Journal of Reports in Pharmaceutical Sciences
Volume 13 · Issue 1 · e156394

OPEN PEER REVIEW

Effects of Pinoresinol from Vietnamese *Gnetum montanum* Markgr Inhibits the Inflammation in Macrophages

Cuong Trinh Tat, Duong Duc Thien, Nguyen Thi Hanh Tran, Nguyen Anh Duc, Hoang Duc Manh, Nguyen Quang Huy, Ly Huyen Ngo, Nguyen Quang Nguyen

SUBMITTED	REVISED	ACCEPTED	DOI
28 Sep 2024	12 Apr 2025	22 Apr 2025	10.5812/jrps-156394

About this report

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

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

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1 Original submission

2 comments · 23 Jan 2025 – 23 Jan 2025

Associate Editor

MAJOR REVISION 23 Jan 2025

Dear Authors,

We hope this message finds you well. We appreciate your submission of the manuscript titled "Effects of Pinoresinol from Vietnamese Gnetum montanum Markgr Inhibits the Inflammation in Macrophages" to the Journal of Reports in Pharmaceutical Sciences. Following a thorough review, we have identified several areas that require major revisions. Please address the following points raised by our reviewers point by point and highlight the changes in the text.
Sincerely,
E.I.C

Editor-in-Chief

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2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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

On behalf of the author group, I would like to thank the comments and authors edited the manuscript in accordance with the comments.

Reviewer 1:

Dear Editor

Review of: 156394

The respected authors have tried to show the anti-inflammatory activity of pinoresinol on the Raw 264.7 cell. Pinoresinol appears to has the strongest antiinflammatory properties (https://pubmed.ncbi.nlm.nih.gov/22955517/).

The authors are required to provide more clarified data on the identity and purity of the pinoresinol. In this regard, the quality of all sections of Figure 1 is unacceptable.

According to the TLC (figure 1b) the purity of the compound is questionable.

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The provided western blotting data should be done in triplicates. Please show/claim/declare this in the supplementary data. Also, please provide raw data of figure 4 with no excision/cropping.

Complete disappearance of p-P38 under the effect of pinoresinol in Figure 4 is so strange.

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The biggest concern of this reviewer is about work innovation/novelty. Another group has researched this issue more deeply.

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Pinoresinol targets NF-κB alongside STAT3 pathway to attenuate IL-6-induced inflammation

The observations made in Fig 5: why the cells in the pinoresinol treatment group are so different in color. Logically, the amount of ROS should be decreased in all cell populations (the intensity of the color should be changed). The authors should carefully describe/interpret the obtained results.

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Ref.

https://onlinelibrary.wiley.com/doi/full/10.1002/hsr2.998

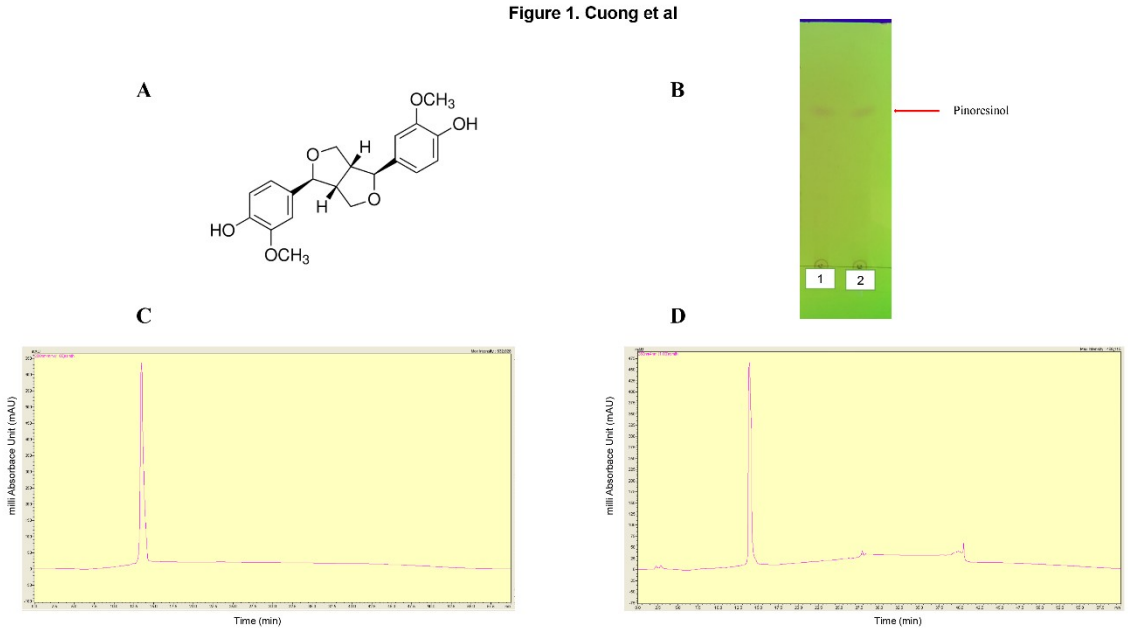
https://pubmed.ncbi.nlm.nih.gov/20600612/

