration in Dialysis Fluid on Hemodynamic Parameters in Chronic Hemodialysis Patients

* Reason: This study is highly relevant as it provides direct, clinical trial evidence on a key therapeutic parameter (dialysate sodium) for managing cardiovascular risk in

CKD patients, a population with inherently high oxidative stress. Citing it would ground your research in a critical clinical context, suggesting that your compound's antioxidant effects could complement standard care to address the underlying oxidative damage that contributes to cardiovascular complications in hemodialysis patients.

** Link: <https://brieflands.com/articles/jjcdc-150516>

Final Decision: Major Revision

Kind Regards | Publishing Assistant

REFeree : Reviewer

6 Oct 2025

I believe the core idea is promising and addresses a critical area of cardiovascular health research. The findings regarding Vanillic Acid's (VA) effect on serum biomarkers, oxidative stress, and the NF- κ B/PPAR γ pathways are significant. However, the manuscript currently requires major and comprehensive revisions across all sections, including the title, study design, methods, results presentation, and discussion to meet the necessary scientific and editorial standards for publication.

1. The current title describes the study's aim, while the title should reflect the primary findings. A results-oriented title that highlights the dual protective mechanism (antioxidant/anti-inflammatory) or the key molecular targets (PPAR γ /NF- κ B) can be more impactful and consistent with the main conclusions.
2. The Abstract (and its language throughout the paper) needs a thorough grammatical and editing review to improve clarity and professionalism.
3. The Study Design needs to be critically reviewed as several crucial tests are potentially missing for a comprehensive cardiotoxicity study. (A) (MAJOR) Cardiac function assessment: The study's conclusion of "cardioprotective effects" is only based on serum biomarkers, oxidative stress, inflammation, and histopathology. The methodology lacks any functional cardiac assessment, such as Echocardiography (Echo) or Electrocardiography (ECG). These tests are essential to demonstrate functional recovery, which is a key measure of cardioprotection. (B) Dose-Response of VA: The two doses of VA were only given during the final two weeks of the eight-week SA exposure. This is a treatment protocol, not a prevention protocol, which should be clarified in the design description. Consideration should be given to including a prophylactic group (VA given before and during SA) or a more detailed time-course study. (C) Arsenite-induced cardiotoxicity is often mediated by programmed cell death (apoptosis). Measuring the activity of the key executioner caspase (Caspase-3) in cardiac tissue homogenates is a direct way to quantify the degree of apoptosis. A Western blot or qPCR for the Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) proteins, and calculating the Bax/Bcl-2 ratio, would provide molecular evidence of VA's ability to shift the heart toward cell survival. The current Western blot only focused on PPAR γ and NF- κ B. (D) Measurement of Myeloperoxidase (MPO) Activity: MPO is a specific enzyme released by activated neutrophils and is a strong indicator of inflammation and tissue damage. This would strengthen the finding that VA reduces inflammation.

4. The presentation of each step in the Materials and Methods section requires greater detail and standardization. (A) Animal Protocols: All procedures must be detailed. For the SA exposure (50 ppm in drinking water), the study should confirm the volume of water consumed daily to estimate the daily SA intake (body weight) for better reproducibility and comparison with other studies. (B) Western Blot Details: While the Western Blot procedure is generally described, essential details are missing, such as the catalog numbers and suppliers of the primary antibodies (This is an issue in all other parts of the article) and the dilution ratios used. (C) Histology Procedure: While the fixation, embedding, and staining are mentioned, the specific criteria used for the Pathological analysis must be clearly defined (e.g., a scoring system for inflammation, necrosis, or myofibrillar disarray) to add quantitative rigor to the qualitative findings.
5. The Results section is presented clearly with statistical data, but the Histopathological figures are "not good" and the following improvements are critical: A- Instead of relying only on a descriptive account ("SA-treated mice suffered significant damage, and VA-treated mice exhibited partial recovery"), the results must include a quantitative assessment of the histopathology. This is typically done with a blinded scoring system (e.g., a five-point scale for edema, necrosis, or inflammation) to provide objective data. B- There is no scale bar on the HP figures. C- The figure legends are not informative and contain basic introduction. More details are needed.
6. The discussion is currently basic and relies heavily on summarizing the literature that supports the findings. A major revision needs to elevate the quality of the discussion by: (A) The discussion should discuss the direct molecular connection between Vanillic Acid and specific pathways. How might VA be directly regulating the expression of certain transcription factors, and how does this, in turn, reduce NF-kB and the inflammatory response? (B) The discussion should clearly articulate the novelty of the current study in the context of existing literature. It must discuss what this paper contributes beyond what is already known from other studies on VA's cardioprotection (e.g., against doxorubicin or isoproterenol). (C) Translational Significance: The discussion needs to move beyond mice and touch on the potential clinical relevance of these findings, addressing questions about safe and effective human dosages or sources of Vanillic Acid.

