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OPEN PEER REVIEW

Selegiline's Protective Role Against Elastase-Induced Oxidative Stress in A549 Cells via NRF2/Keap1 Pathway

Parisa Rashidi, Mohammad Ali Khaksar, Narges Atefipour, Maryam Radan, Fereshteh Nejaddehbashi, Khojasteh Hoseinynejad, Zahra Mansouri

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About this report

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1 Original submission

2 comments · 21 Mar 2025 – 21 Mar 2025

Soheil Abbaspour-Ravasjani Reviewer

MAJOR REVISION 21 Mar 2025

Abstract

1. Revise COPD Description: Replace "characterized by oxidative stress and inflammation followed by lung tissue" with "characterized by oxidative stress, inflammation, and progressive lung tissue damage" for a more accurate and concise depiction of COPD pathology.
2. Detail Elastase's Contribution: Clarify elastase's role by revising "Elastase contributes to COPD progression by increasing reactive oxygen species (ROS) production and degrading elastin" to "Elastase, a protease, exacerbates COPD by degrading elastin and increasing ROS production."
3. Sharpen Study Objectives: Refine "This study aims to investigate its effects on lung cells, focusing on reducing oxidative stress through the NRF2/Keap1 pathway" to "This study investigates selegiline's ability to reduce oxidative stress in lung cells via the NRF2/Keap1 pathway" for a clearer statement of purpose.
4. Improve Results Precision: Revise "This study found that elastase-induced oxidative stress through ROS production, followed by lipid peroxidation, suppressed anti-oxidant activity" to "Elastase increased ROS and lipid peroxidation while suppressing antioxidant activity via the NRF2/Keap1 pathway. Selegiline reduced oxidative stress and improved NRF2/Keap1 gene expression" for specificity and flow.
5. Strengthen Conclusion: Enhance "demonstrate the ability of selegiline as an MAO-B inhibitor to reduce oxidative stress and increase cell viability" to "Selegiline mitigates elastase-induced damage, suggesting its potential as a therapeutic agent for COPD" to highlight clinical relevance.

Introduction

6. Broaden COPD Context: Expand "Chronic obstructive pulmonary disease (COPD) is a leading global health concern, characterized by conditions such as emphysema, chronic bronchitis, and small airway disease" to "COPD encompasses emphysema and chronic bronchitis, driven by inflammation, oxidative stress, and protease-antiprotease imbalance" for a comprehensive overview.
7. Define Oxidative Stress Role: Clarify "When the balance between oxidation and the antioxidant defense system is disrupted" to "Oxidative stress, an imbalance favoring ROS production, damages cellular components and impairs lung function" for precision.
8. Explain NRF2/Keap1 Function: Revise "The Keap1-NRF2 pathway regulates cellular protection genes" to "The NRF2/Keap1 pathway regulates antioxidant gene expression, with NRF2 activation promoting cytoprotection" to detail its mechanism.
9. Correct Selegiline's Mechanism: Fix "selegiline enhances antioxidant defenses by decreasing the activity of superoxide dismutase (SOD) and CAT" to "Selegiline, an MAO-B inhibitor used in Parkinson's disease, exhibits antioxidant properties by enhancing SOD and CAT activity" as the original statement contradicts known effects.
10. State Hypothesis Clearly: Refine "this study aims to investigate selegiline's protective role" to "We hypothesized that selegiline reduces elastase-induced oxidative damage in lung cells by modulating the NRF2/Keap1 pathway" for a direct hypothesis statement.

Methods

11. Detail Cell Culture Conditions: Expand "cultivated human lung epithelial A549 cells in DMEM with 10% FBS and antibiotics" to "A549 cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO_2" for reproducibility.
12. Correct MTT Assay Timing: Adjust "MTT analysis was performed 24, 48, and 72 hours after adding the exosomes" to "MTT assay was performed at 24 hours post-treatment to assess cell viability" as the original

mentions irrelevant time points and exosomes.
13. Specify ROS Methodology: Revise "Cells were treated with DCFH-DA at 37°C for 30 minutes" to "ROS levels were measured using DCFH-DA and flow cytometry after 24-hour treatment" for methodological accuracy.
14. Clarify Oxidative Stress Assays: Refine "the level of CAT and MDA were evaluated in all experimental groups using specific assay kits" to "MDA and CAT levels were quantified in cell supernatants using spectrophotometric assays" for specificity.
15. Detail Gene Expression Process: Expand "RNA was quantified in cell lysates using the RNeasy Plus Mini Kit" to "RNA was extracted, reverse-transcribed, and NRF2/Keap1 expression was analyzed via qRT-PCR" for a complete description.

Results
16. Detail Oxidative Stress Markers: Revise "A substantial rise in the level of MDA was evaluated" to "MDA increased 3-fold with elastase ($p < 0.001$), and CAT decreased by 50% ($p < 0.001$). Selegiline reversed these effects dose-dependently" for quantitative clarity.

Discussion
17. Connect to COPD Pathology: Revise "Porcine pancreatic elastase is a specific type of protease" to "Elastase-induced oxidative stress mirrors COPD pathology, where protease imbalance and ROS drive lung damage" to link findings to disease context.
18. Elaborate Selegiline's Mechanism: Adjust "selegiline significantly reduced the production of reactive oxygen species" to "Selegiline's antioxidant effects likely stem from MAO-B inhibition and direct ROS scavenging, enhancing cellular defenses" for mechanistic depth.
19. Clarify Pathway Modulation: Refine "resulted in a reduction of NRF2 gene expression while simultaneously increasing the expression levels of the KEAP1 gene" to "By upregulating NRF2 and downregulating Keap1, selegiline promotes antioxidant gene expression, counteracting oxidative stress" for clarity.
20. Suggest Clinical Implications: Enhance "The findings underscore the therapeutic potential of selegiline" to "These findings support selegiline's potential as an adjunct therapy in COPD to mitigate oxidative damage" for practical relevance.

Conclusion
21. Summarize Findings Concisely: Revise "selegiline, an MAO-B inhibitor, can mitigate oxidative stress and enhance cell viability" to "Selegiline reduced elastase-induced oxidative stress and improved cell viability via NRF2/Keap1 modulation" for a succinct summary.
22. Emphasize Significance: Strengthen "The findings underscore the therapeutic potential of selegiline" to "This study suggests selegiline could be repurposed for COPD treatment, warranting further research" to highlight future directions.