https://applbiolchem.springeropen.com/articles/10.3839/jksabc.2011.027

https://onlinelibrary.wiley.com/doi/10.1155/2020/8704146#:~:text=Isoorientin%20Inhibits%20Inflammation%20in%20Macrophages,of%20Inflammation%20%2D%20Wiley%20Online%20Library

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[20] Goels, T., Eichenauer, E., Langeder, J., Hoeller, F., Sykora, C., Tahir, A., ... & Glasl, S. (2020). Norway spruce balm: Phytochemical composition and ability to enhance re-epithelialization in vitro. Planta medica, 86(15), 1080-1088.

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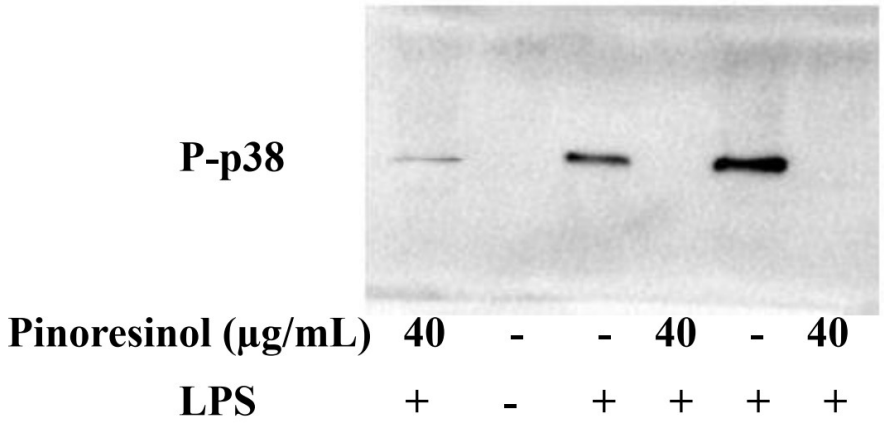
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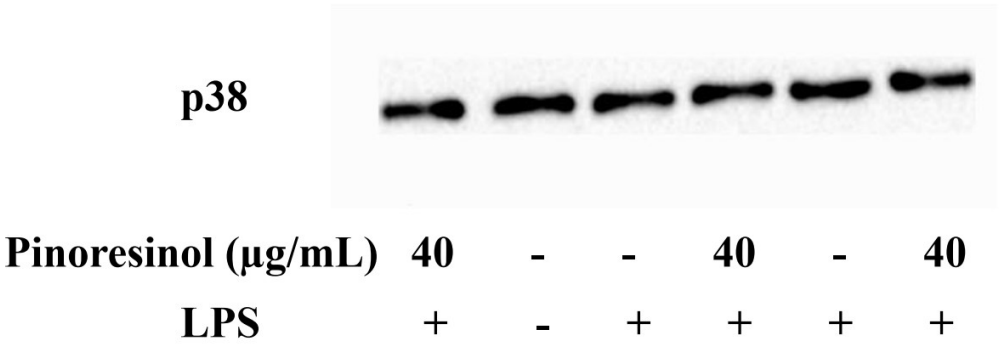
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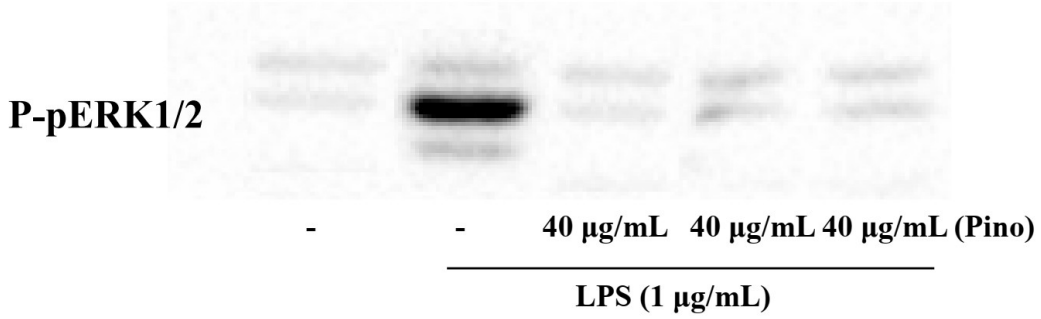
The author group has added