REFEREE : Associate Editor

7 Oct 2025

The Ms needs some major revision before suitable for publication in JJNPP

REFEREE : EIC

7 Oct 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

Thank You for Your Consideration

Thank you for your consideration and attention in reviewing our manuscript titled “**Protective role of Vanillic Acid Against Sodium Arsenite-Induced Cardiotoxicity in Male Mice: A Focus on Antioxidant and Anti-Inflammatory Properties**” We appreciate the time and effort you and the reviewers have dedicated to this process.

If there are any further questions or if additional information is required, please do not hesitate to reach out.

Thank you once again for your support.

Best regards,

Maryam Shirani

Toxicology Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Email: mshirani86@yahoo.com

Reviewer 1:
This manuscript [Unveiling the Power of Vanillic Acid Against Sodium arsenite -Induced Cardiotoxicity;] has been reviewed for its methodological and statistical aspects. The study is described as an experimental animal study involving five distinct treatment groups, with an allocation ratio resulting in (n=6) animals per group. While this fits the definition of a controlled laboratory experiment, the manuscript frequently employs terminology traditionally associated with higher-level clinical research (e.g., using phrases suggesting high efficacy or clinical relevance) without the corresponding methodological rigor or justification required for such claims.

Response: Thanks for your valuable feedback about our use of clinical terminology, we appreciate the opportunity to clarify our intentions. While we used terms like "high efficacy" and "clinical relevance," our aim was to emphasize the potential implications of our findings rather than to claim immediate applicability to clinical settings. Our study was carefully designed with appropriate methodological rigor for an experimental animal study, using six animals per treatment group, which is a standard practice for preliminary research. We conducted thorough statistical analyses to ensure the reliability of our results and acknowledge the inherent limitations of animal studies in translating findings to humans. Ultimately, we hope that our research serves as a valuable foundation for future studies that can explore the therapeutic potential of vanillic acid, paving the way for subsequent clinical investigations.

- Absence of Blinding Documentation: There is no explicit statement confirming that investigators were blinded during any critical stage of the study, including:
 - o Administration of treatments (if applicable to the blinding schedule).
 - o Data collection (e.g., behavioral observations, if any).
 - o Biochemical assay interpretation (e.g., spectrophotometer readings or ELISA plate analyses).
 - o Histopathological scoring (where subjective interpretation is highly susceptible to experimenter expectation).

Response: Thanks for your valuable comment. In response to the concern regarding the absence of blinding documentation in our study, we would like to clarify that while explicit statements regarding blinding were not included in the manuscript, we implemented rigorous protocols to minimize bias throughout the research process. Specifically, the administration of treatments and collection of data were conducted by trained personnel who were not involved in the subsequent analysis of the biochemical assays. This separation of duties was designed to reduce the potential for bias in interpreting results. We appreciate the reviewer's feedback and will incorporate clearer documentation of these procedures in the revised manuscript to enhance transparency and rigor in our methodology.

- No sample size calculation or a priori power analysis is reported. Ethical guidelines mandate that researchers justify the number of animals used, ensuring that neither an excessive number is used (wasting resources and animal lives) nor too few are used (rendering the study statistically inconclusive, which is also unethical).

Response: Thank you for your valuable feedback. In response to the reviewer's concern regarding the lack of sample size calculation or a priori power analysis, we acknowledge the importance of these considerations in ensuring ethical research practices. The number of animals used in our study was determined based on previous studies (1, 2). We aimed to use a sufficient number of subjects to achieve statistically significant results while adhering to ethical guidelines to avoid unnecessary use of animals. In our revised manuscript, we will provide a more detailed justification for our sample size selection, referencing relevant literature to support our approach.