General Comments
23. Standardize Pathway Notation: Consistently use "NRF2/Keap1" throughout instead of variations like "NRF2/keap1" or "Keap1-NRF2" for uniformity.
24. Fix Grammatical Errors: Correct phrases like "followed by lung tissue" to "in lung tissue" and "markedly disparity" to "marked difference" for proper grammar.
25. Enhance Figure Legends: Revise "Figure 1. Effect of Selegiline on Cell viability in groups" to "Figure 1: Cell viability in A549 cells after 24-hour treatment with elastase and selegiline (mean ± SEM, n=3)" for detailed, informative captions.
26. Validate References: Ensure all cited studies (e.g., Hou et al., Bayati et al.) align with claims and are formatted per journal guidelines.
27. Clarify Ethical Statements: Retain "Ethical Code: IR.AJUMS.REC.1402.652" and add a note if no human/animal data were used, e.g., "This study used only cell lines, requiring no additional ethical approvals beyond institutional standards."

Here are useful references for the authors' observation:
https://apb.tbzmed.ac.ir/Article/apb-39158
https://www.sciencedirect.com/science/article/pii/S0378517322005828

Referee 1 Reviewer

MAJOR REVISION 21 Mar 2025

Dear Editor
Have a nice day.
The author investigated “Selegiline and Pulmonary Protection: A novel approach to mitigate elastase-induced cell toxicity through the Nrf2/Keap1 pathway”.
I would suggest reconsidering the manuscript for publication only after the following major revisions.
1. Mention the quantitative results obtained in the abstract.
2. The title needs changes.
3. In the background section, explain more about the importance and novelty of the title.
4. The sources of all materials used must be specified.

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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.

We sincerely appreciate the time and effort you and the esteemed reviewers have devoted to evaluating our manuscript, ***Protective Effects of Selegiline Against Elastase-Induced Oxidative Stress in Lung Epithelial Cells Through Modulation of the NRF2/Keap1 Signaling Pathway***. We are grateful for the insightful and constructive comments, which have significantly improved the clarity and scientific quality of our work. Below, we provide point-by-point responses to each reviewer's comments. All changes made in the revised manuscript are highlighted for clarity.

Reviewer 1

1. Quantitative results added to the abstract.
2. Title revised for clarity and relevance. *(Protective Effects of Selegiline Against Elastase-Induced Oxidative Stress in Lung Epithelial Cells Through Modulation of the NRF2/Keap1 Signaling Pathway)*
3. Background expanded to highlight novelty.
4. Source of materials specified in the Methods section.

Reviewer 2

1. Language polished using editing software and expert review.
2. COPD description revised as suggested.
3. Rationale for using selegiline added to the abstract.
4. Number of replicates and independent experiments specified.
5. NRF2/Keap1 capitalization standardized throughout.
6. Antioxidant enzyme activity description corrected.
7. Elastase's role in ROS production clarified.
8. Reference for elastase-induced ROS added.
9. H_2O_2 treatment description clarified.
10. The MTT assay protocol was completed with seeding and volume details.
11. Irrelevant exosome mention removed.
12. Oxidative stress assay kits specified.
13. Gene expression methodology is detailed.
14. Grammar errors in Results corrected.
15. Concentration units standardized to µM.
16. High-dose selegiline effects explained.
17. Results compared with previous studies.
18. Study limitations acknowledged.

Reviewer 3

Abstract Revisions:

1. COPD description revised as suggested.
2. Elastase's role rephrased for clarity.
3. Study objective clarified.
4. Results restated with more specificity.
5. Conclusion enhanced to highlight therapeutic potential.

Introduction Improvements:

6. Broader COPD context provided.
7. Oxidative stress definition clarified.
8. NRF2/Keap1 pathway description improved.
9. Selegiline's mechanism of action is corrected.
10. Hypothesis stated explicitly.

Methods Section:

11. Cell culture conditions expanded.
12. MTT assay timing corrected.
13. ROS detection method clarified.
14. Details of MDA and CAT assays included.
15. The qPCR process is fully detailed.

Results and Discussion:

16. Quantitative results (MDA, CAT) added.
17. COPD linkage elaborated.
18. The mechanism of selegiline is discussed further.
19. Clarified gene expression changes.
20. Clinical implications discussed.

Conclusion and General Comments:

21. Conclusion revised for precision.
22. Emphasized the potential for repurposing selegiline.
23. Standardized NRF2/Keap1 usage throughout.
24. Grammar errors corrected across the manuscript.
25. Figure legends revised for clarity and completeness.
26. References checked and updated per journal style.
27. Ethical statement clarified:
    This study was conducted using cell lines only. No human or animal data were involved. Ethical approval: IR.AJUMS.REC.1402.652.

We hope that the revised manuscript adequately addresses all reviewer concerns and meets the journal's publication standards. Thank you once again for the opportunity to revise and improve our work.

Sincerely,
Zahra Mansouri

2 Revision 1

2 comments · 16 Apr 2025 – 18 Apr 2025

Referee 1 Reviewer

ACCEPT 16 Apr 2025

Dear Editor
Have a nice day.
The addressed issues and concerns have been effectively resolved. Consequently, the reviewer agrees to proceed with the publication of this paper in its current state.

Soheil Abbaspour-Ravasjani Reviewer

MINOR REVISION 18 Apr 2025

Abstract
1. The objectives statement is concise but could include the specific quantitative endpoints assessed (e.g., percentage change in ROS or fold-change in NRF2/Keap1 expression) for greater clarity and impact.
2. The background repeats the therapeutic potential of selegiline twice in the concluding sentences; consider consolidating to avoid redundancy.
3. The phrase "according to the results" is vague—replace it with direct numeric findings or specific observations to strengthen the claim.
4. Verb-tense shifts (e.g., "investigates" vs. "were investigated") should be standardized to past tense for consistency in reporting completed research.
5. The term "NRF2/Keap1 gene expression" is imprecise; specify that mRNA levels were measured by qRT-PCR and identify the reference gene used.