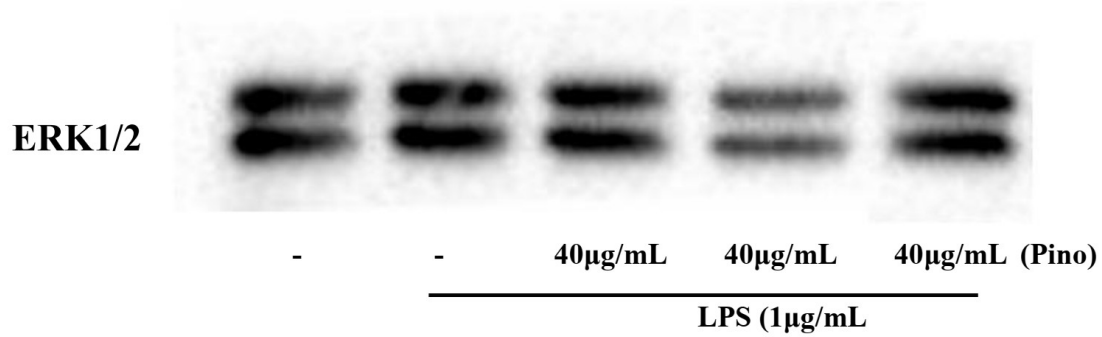
+The provided western blotting data should be done in triplicates. Please show/claim/declare this in the supplementary data. Also, please provide raw data of figure 4 with no excision/cropping.

As following: the western blot results of p-p38 and p-ERK1/2 for 3 times of the concentration of 40 μg/mL for Pinoresinol