- The manuscript provides no evidence or reporting of tests conducted to verify these assumptions for any of the dependent variables analyzed (e.g., serum enzyme levels, protein expression ratios). Standard checks, such as the Shapiro–Wilk test (for normality) and Levene’s test or Bartlett’s test (for homogeneity), must be performed and reported.

Response: Thank you for your valuable feedback. Most of the tests were conducted following the protocols established by the respective companies. To avoid redundancy and reduce the similarity index of the manuscript, we have summarized these methods briefly. Additionally, where tests were performed according to manual guidelines, we have provided relevant references to support our methodology. This approach ensures that we maintain a high standard of scientific rigor while also adhering to ethical considerations regarding the presentation of data. We appreciate your understanding and will ensure that our manuscript clearly reflects these practices.

Prism 9 automatically performs Bartlett's test for the homogeneity of variances, alongside the Brown-Forsythe test, when we run a one-way ANOVA. This test checks if the variances of our groups are equal, which is an assumption for ANOVA. We don't need to manually run it; Prism runs it by default when we have at least five values per group and reports the results, which include a P value. Shapiro–Wilk test for normality was checked.

- No adjustment for multiple comparisons (e.g., using the Bonferroni correction, or controlling the False Discovery Rate (FDR) via Benjamini-Hochberg procedure) has been applied or documented. This methodological oversight means that reported significant findings might simply be artifacts of repeated testing.

Response: Thanks for your comment. For the multiple comparisons, the Tukey post hoc test was recommended, which we performed as mentioned in the text.

- Effect sizes (e.g., Cohen's (d) for pairwise comparisons, or (η^2) (eta-squared) for ANOVA results) are mandatory. Effect sizes quantify the magnitude of the difference or relationship, independent of sample size. A small (p)-value with a trivially small effect size is less informative than a moderate (p)-value with a large effect size.

Response: Thanks for your comment. The Eta squared test was also conducted for effect size on the ANOVA data.

- Figures should not simply display data points or means with error bars. They must clearly indicate the results of the statistical comparisons performed (e.g., using asterisks or letters above the bars corresponding to significance levels determined by the post-hoc tests). Exact (p)-values or CI bounds should ideally be presented alongside the figure or in accompanying tables.

Response: With respect to the opinion of the respected referee, the graphs include comparisons that are marked with a hashtag and a star, and the values and comparisons are clearly stated below the figure.

- A common error is the inconsistent use of error bars. All error bars must be explicitly defined in the figure legend as representing either Standard Deviation (SD) or Standard Error of the Mean (SEM). For describing the data variability of the sample, SD is generally preferred; for inferential statistics presentation, SEM is often used, but consistency is vital.

Response: Thanks for your comment. In response to your request, we have standardized the error bars in our figures to represent Standard Deviation (SD). All figure legends have been updated accordingly to clarify that SD is used to depict data variability. This change ensures consistency throughout our presentation of the results.

Reviewer 2:

I believe the core idea is promising and addresses a critical area of cardiovascular health research. The findings regarding Vanillic Acid's (VA) effect on serum biomarkers, oxidative stress, and the NF-kB/PPAR gamma pathways are significant. However, the manuscript currently requires major and comprehensive revisions across all sections, including the title, study design, methods, results presentation, and discussion to meet the necessary scientific and editorial standards for publication.

1. The current title describes the study's aim, while the title should reflect the primary findings. A results-oriented title that highlights the dual protective mechanism (antioxidant/anti-inflammatory) or the key molecular targets (PPAR gamma/NF- κ B) can be more impactful and consistent with the main conclusions.

Response: Thanks for your valuable comment, it was revised.

2. The Abstract (and its language throughout the paper) needs a thorough grammatical and editing review to improve clarity and professionalism.

Response: Thanks for your comment, it was revised.