Introduction
1. The opening sentence broadly defines COPD; streamline by focusing immediately on oxidative stress and protease–antiprotease imbalance to lead directly into your study's rationale.
2. In describing the NRF2/Keap1 pathway, add a brief mechanistic note on how oxidative stress disrupts the NRF2–Keap1 interaction to activate downstream antioxidant genes.
3. Several sentences are run-on (e.g., "COPD encompasses… imbalance"); splitting these will improve readability and flow.
4. Introduce selegiline's antioxidant rationale earlier—move the sentence on its MAO-B inhibition and ROS-scavenging activity up to better contextualize your hypothesis.
5. The hypothesis statement is clear but would be stronger if you specify expected effects (e.g., "we hypothesized that selegiline would upregulate NRF2 and downregulate Keap1, resulting in reduced ROS").

Materials
1. Suppliers are listed, but catalog and lot numbers for key reagents (selegiline, elastase, RNA kits) should be provided to ensure reproducibility.
2. Indicate the purity grade of selegiline and elastase powders (e.g., ≥98% purity) to clarify reagent quality.
3. Specify whether FBS was heat-inactivated and state exact antibiotic concentrations (e.g., 100 U/mL penicillin, 100 µg/mL streptomycin).

Methods
1. For the MTT assay, include the microplate reader model and wavelength bandwidth, and clarify whether absorbance readings were taken in duplicate or triplicate wells.
2. The ROS detection protocol omits DCFH-DA concentration and incubation time; add these details so others can replicate the assay.

3. Describe your flow-cytometry gating strategy and controls (e.g., unstained, H_2O_2-treated positive control) to validate ROS measurements.
4. In the qRT-PCR section, list primer sequences for NRF2, Keap1, and the housekeeping gene, along with amplicon sizes and thermal cycling conditions.
5. Under statistical analysis, state how normality was assessed, specify the exact post hoc test used (e.g., Tukey's), and describe any corrections for multiple comparisons.

Results
1. Figures 1–4 lack fully detailed axis labels and units (e.g., "Fold-change" or "RFU"); adding these will make the data presentation clearer.
2. Statistical annotation symbols are defined in the captions but not consistently applied to all data points; ensure every comparison shows its corresponding symbol.
3. When stating "no statistically significant difference" for 40 µM selegiline, include the actual p-value to improve transparency.
4. Consider reporting effect sizes (e.g., Cohen's d) alongside p-values for key comparisons to convey the magnitude of selegiline's effects.
5. Consolidate descriptions of MDA and CAT results into a single paragraph to reduce redundancy and improve narrative flow.

Discussion
1. The opening paragraph reiterates COPD pathology; shorten this and shift focus to how your elastase model parallels clinical oxidative stress.
2. When referencing prior studies (e.g., Hou et al., Bayati et al.), briefly summarize their key findings and directly compare them to your own data.
3. Some sentences encompass multiple concepts (e.g., lines 181–184); dividing these will enhance clarity and prevent reader fatigue.
4. Cross-reference Figure 5 explicitly in the text when discussing your proposed mechanism to help readers connect narrative and illustration.
5. The limitations section notes only the need for further studies; expand by addressing potential confounders (e.g., cell-line specificity, absence of in vivo validation).

Conclusion
1. The conclusion largely echoes the abstract; rephrase to emphasize your novel contributions and the translational potential of repurposing selegiline for COPD.
2. Propose concrete next steps—such as testing in animal models of elastase-induced emphysema or examining combination therapies targeting NRF2/Keap1—to guide future research.

Here are useful references for the authors' observation:
doi: 10.34172/apb.2023.077
https://doi.org/10.1016/j.ijpharm.2022.122027

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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

We thank you for your valuable comments and suggestions concerning our manuscript entitled '***Protective Effects of Selegiline Against Elastase-Induced Oxidative Stress in Lung Epithelial Cells Through Modulation of the NRF2/Keap1 Signaling Pathway***'. We have carefully considered all the comments and suggestions, and wherever possible, incorporated them into the revised manuscript. Point-to-point responses to the reviewers' comments are provided below. All revisions made in response to each reviewer's comment are highlighted in a specific color.

We hope that the revisions in the manuscript, shown in highlight, along with our accompanying responses, will be sufficient to make our manuscript suitable for publication in ***Jundishapur Journal of Natural Pharmaceutical Products***. If you have any further queries, please do not hesitate to contact us

Kind regards,

Z.M

REVIEW COMMENTS:

Reviewer(s)' Comments to Author:

Reviewer 1

Response: We appreciate the time and effort that the reviewer has dedicated to providing his/her valuable feedback on our manuscript.

Reviewer 2

Response: Your valuable input has significantly contributed to the improvement of my project, and I am thankful for your time and effort.

Comments to the Author

1. The objectives statement is concise but could include the specific quantitative endpoints assessed (e.g., percentage change in ROS or fold-change in NRF2/Keap1 expression) for greater clarity and impact.

Thank you for your valuable comment. We have revised the objectives statement in response to the reviewer's suggestion.

2. The background repeats the therapeutic potential of selegiline twice in the concluding sentences; consider consolidating to avoid redundancy.

Thank you for the reviewer's valuable comment. The necessary revisions have been made.

3. The phrase "according to the results" is vague—replace with direct numeric findings or specific observations to strengthen the claim.

Thank you for the reviewer's valuable comment. The necessary revisions have been made.

4. Verb-tense shifts (e.g., "investigates" vs. "were investigated") should be standardized to past tense for consistency in reporting completed research.

Thank you for the reviewer's valuable comment. The necessary revisions have been made.

5. The term "NRF2/Keap1 gene expression" is imprecise; specify that mRNA levels were measured by qRT-PCR and identify the reference gene used.

Thank you for the reviewer's valuable comment. Accordingly, we have revised the text to specify that the mRNA levels of Nrf2 and Keap1 were measured using qRT-PCR. We have also included the reference gene (GAPDH) used for normalization.

Introduction

1. The opening sentence broadly defines COPD; streamline by focusing immediately on oxidative stress and protease–antiprotease imbalance to lead directly into your study's rationale.

Thank you for the comment. As suggested, the opening sentence has been revised to directly address oxidative stress and protease–antiprotease imbalance.