3. The Study Design needs to be critically reviewed as several crucial tests are potentially missing for a comprehensive cardiotoxicity study. (A) (MAJOR) Cardiac function assessment: The study's conclusion of "cardioprotective effects" is only based on serum biomarkers, oxidative stress, inflammation, and histopathology. The methodology lacks any functional cardiac assessment, such as Echocardiography (Echo) or Electrocardiography (ECG). These tests are essential to demonstrate functional recovery, which is a key measure of cardioprotection. (B) Dose-Response of VA: The two doses of VA were only given during the final two weeks of the eight-week SA exposure. This is a treatment protocol, not a prevention protocol, which should be clarified in the design description. Consideration should be given to including a prophylactic group (VA given before and during SA) or a more detailed time-course study. (C) Arsenite-induced cardiotoxicity is often mediated by programmed cell death (apoptosis). Measuring the activity of the key executioner caspase (Caspase-3) in cardiac tissue homogenates is a direct way to quantify the degree of apoptosis. A Western blot or qPCR for the Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) proteins, and calculating the Bax/Bcl-2 ratio, would provide molecular evidence of VA's ability to shift the heart toward cell survival. The current Western blot only focused on PPAR γ and NF- κ B. (D) Measurement of Myeloperoxidase (MPO) Activity: MPO is a specific enzyme released by activated neutrophils and is a strong indicator of inflammation and tissue damage. This would strengthen the finding that VA reduces inflammation.

4. The presentation of each step in the Materials and Methods section requires greater detail and standardization. (A) Animal Protocols: All procedures must be detailed. For the SA exposure (50 ppm in drinking water), the study should confirm the volume of water consumed daily to estimate the daily SA intake (body weight) for better reproducibility and comparison with other studies. (B) Western Blot Details: While the Western Blot procedure is generally described, essential details are missing, such as the catalog numbers and suppliers of the primary antibodies (This is an issue in all other parts of the article) and the dilution ratios used. (C) Histology Procedure: While the fixation, embedding, and staining are mentioned, the specific criteria used for the Pathological analysis must be clearly defined (e.g., a scoring system

for inflammation, necrosis, or myofibrillar disarray) to add quantitative rigor to the qualitative findings.

Response: Thank you for your valuable feedback regarding our study on the cardioprotective effects of vanillic acid (VA) against sodium arsenite (SA)-induced cardiotoxicity. We acknowledge that the absence of functional cardiac assessments, such as Echocardiography (Echo) and Electrocardiography (ECG), limits our conclusions about cardioprotection. Due to constraints in resources and time, we were unable to implement these essential evaluations in our methodology. We recognize the importance of these assessments for a comprehensive understanding of cardiac function and recovery and plan to address this limitation in future research. Your insights are greatly appreciated and will guide our efforts to enhance the rigor of our studies going forward.

In response to your critique regarding the dose-response protocol and the design of our study on the protective effects of vanillic acid (VA) against sodium arsenite (SA)-induced cardiotoxicity, I want to emphasize that the two doses of VA were administered only during the final two weeks of the eight-week SA exposure. This protocol was designed as a treatment protocol to evaluate the therapeutic effects of VA after the onset of SA exposure, reflecting real-world scenarios where intervention occurs post-exposure to toxic substances. However, we acknowledge the value of your suggestion to include a prophylactic group, where VA could be administered before and during SA exposure. This approach could be explored in future studies to better understand the efficacy of VA in both preventive and therapeutic contexts.

Due to the low quality of the images, the research team decided to remove the photos from the manuscript.

5. The Results section is presented clearly with statistical data, but the Histopathological figures are "not good" and the following improvements are critical: A- Instead of relying only on a descriptive account ("SA-treated mice suffered significant damage, and VA-treated mice exhibited partial recovery"), the results must include a quantitative assessment of the histopathology. This is typically done with a blinded scoring system (e.g., a five-point scale for edema, necrosis, or inflammation) to provide objective data. B- There is no scale bar on the HP figures. C- The figure legends are not informative and contain basic introduction. More details are needed.

Response: Thanks for the reviewer's comment. Due to the low quality of the images, the research team decided to remove the photos from the manuscript.

6. The discussion is currently basic and relies heavily on summarizing the literature that supports the findings. A major revision needs to elevate the quality of the discussion by: (A) The discussion should discuss the direct molecular connection between Vanillic Acid and specific pathways. How might VA be directly regulating the expression of certain transcription factors, and how does this, in turn, reduce NF- κ B and the inflammatory response? (B) The discussion should clearly articulate the novelty of the current study in the context of existing literature. It must discuss what this paper contributes beyond what is already known from other studies on VA's cardioprotection (e.g., against doxorubicin or isoproterenol). (C) Translational Significance: The discussion needs to move beyond mice and touch on the potential clinical relevance of these findings, addressing questions about safe and effective human dosages or sources of Vanillic Acid.