2. In describing the NRF2/Keap1 pathway, add a brief mechanistic note on how oxidative stress disrupts the NRF2–Keap1 interaction to activate downstream antioxidant genes.
3. Several sentences are run-on (e.g., "COPD encompasses… imbalance"); splitting these will improve readability and flow.

Thank you for the comment. The revisions have been highlighted in the manuscript.

4. Introduce selegiline's antioxidant rationale earlier—move the sentence on its MAO-B inhibition and ROS-scavenging activity up to better contextualize your hypothesis.

Thank you for the comment. As suggested, the sentence regarding selegiline's MAO-B inhibition and ROS-scavenging activity has been moved earlier in the manuscript to better contextualize the hypothesis.

5. The hypothesis statement is clear but would be stronger if you specify expected effects (e.g.,

"we hypothesized that selegiline would upregulate NRF2 and downregulate Keap1, resulting in reduced ROS").

Thank you for the suggestion. The expected effects have been added to the hypothesis statement as recommended.

Materials

1. Suppliers are listed, but catalog and lot numbers for key reagents (selegiline, elastase, RNA kits) should be provided to ensure reproducibility.

We thank the reviewer for this important comment. The catalog numbers for key reagents, including selegiline, elastase, and RNA extraction kits, have been added to the manuscript to enhance reproducibility. However, as the reagents were used during earlier stages of the project and the original packaging is no longer available, lot numbers could not be retrieved. We acknowledge the importance of this information and will ensure its inclusion in future studies.B

2. Indicate the purity grade of selegiline and elastase powders (e.g., ≥98% purity) to clarify reagent quality

Thank you for the comment. As requested, the purity grades of selegiline and elastase powders have been added to the manuscript.

3. Specify whether FBS was heat-inactivated and state exact antibiotic concentrations (e.g., 100 U/mL penicillin, 100 µg/mL streptomycin).

Thank you for the comment. The manuscript has been revised to specify that non–heat-inactivated FBS was used, and the exact concentrations of antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) have been added.

Methods

1. For the MTT assay, include the microplate reader model and wavelength bandwidth, and clarify whether absorbance readings were taken in duplicate or triplicate wells.

Thank you for the comment. In the Methods section, we clarified that the assay was performed in triplicate wells and absorbance was measured using a Bio-Rad microplate reader (bandwidth 10 nm) at 570 nm.

2. The ROS detection protocol omits DCFH-DA concentration and incubation time; add these details so others can replicate the assay.

Thank you for pointing this out. The DCFH-DA concentration (10 µM) and incubation time (30 minutes at 37°C in the dark) have now been added to the Methods.

3. Describe your flow-cytometry gating strategy and controls (e.g., unstained, H_2O_2-treated positive control) to validate ROS measurements.

Thank you for your valuable comment. The Methods section has been revised to clearly describe our gating strategy and controls. Unstained cells were used as negative controls, and H_2O_2-treated cells (100 µM) served as positive controls. A standard gating strategy was applied to exclude cell debris and doublets, ensuring accurate detection of ROS-positive cells based on FITC fluorescence.

4. In the qRT-PCR section, list primer sequences for NRF2, Keap1, and the housekeeping gene, along with amplicon sizes and thermal cycling conditions.

Thank you for your helpful comment. The qRT-PCR section has been revised to include the primer sequences, amplicon sizes, and NCBI accession numbers for NRF2, Keap1, and the housekeeping gene (GAPDH), along with the thermal cycling conditions. All reactions were performed in triplicate.

5. Under statistical analysis, state how normality was assessed, specify the exact post hoc test used (e.g., Tukey's), and describe any corrections for multiple comparisons.

Thank you for your comment. The statistical analysis section has been revised to specify that normality was assessed using the Shapiro–Wilk test. Differences between experimental groups were analyzed using a one-way ANOVA followed by a post-hoc Tukey's test. Additionally, corrections for multiple comparisons were applied using Tukey's test. P-values less than 0.05 were considered statistically significant.

Results

1. Figures 1–4 lack fully detailed axis labels and units (e.g., "Fold-change" or "RFU"); adding these will make the data presentation clearer.

Thank you for your suggestion. The axis labels and units in Figures 1–4 have been revised to include full details (e.g., "Fold change", "RFU", "%", "µmol/L", "U/mg protein") to enhance data clarity and consistency.

2. Statistical annotation symbols are defined in the captions but not consistently applied to all data points; ensure every comparison shows its corresponding symbol.

We appreciate the reviewer's comment. Statistical annotation symbols have been reviewed and consistently applied in all figures. Comparisons without symbols indicate non-significant differences.

3. When stating "no statistically significant difference" for 40 µM selegiline, include the actual p-value to improve transparency.

We thank the reviewer for the suggestion. The actual p-value for the 40 µM selegiline group has been added to the text.

4. Consider reporting effect sizes (e.g., Cohen's d) alongside p-values for key comparisons to convey the magnitude of selegiline's effects.

We appreciate the reviewer's insightful suggestion. However, as this study was performed on a single cell line with triplicate experiments under tightly controlled in vitro conditions, calculating effect sizes such as Cohen's d was deemed not applicable. In such cell-based assays, statistical significance testing is typically the standard method for interpreting results. Nonetheless, we

acknowledge the value of effect size metrics and will consider their inclusion in future studies with larger sample sizes or in vivo models.

5. Consolidate descriptions of MDA and CAT results into a single paragraph to reduce redundancy and improve narrative flow.

We thank the reviewer for the helpful suggestion. The descriptions of MDA and CAT results have now been consolidated into a single paragraph to reduce redundancy and improve narrative flow**.**

Discussion

1. The opening paragraph reiterates COPD pathology; shorten this and shift focus to how your elastase model parallels clinical oxidative stress.

We thank the reviewer for the suggestion.The opening paragraph has been revised to focus more on how elastase-induced oxidative stress mirrors the clinical pathology of COPD, as per your recommendation.

2. When referencing prior studies (e.g., Hou et al., Bayati et al.), briefly summarize their key findings and directly compare them to your own data.

We thank the reviewer for the suggestion. All referenced studies (e.g., Hou et al., Bayati et al.) have been included to support our findings, with a summary of their key results.

3. Some sentences encompass multiple concepts (e.g., lines 181–184); dividing these will enhance clarity and prevent reader fatigue.

We thank the reviewer for the suggestion. The abovementioned sentences were revised.

4. Cross-reference Figure 5 explicitly in the text when discussing your proposed mechanism to help readers connect narrative and illustration.

Thank you for your valuable suggestion. We have revised the manuscript to explicitly reference Figure 5 in the text when discussing the proposed mechanism of Selegiline

5. The limitations section notes only the need for further studies; expand by addressing potential confounders (e.g., cell-line specificity, absence of in vivo validation).

We thank the reviewer for the suggestion. The limitations section has been revised to address potential confounders, including cell-line specificity and the absence of in vivo validation." Conclusion

1. The conclusion largely echoes the abstract; rephrase to emphasize your novel contributions and the translational potential of repurposing selegiline for COPD.

We thank the reviewer for the valuable comment. The conclusion has been revised to emphasize the novel contributions of the study and the translational potential of repurposing selegiline for COPD.

2. Propose concrete next steps—such as testing in animal models of elastase-induced emphysema or examining combination therapies targeting NRF2/Keap1—to guide future research.

We thank the reviewer for the helpful suggestion. A statement has been added to the conclusion to propose future directions, including in vivo studies using animal models of elastase-induced emphysema .

3 Revision 2

3 comments · 11 May 2025 – 11 May 2025

Soheil Abbaspour-Ravasjani Reviewer

MAJOR REVISION 11 May 2025

Title

1. Replace "Protective Effects" with "Protective Role" to emphasize the mechanistic focus of the study.
2. Specify the cell type in the title, e.g., "in A549 Lung Epithelial Cells," to enhance clarity and specificity.
3. Shorten to "Selegiline's Protective Role Against Elastase-Induced Oxidative Stress in A549 Cells via NRF2/Keap1 Pathway" for conciseness.

Abstract

4. Clarify "lung cells" as "A549 lung epithelial cells" to specify the cell line used.
5. Quantify ROS reduction, e.g., "reduced ROS levels by approximately 30% at 20 µM," to provide a concrete result.
6. Correct "NRF2 and Keap1 gene expression" to "NRF2 and Keap1 mRNA expression" for precision.
7. Replace "may offer therapeutic benefits" with "demonstrates potential therapeutic benefits" to reflect the study's findings.
8. Add a sentence on limitations, e.g., "Further in vivo studies are needed to confirm clinical relevance," to balance the conclusions.

Keywords

9. Add "antioxidant" to reflect the study's focus on oxidative stress mitigation.
10. Replace "A549" with "lung epithelial cells" to broaden applicability while maintaining specificity.
11. Include "elastase-induced damage" to highlight the experimental model.

Introduction (Pages 12-13)

12. Update COPD prevalence with a 2025 statistic, e.g., "COPD affects over 380 million people globally (WHO, 2025)."
13. Correct "both of which contribute" to "which collectively contribute" for grammatical flow.
14. Specify ROS types, e.g., "superoxide, hydrogen peroxide, and hydroxyl radicals," to enhance scientific detail.
15. Provide a reference for the claim that catalase is overwhelmed in COPD.
16. Clarify "disrupts the protease-antiprotease balance" by adding "through excessive elastin degradation."
17. Correct "to this end" to "therefore" for formal tone and clarity.
18. Quantify selegiline's antioxidant effects, e.g., "previously shown to reduce ROS by 20-40% in neuronal cells [6]."
19. Strengthen the hypothesis by specifying outcomes, e.g., "reduce ROS levels by at least 25%."
20. Correct "would upregulate NRF2 expression" to "would increase NRF2 mRNA expression" for precision.

Materials (Page 13)

21. Add catalog numbers for DMEM, FBS, and antibiotics to ensure reproducibility.
22. Specify the lot numbers for selegiline and elastase, or explain their unavailability consistently across all reagents.
23. Clarify "DMEM" as "Dulbecco's Modified Eagle Medium" on first use for non-specialist readers.
24. Confirm the CAS number for selegiline (listed as 14611520) is correct, as it appears incomplete; it should be 14611-51-9.
25. Specify the storage conditions for reagents, e.g., "selegiline stored at -20°C," to enhance methodological detail.

Methods (Pages 13-15)

26. Specify the passage number range of A549 cells used, e.g., "passages 5-10," to ensure consistency.
27. Clarify "randomly divided" by describing the randomization method, e.g., "assigned using a random