Response: Thanks for the reviewer's comment. VA may regulate the expression of certain transcription factors such as PPAR γ and NF- κ B. By activating PPAR γ , VA can inhibit the NF- κ B pathway, which is crucial in the inflammatory response. This inhibition leads to a decrease in the expression of pro-inflammatory cytokines, thereby reducing inflammation. Understanding these mechanisms can provide insights into how VA exerts its cardioprotective effects at the molecular level.

The present study contributes novel insights into the cardioprotective effects of VA specifically against SA-induced cardiac damage. While previous research has established VA's protective role against cardiotoxic agents like doxorubicin and isoproterenol, this study uniquely focuses on the mechanism by which VA mitigates oxidative stress and inflammation specifically induced by SA. The findings highlight VA's ability to activate PPAR γ , leading to the suppression of NF- κ B signaling, which is a pathway not extensively explored in the context of SA-induced toxicity. This

novel angle not only enhances our understanding of VA's multifaceted role but also underscores its potential as a therapeutic agent in cases of environmental SA-induced cardiac injury.

The current study presents promising findings regarding the cardioprotective effects of VA against SA-induced cardiac damage; however, it is crucial to acknowledge its limitations, particularly in terms of clinical applicability. The research primarily utilizes rodent models, which may not accurately reflect human physiological responses. Future research must prioritize clinical trials to validate these findings in human populations, establish appropriate dosages, and assess long-term safety and efficacy to ensure that the benefits of VA can be effectively translated into clinical practice. Limitation text was also added to the discussion section.

Reviewer 3:

Dear AE,

Thank you very much for giving me the opportunity to review this manuscript.

Here are my comments:

1. The abstract omits critical information required to assess the study's validity. The full manuscript must specify the animal model (e.g., strain of mice), the doses of both SA and VA used, the group design and sample size (n) per group.

Response: thanks for your valuable comment it was revised.

2. The results are described qualitatively ("significantly elevated," "notably reduced"). The full manuscript must provide the actual numerical data (mean $\pm$ SD), report the results of statistical tests (e.g., p-values), and specify the number of replicates.

Response: thanks for your valuable comment it was revised.

3. The conclusion that VA is a "therapeutic agent" is premature without discussing the dose used relative to a feasible human equivalent intake and without commenting on the pharmacokinetics or potential toxicity of VA itself.

Response: The current study presents promising findings regarding the cardioprotective effects of VA against SA-induced cardiac damage; however, it is crucial to acknowledge its limitations, particularly in terms of clinical applicability. The research primarily utilizes rodent models, which may not accurately reflect human physiological responses. Future research must prioritize clinical trials to validate these findings in human populations, establish appropriate dosages, and assess long-term safety and efficacy to ensure that the benefits of VA can be effectively translated into clinical practice. Limitation text was also added to the discussion section.

4. As part of the review process, we would like to draw the authors' attention to several recent publications that may be relevant to their study. These references are suggested to help strengthen the manuscript's background, contextualize its findings within the broader literature, and ensure that it reflects the most up-to-date research in the field. These references are provided solely as suggestions, and citing them is not mandatory. The aim is to enhance the academic rigor and comprehensiveness of the manuscript.

These article(s) are as below:

A. High-intensity Interval Training Protectively Modulates Doxorubicin-Induced Changes in Cardiac Mitochondrial Dynamics and Autophagy Process

* Reason: This study is highly relevant as it provides direct mechanistic evidence that a non-pharmacological intervention (HIIT) protects against doxorubicin cardiotoxicity by modulating key cellular processes, offering a strong comparative strategy to your pharmacological approach with vanillic acid. Citing it would powerfully position your research within the broader field of cardioprotection, highlighting that both exercise and antioxidant compounds target similar pathways (oxidative stress, mitochondrial function) to counteract drug-induced cardiac injury.

** Link: <https://brieflands.com/articles/jcrps-159586>

B. Impact of Low Sodium Concentration in Dialysis Fluid on Hemodynamic Parameters in Chronic Hemodialysis Patients

* Reason: This study is highly relevant as it provides direct, clinical trial evidence on a key therapeutic parameter (dialysate sodium) for managing cardiovascular risk in CKD patients, a population with inherently high oxidative stress. Citing it would ground your research in a critical clinical context, suggesting that your compound's antioxidant effects could complement standard care to address the underlying oxidative damage that contributes to cardiovascular complications in hemodialysis patients.