number generator."
28. Provide the source and catalog number for the MTT assay kit or reagents.
29. Correct "5,000 cells were seeded" to "5,000 cells/well were seeded" for precision.
30. Specify the flow cytometer model and software, e.g., "BD FACSCalibur with CellQuest Pro," for reproducibility.
31. Clarify "standard gating strategy" by describing gate parameters, e.g., "FSC/SSC gates to exclude debris, singlet gate for doublets."
32. Provide the catalog number for the DCFH-DA reagent used in ROS detection.
33. Specify the spectrophotometer model and wavelength for MDA and CAT assays, e.g., "SpectraMax at 532 nm for MDA."
34. Correct "Quantitative Reverse Transcriptase kit" to "cDNA Synthesis Kit (Pars Tous, CAT NO A101162)" for consistency.
35. Add NCBI accession numbers for NRF2, Keap1, and GAPDH to validate primer specificity.
36. Clarify "appropriate concentrations" of primers by specifying, e.g., "0.5 µM each."
37. Correct "Prism 8 software was utilized" to "GraphPad Prism 8 was used" for formal tone.
38. Specify the ANOVA model, e.g., "one-way ANOVA with repeated measures," to clarify the statistical approach.
Results (Pages 15-17)
39. Add units to Figure 1 axis labels, e.g., "Cell Viability (% of Control)" for clarity.
40. Quantify cell viability changes, e.g., "H_2O_2 reduced viability by 40%," to provide specific results.
41. Correct "marked decline" to "significant reduction" for formal scientific tone.
42. In Figure 2, specify RFU units as "Relative Fluorescence Units (RFU)" in the axis label.
43. Clarify "substantial rise in ROS levels" at 40 µM selegiline by quantifying, e.g., "increased ROS by 15%."
44. Consolidate MDA and CAT results into one paragraph, e.g., "Elastase increased MDA levels 3-fold and reduced CAT activity by 50%, while selegiline (20 µM) restored these parameters ($P<0.001$)."
45. In Figure 3, add units to MDA (e.g., "µmol/L") and CAT (e.g., "U/mg protein") axis labels.
46. Correct "notable elevation" in ROS to "significant increase" for consistency.
47. In Figure 4, clarify "Fold change" as "Fold Change Relative to Control" in axis labels.
48. Quantify NRF2 and Keap1 expression changes, e.g., "NRF2 mRNA increased 2.5-fold at 20 µM selegiline."
49. Correct "considerable distinction" to "significant difference" for formal tone.
Discussion (Pages 18-21)
50. Shorten the COPD pathology recap by focusing on elastase's role, e.g., "Elastase drives oxidative stress and emphysema, mirroring COPD pathology."
51. Provide specific findings from Hou et al., e.g., "Hou et al. reported elastase-induced apoptosis via JNK/p38 MAPK pathways [7]."
52. Correct "Recent research has identified" to "Recent studies have established" for formal tone.
53. Split the run-on sentence (lines 183-184) into two, e.g., "Elastase increased ROS production. This was associated with elevated MDA levels."
54. Clarify "various types of neurons" by specifying, e.g., "dopaminergic and cortical neurons."
55. Add a reference for the claim that selegiline enhances glutathione-dependent enzymes.
56. Quantify selegiline's effects in prior studies, e.g., "Qin et al. reported a 30% reduction in cardiac ROS [16]."
57. Correct "PPE" to "porcine pancreatic elastase (PPE)" on first use in the discussion for clarity.
58. Expand the limitations section to include potential off-target effects of selegiline, e.g., "MAO-B inhibition may affect neurotransmitter levels."
59. Cross-reference Figure 5 earlier, e.g., "Selegiline's mechanism (Figure 5) involves NRF2 upregulation."
Conclusion (Page 21)

60. Rephrase to avoid abstract repetition, e.g., "Selegiline protects A549 cells from elastase-induced oxidative stress by enhancing NRF2-mediated antioxidant defenses."
61. Specify translational potential, e.g., "Repurposing selegiline could offer a novel adjunct therapy for COPD."
62. Correct "give evidence" to "provide evidence" for grammatical accuracy.
63. Add a specific future direction, e.g., "Clinical trials should evaluate selegiline's efficacy in COPD patients with high oxidative stress."

Associate Editor MAJOR REVISION 11 May 2025

The Ms still needs some revisions before it is suitable for publication in JJNPP.

Editor-in-Chief MAJOR REVISION 11 May 2025

The editorial team has reviewed the latest version of your submission alongside the reviewers' comments. *This will be your last opportunity to address the reviewers' concerns* comprehensively. Please ensure:

1. *Point-by-point responses* to *all* reviewer comments, clearly indicating how each concern has been addressed.
2. Revisions highlighted in the manuscript (tracked changes or colored text).
3. Strict adherence to the journal's formatting guidelines and citation style.
4. Submission of all files (*revised manuscript*, response letter, supplementary materials).

Note: Failure to fully address the reviewers' feedback may result in the rejection of your manuscript.

We appreciate your diligence and look forward to receiving your final revision.

4

Revision 3

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

We thank you for your valuable comments and suggestions concerning our manuscript entitled '***Protective Effects of Selegiline Against Elastase-Induced Oxidative Stress in Lung Epithelial Cells Through Modulation of the NRF2/Keap1 Signaling Pathway***'. We have carefully considered all the comments and suggestions and incorporated them wherever possible into the revised manuscript. Point-to-point responses to the reviewers' comments are provided below. All revisions in response to the reviewer's comments are highlighted in a specific color.

We hope that the revisions in the manuscript, shown in highlight, along with our accompanying responses, will be sufficient to make our manuscript suitable for publication in ***Jundishapur Journal of Natural Pharmaceutical Products***. If you have any further queries, please do not hesitate to contact us.

Kind regards,

Z.M

We appreciate the time and effort the reviewer has dedicated to providing his/her valuable feedback on our manuscript.

1. Replace "Protective Effects" with "Protective Role" to emphasize the mechanistic focus of the study.

Thank you for the insightful comment. The title has been revised to use 'Protective Role', which better reflects the mechanistic emphasis.

2. Specify cell type in the title, e.g., "in A549 Lung Epithelial Cells," to enhance clarity and specificity.

We appreciate the suggestion. The cell type 'A549 Lung Epithelial Cells' has been specified in the title for improved clarity.

3. *Shorten to "Selegiline's Protective Role Against Elastase-Induced Oxidative Stress in A549 Cells via NRF2/Keap1 Pathway" for conciseness.*

Thank you for the helpful suggestion. The title has been shortened accordingly to improve conciseness.

4. To specify the cell line used, clarify "lung cells" as "A549 lung epithelial cells."

Thank you for pointing this out. The abstract now says 'A549 lung epithelial cells' instead of 'lung cells.'

5. Quantify ROS reduction, e.g., "reduced ROS levels by approximately 30% at 20 µM," to provide a concrete result.

Thank you for the recommendation. The ROS level changes have been quantified and incorporated into the Results section for precision.

6. Correct "NRF2 and Keap1 gene expression" to "NRF2 and Keap1 mRNA expression" for precision.

Thank you for pointing this out. We have corrected the terminology throughout the text to 'NRF2 and Keap1 mRNA expression'.

7. Replace "may offer therapeutic benefits" with "demonstrates potential therapeutic benefits" to reflect the study's findings.

Thank you for the suggestion. To reflect our findings more accurately, the phrase has been revised to 'demonstrates potential therapeutic benefits'.

8. Add a sentence on limitations, e.g., "Further in vivo studies are needed to confirm clinical relevance," to balance the conclusions.

Thank you for the suggestion. A sentence highlighting the need for further in vivo studies has been added to the limitations.

9. Add "antioxidant" to reflect the study's focus on oxidative stress mitigation.

Thank you for the comment. 'Antioxidant' has been added to the keywords to highlight the focus on oxidative stress.

10. Replace "A549" with "lung epithelial cells" to broaden applicability while maintaining specificity.

Thank you for the suggestion. As suggested, 'A549' has been replaced with 'lung epithelial cells' in the keyword section.

11. Include "elastase-induced damage" to highlight the experimental model.

Thank you for the suggestion. To emphasize the experimental model, 'Elastase-induced damage' has been added to the keywords.

12. Update COPD prevalence with a 2025 statistic, e.g., "COPD affects over 380 million people globally (WHO, 2025).
Thank you for the suggestion. We have updated the COPD prevalence statistic to reflect the latest data: "COPD affects over 380 million people globally (WHO, 2025)," ensuring that the manuscript reflects the most current epidemiological figures.

13. Correct "both of which contribute" to "which collectively contribute" for grammatical flow.

We appreciate the grammatical recommendation. To improve the sentence's flow and

clarity, the phrase has been revised from "both of which contribute" to "which collectively contribute.

14. Specify ROS types, e.g., "superoxide, hydrogen peroxide, and hydroxyl radicals," to enhance scientific detail.

Thank you for the suggestion to increase scientific precision. To provide greater detail, we have specified the reactive oxygen species as "superoxide, hydrogen peroxide, and hydroxyl radicals.

15. Provide a reference for the claim that catalase is overwhelmed in COPD.
We are grateful for this observation. A relevant reference has been included to support the claim that catalase activity is overwhelmed in COPD, strengthening the background rationale.

16. Clarify "disrupts the protease-antiprotease balance" by adding "through excessive elastin
Thank you for this clarification. We have revised the sentence to read: "disrupts the protease-antiprotease balance through excessive elastin degradation," which better describes the underlying pathological mechanism.

*17. Correct "to this end" to "*therefore*" for formal tone and clarity.*
We appreciate the suggestion to enhance formality. For improved clarity and tone, the phrase "to this end" has been replaced with “therefore”.

18. Quantify selegiline’s antioxidant effects, e.g., "previously shown to reduce ROS by 20-40% in neuronal cells
Thank you for the insightful comment. We have added quantification to strengthen support for its antioxidant effects.

19. Strengthen the hypothesis by specifying outcomes, e.g., "reduce ROS levels by at least 25%.
We agree that specificity strengthens the hypothesis. The ROS level changes have been quantified and incorporated into the Results section for precision.

20. Correct "would upregulate NRF2 expression" to "would increase NRF2 mRNA expression" for precision.
Thank you for this important correction. The wording has been updated to "would increase NRF2 mRNA expression" to reflect the precise molecular measurements performed.

21. Add catalog numbers for DMEM, FBS, and antibiotics to ensure reproducibility.
Thank you for this suggestion. To improve reproducibility, we have added the catalog numbers for DMEM, FBS, and antibiotics to the revised manuscript.

22. Specify the lot numbers for selegiline and elastase, or explain their unavailability consistently across all reagents.
We appreciate this comment. The Methods section consistently clarifies the unavailability of lot numbers for selegiline and elastase.

23. Clarify "DMEM" as "Dulbecco's Modified Eagle Medium" on first use for non-specialist readers.
Thank you for this suggestion. This has been addressed by expanding the abbreviation upon first mention.

24. Confirm the CAS number for selegiline (listed as 14611520) is correct, as it appears incomplete; it should be 14611-51-9.
Thank you for catching this. The CAS number has been corrected to 14611-51-9 as requested.

25. Specify the storage conditions for reagents, e.g., "selegiline stored at -20°C," to enhance methodological detail.
Thank you for pointing this out. Per the reviewer's suggestion, storage conditions for key reagents, including selegiline, have been added.

26. Specify the passage number range of A549 cells used, e.g., "passages 5-10," to ensure consistency.
Thank you for this suggestion. Passage numbers of A549 cells have been specified in the revised manuscript.

27. Clarify "randomly divided" by describing the randomization method, e.g., "assigned using a random number generator."
Thank you for this suggestion. As no formal randomization was applied, the term "randomly" has been removed to avoid confusion.

28. Provide the source and catalog number for the MTT assay kit or reagents.
Thank you for the insightful comment. We have included the source and catalog number of the MTT assay kit.

29. Correct "5,000 cells were seeded" to "5,000 cells/well were seeded" for precision.
Thank you for this suggestion. This sentence has been corrected as suggested.

30. Specify the flow cytometer model and software, e.g., "BD FACSCalibur with CellQuest Pro," for reproducibility.

Thank you for this suggestion. The flow cytometer model and analysis software details have been added.

31. Clarify "standard gating strategy" by describing gate parameters, e.g., "FSC/SSC gates to exclude debris, singlet gate for doublets."
Thank you for the insightful comment. The gating strategy has been detailed as recommended.

32. Provide the catalog number for the DCFH-DA reagent used in ROS detection.
Thank you for this suggestion. The catalog number for DCFH-DA reagent has been added accordingly.

33. Specify the spectrophotometer model and wavelength for MDA and CAT assays, e.g., "SpectraMax at 532 nm for MDA".

We sincerely appreciate the reviewer's valuable comment. The spectrophotometer model and wavelength for the MDA and CAT assays have now been specified in the Methods section for clarity and reproducibility.

34. Correct "Quantitative Reverse Transcriptase kit" to "cDNA Synthesis Kit (Pars Tous, CAT NO A101162)" for consistency.

We are grateful for the reviewer's insightful suggestion. The kit name has been corrected accordingly for consistency throughout the manuscript.

35. Add NCBI accession numbers for NRF2, Keap1, and GAPDH to validate primer specificity.

We thank the reviewer for their careful attention to detail. To validate their specificity, we have added the NCBI accession numbers for the primers used.

36. Clarify "appropriate concentrations" of primers by specifying, e.g., "0.5 μM each."

We appreciate the reviewer's recommendation to clarify primer concentrations. The exact concentrations (0.5 μM each) have been specified in the Methods section.

37. Correct "Prism 8 software was utilized" to "GraphPad Prism 8 was used" for formal tone.

We are thankful for the reviewer’s suggestion regarding formal language. To improve clarity and professionalism, the phrase has been updated to “GraphPad Prism 8 was used.”

38. Specify the ANOVA model, e.g., "one-way ANOVA with repeated measures," to clarify the statistical approach.

We sincerely thank the reviewer for emphasizing statistical rigor. The ANOVA model has now been clearly specified as “one-way ANOVA with repeated measures.”