** Link: <https://brieflands.com/articles/jjcdc-150516>

Response: I appreciate your valuable suggestions. I apologize for not including references in the manuscript due to the journal's restrictions on the number of citations.

Final Decision: Major Revision

Kind Regards | Publishing Assistant

Reviewer 5:

Dear Editor,

The manuscript titled "Unveiling the Power of Vanillic Acid Against Sodium arsenite -Induced Cardiotoxicity" was reviewed as follows:

1- The Introduction should specify the type and tolerable level of human exposure to sodium arsenite.

Response: Thanks for your attention. In 2017, the World Health Organization (WHO), through the Joint FAO/WHO Expert Committee on Food Additives (JECFA), established a provisional

drinking water guideline for inorganic sodium arsenite set at 10 µg/L.

2- Please clarify the rationale for the chosen dosages of compounds in the treatment groups.

Response: Thanks for your comment, the dosage references were added.

3- The Discussion mentions a dose-dependent response of VA. Could a VA dosage be determined that effectively eliminates SA's negative effects without causing side effects?

Response: Thanks for your comment. Determining an appropriate dosage of VA that effectively mitigates the negative effects of SA without causing side effects is a complex challenge. Future research should focus on this issue, as it represents a significant limitation of current studies. Additionally, conducting long-term studies may provide insights into the sustained effects of VA over time. This area of research requires further exploration to reach definitive and reliable conclusions.

4- For improved clarity, please consolidate multi-part images (e.g., Image 2). Image 4's description should include A, B, C labels. Figures should use symbols that highlight key results; for instance, Figure 3 should indicate the significant difference between both VA and SA dose groups and the control group.

Response: Thanks for your attention. All figures are labeled. Figure 3 is just a single image and does not require labeling. Figure 4 caption has been modified. According to the study, the results of the SA group were compared with the control group, and the treatment groups were also compared with SA. If the mentioned comparisons were not significantly different, they will not be marked.

5- In Figure 5, the difference in damage between the two VA and SA groups is not readily apparent.

Response: Thanks for the reviewer's comment. Due to the low quality of the images, the research team decided to remove the photos from the manuscript.

References

1. Song L, Srilakshmi M, Wu Y, Saleem TM. Sulforaphane Attenuates Isoproterenol-Induced Myocardial Injury in Mice. *BioMed Research International*. 2020;2020(1):3610285.
2. Obeidat HM, Althunibat OY, Alfwuaires MA, Aladaileh SH, Algefare AI, Almuqati AF, et al. Cardioprotective effect of taxifolin against isoproterenol-

induced cardiac injury through decreasing oxidative stress, inflammation, and cell death, and activating Nrf2/HO-1 in mice. *Biomolecules*. 2022;12(11):1546.

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer

9 Nov 2025

1. Please check the entire manuscript grammatically, especially for punctuation.
2. In the introduction after reference 19, please mention the potential of vallinic acid as an antioxidant and mention previous studies that showed that this agent had antioxidant activity. Then, they explained why this study should be conducted and what makes the current work novel.
3. Please combine the cardiac oxidative stress and cytokine together. Also, please add more detail about the methods of assay, for example, the ELISA reader used or the spectrophotometer?
4. What is the positive antioxidant control to compare the vanillic acid antioxidant potential in this article? Please see this article "Cardioprotective Effects of Fisetin Against Sodium Arsenite-Induced Toxicity in Experimental Rats: Roles of the Nrf2/HO-1 and Bax/Bcl-2/Caspase-3 Pathways"
5. Why do authors not use Real-time to precisely show the alteration in RNA expression and then validate by western blot, as the western blot is not a quantitative method? Please add the plot of quantification of the western blot in Figure 4.
6. Please compare the VA antioxidant potential with other antioxidants, such as those in this article "Cardioprotective Effects of Fisetin Against Sodium Arsenite-Induced Toxicity in Experimental Rats: Roles of the Nrf2/HO-1 and Bax/Bcl-2/Caspase-3 Pathways" in the discussion.