39. Add units to Figure 1 axis labels, e.g., "Cell Viability (% of Control)" for clarity.

We appreciate the reviewer’s constructive feedback on the figure's clarity. To improve understanding, units have been added to all relevant axis labels in Figure 1.

40. Quantify cell viability changes, e.g., "H_2O_2 reduced viability by 40%," to provide specific results.

We are grateful for the reviewer’s request for quantitative details. Cell viability changes have been quantified and incorporated into the Results section for precision.

41. Correct "marked decline" to "significant reduction" for a formal scientific tone.

We thank the reviewer for their valuable comment on the scientific tone. The phrase “marked decline” has been replaced with the more formal “significant reduction”.

42. In Figure 2, specify RFU units as "Relative Fluorescence Units (RFU)" in the axis label.

We appreciate the reviewer’s attention to detail in labeling. The axis label in Figure 2 now clearly specifies “Relative Fluorescence Units (RFU)”.

43. Clarify "substantial rise in ROS levels" at 40 µM selegiline by quantifying, e.g., "increased ROS by 15%."

We sincerely thank the reviewer for recommending the quantification of ROS changes. The increase in ROS levels at 40 µM selegiline has been quantified as a 15% increase and included in the text.

44. Consolidate MDA and CAT results into one paragraph, e.g., "Elastase increased MDA levels 3-fold and reduced CAT activity by 50%, while selegiline (20 µM) restored these parameters (P<0.001)."

We appreciate the reviewer's helpful suggestion for clarity. The MDA and CAT assays results have been consolidated into a coherent paragraph for improved readability.

45. In Figure 3, add units to MDA (e.g., "µmol/L") and CAT (e.g., "U/mg protein") axis labels.

We are grateful for the reviewer's insightful comment. For clarity, units have been added to the axes of MDA and CAT in Figure 3.

46. Correct "notable elevation" in ROS to "significant increase" for consistency.

We thank the reviewer for their valuable input on terminology consistency. Throughout the manuscript, the phrase "notable elevation" has been changed to "significant increase."

47. In Figure 4, clarify "Fold change" as "Fold Change Relative to Control" in axis labels.

We appreciate the reviewer's suggestion for clearer labeling. The term "Fold Change" in Figure 4 has been clarified as "Fold Change Relative to Control."

48. Quantify NRF2 and Keap1 expression changes, e.g., "NRF2 mRNA increased 2.5-fold at 20 µM selegiline."

We sincerely thank the reviewer for requesting quantification of gene expression. The fold changes in NRF2 and Keap1 mRNA levels have been included with specific values.

49. Correct "considerable distinction" to "significant difference" for formal tone.

We are grateful for the reviewer’s guidance on formal tone. To enhance professionalism, the phrase has been corrected to “significant difference.”

50. Shorten the COPD pathology recap by focusing on elastase’s role, e.g., "Elastase drives oxidative stress and emphysema, mirroring COPD pathology."

We appreciate the reviewer’s suggestion to focus the discussion. The COPD pathology overview has been condensed to emphasize elastase’s critical role.

51. Provide specific findings from Hou et al., e.g., "Hou et al. reported elastase-induced apoptosis via JNK/p38 MAPK pathways [7]."

We thank the reviewer for recommending the inclusion of specific findings. Relevant results from Hou et al. have been added to support the discussion.

52. Correct "Recent research has identified" to "Recent studies have established" for formal tone.

We are grateful for the reviewer’s comment on formal phrasing. The wording has been revised accordingly to improve tone.

53. Split the run-on sentence (lines 183-184) into two, e.g., "Elastase increased ROS production. This was associated with elevated MDA levels."

We appreciate the reviewer’s suggestion for clarity and sentence structure. The run-on sentence has been split into two concise statements.

54. Clarify "various types of neurons" by specifying, e.g., "dopaminergic and cortical neurons."

We thank the reviewer for their insightful comment. The description of neuron types has been specified for precision.

55. Add a reference for the claim that selegiline enhances glutathione-dependent enzymes.

We sincerely appreciate the reviewer's request for additional references. A relevant citation has been added to support this claim.

56. Quantify selegiline's effects in prior studies, e.g., "Qin et al. reported a 30% reduction in cardiac ROS [16]."

We are grateful for the reviewer's recommendation to quantify selegiline's effects. Quantitative data from previous studies have been included.

57. Correct "PPE" to "porcine pancreatic elastase (PPE)" on first use in the discussion for clarity.

We appreciate the reviewer's comment on clarity. PPE has been expanded upon in the first mention for reader comprehension.

58. Expand the limitations section to include potential off-target effects of selegiline, e.g., "MAO-B inhibition may affect neurotransmitter levels."

We thank the reviewer for highlighting the limitations. The limitations section now discusses potential off-target effects of selegiline.

59. Cross-reference Figure 5 earlier, e.g., "Selegiline's mechanism (Figure 5) involves NRF2 upregulation."

We are grateful for the reviewer's suggestion to improve cross-referencing. Figure 5 is now cited earlier to illustrate the mechanism better.

60. Rephrase to avoid abstract repetition, e.g., "Selegiline protects A549 cells from elastase-induced oxidative stress by enhancing NRF2-mediated antioxidant defenses."

We appreciate the reviewer's recommendation to avoid repetition. The conclusion has been rephrased to provide a concise and novel summary.

61. Specify translational potential, e.g., "Repurposing selegiline could offer a novel adjunct therapy for COPD."

We thank the reviewer for encouraging the addition of translational relevance. The potential clinical applications of selegiline have been highlighted.

62. Correct "give evidence" to "provide evidence" for grammatical accuracy.

We are grateful for the reviewer’s attention to grammatical accuracy. The phrase has been corrected accordingly.

63. Add a specific future direction, e.g., "Clinical trials should evaluate selegiline’s efficacy in COPD patients with high oxidative stress."

We sincerely appreciate the reviewer’s suggestion for future research directions. The discussion recommends clinical trials.

4 Revision 3

1 comment · 21 May 2025

Editor-in-Chief

ACCEPT 21 May 2025

We are pleased to inform you that your manuscript has been accepted for publication in JJNPP.