REFEREE : Associate Editor

12 Nov 2025

The Ms still needs some minor revisions before suitable for publication in JJNPP

REFEREE : EIC

12 Nov 2025

Our impression is that the above-mentioned manuscript could become suitable for publication in JJNPP. However, the criticism from the referees requires that we defer a final decision until you have considered their remarks and revised your manuscript accordingly.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear editor in chief

Thank you for your consideration and attention in reviewing our manuscript titled “**Protective role of Vanillic Acid Against Sodium Arsenite-Induced Cardiotoxicity in Male Mice: A Focus on Antioxidant and Anti-Inflammatory Properties**” We appreciate the time and effort you and the reviewers have dedicated to this process.

If there are any further questions or if additional information is required, please do not hesitate to reach out.

Thank you once again for your support.

Best regards,

Saeedeh Shariati

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Email: saeedeh.shariati@yahoo.com

Reviewer 1

1. Please check the entire manuscript grammatically, especially for punctuation.

Response: Thanks for your comment, the issues were revised in manuscript.

2. In the introduction after reference 19, please mention the potential of vanillic acid as an antioxidant and mention previous studies that showed that this agent had antioxidant activity. Then, they explained why this study should be conducted and what makes the current work novel.

Response: Thanks for your comment, the issues were revised in manuscript.

3. Please combine the cardiac oxidative stress and cytokine together. Also, please add more detail about the methods of assay, for example, the ELISA reader used or the spectrophotometer?

Response: Thanks for your comment, for better understanding, it is better not to merge the contents. the issues were revised in manuscript.

4. What is the positive antioxidant control to compare the vanillic acid antioxidant potential in this article? Please see this article “Cardioprotective Effects of Fisetin Against Sodium Arsenite-

Induced Toxicity in Experimental Rats: Roles of the Nrf2/HO-1 and Bax/Bcl-2/Caspase-3 Pathways”

Response: Thanks for your consideration, in most toxicology studies, the study design is to compare the control group with the toxic group and also compare the treatment groups with the toxic group. Also, a vanillic acid group is tested as a single group to ensure that vanillic acid does not have negative effects on the study. Therefore, the study was designed by the research team based on toxicology knowledge, resources, and the number of animals available.

5. Why do authors not use Real-time to precisely show the alteration in RNA expression and then validate by western blot, as the western blot is not a quantitative method? Please add the plot of quantification of the western blot in Figure 4.

Response: Thank you for your insightful question regarding the choice of methods for assessing RNA expression levels. After careful consideration of our budget constraints, we determined that focusing on protein quantification through western blotting was the most practical approach for our study. While Real-time PCR is a valuable tool for quantifying RNA, in this context, validating protein expression provides more immediate insights into biological relevance. Western blotting allows us to confirm the presence and size of proteins, as well as any post-translational modifications, which are crucial for understanding the functional consequences of gene expression. Additionally, we believe that the integration of these techniques enhances the robustness of our findings. Regarding your request for quantification of the western blot in Figure 4, we appreciate your feedback and will consider including this data to strengthen our analysis; quantitative values along with western blot images have been included in the manuscript in the form of a figure. Thank you again for your thoughtful input.

6. Please compare the VA antioxidant potential with other antioxidants, such as those in this article ”Cardioprotective Effects of Fisetin Against Sodium Arsenite-Induced Toxicity in Experimental Rats: Roles of the Nrf2/HO-1 and Bax/Bcl-2/Caspase-3 Pathways” in the discussion.

Reponses: With respect to the opinion of the reviewer, we are sorry to compare the mentioned study with the present study due to the lack of credibility of the journal of the mentioned article. The article in question was published in the “International Journal of Pharmacology”. The journal mentioned is also on the Ministry of Health's blacklist. However, this journal is not recognized as credible by the authoritative website of the Ministry of Health of Iran (NOPA) and lacks presence in reputable indexing services. Given that the journal's policies emphasize the importance of citing credible sources in its manuscripts, the aforementioned article cannot be utilized in the current manuscript. This raises significant concerns regarding the reliability of the findings presented in the article. In academic and clinical research, the credibility of the journals from which we draw references is paramount. Journals that are not indexed in recognized databases or do not meet the standards set by authoritative health organizations may compromise the integrity of the research. Therefore, it is essential to rely on sources that have been validated by established institutions to ensure that our work is built on a solid foundation of trustworthy evidence.

OPEN PEER REVIEW

Revision (2)

REFEREE : EIC

16 Nov 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.