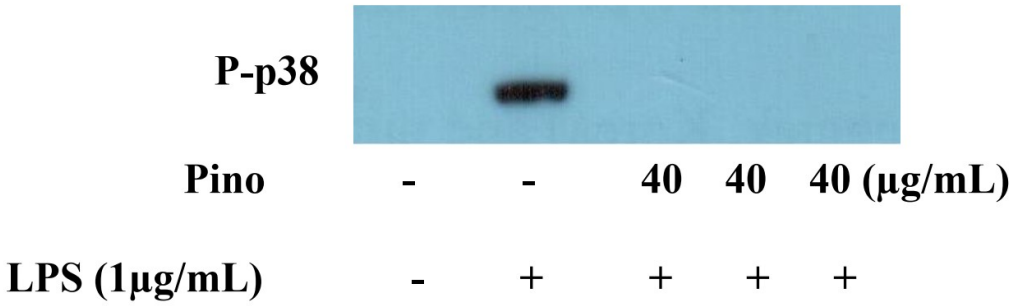


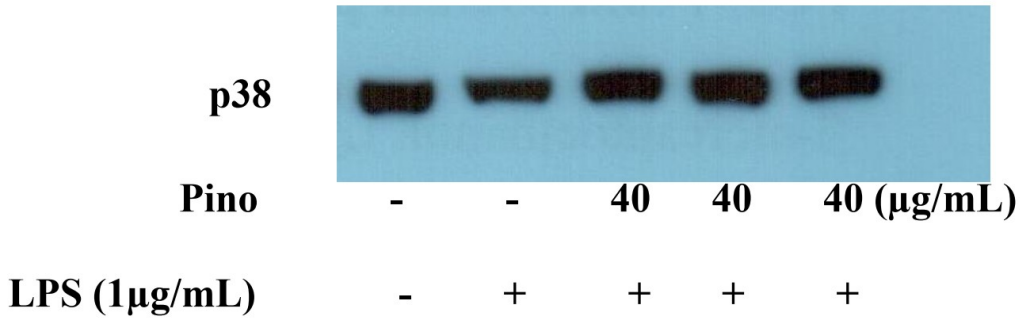




+ Complete disappearance of p-P38 under the effect of pinoresinol in Figure 4 is so strange.

With this comment, authors re-evaluated the western blot of P-p38 on film for the following results:





+ The mechanism of action of the compound should be discussed and authors are required to show that using a schematic presentation.

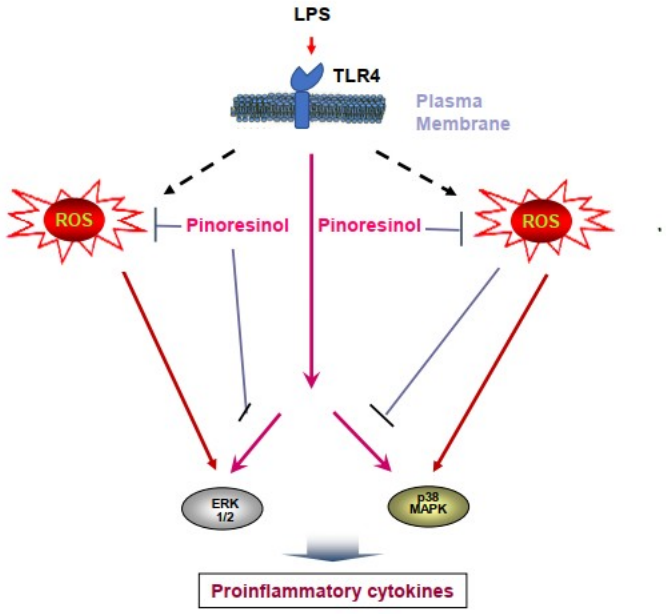


Figure 6. Schematic diagram of the anti-inflammatory and ROS mechanism of pinoresinol in macrophage cells.
LPS induces MAPK and ROS through Toll-like receptor (TLR) 4. Pre-treatment with pinoresinol effectively abrogates the phosphorylation of p38, ERK1/2, and ROS production by pre-treatment with Pinoresinol.

+ Why have the authors selected the mentioned parameters (p-ERK/p-P38) and why not other mediators of inflammation?

The p38 MAPK pathway is identified as a major regulator of the pro-inflammatory cytokine production of myeloid cells. Many anti-inflammatory drugs target this factor for evaluation on cellular, preclinical and clinical models of inflammatory disease.
Extracellular signal-associated kinases 1 and 2 (ERK1/2) are the final component of the mitogen-activated protein kinase phosphorylated layer (MAPK), an integral module in a variety of different signaling pathways to shape cell behavior and fate. ERK1/2 plays a role in the mechanism of action of immune receptors for inflammatory gene expression in response to infection and cell or tissue damage. In particular, the Toll receptor activates ERK1/2 which leads to dysfunction in the inflammatory response and is also a potential treatment for inflammatory diseases.
Currently, the inflammatory inhibition pathway of pinoresinol through NK-BF has been demonstrated by several studies. However, the effect of this substance on ROS during inflammation has not been published. From the above reasons, the authors chose p38 and ERK1/2 to research.

The biggest concern of this reviewer is about work innovation/novelty. Another group has researched this issue more deeply.

Reactive oxygen species (ROS) are signaling molecules that play an important role in the progression of inflammatory disorders. Enhancement of ROS production by leukocytes at the site of inflammation causes endothelial dysfunction and tissue damage. The past and current research in the field of inflammation, with a particular emphasis on oxidative stress-mediated signaling mechanisms that are associated with inflammation and tissue damage, which have great potential to find inflammatory active substances to aid in the treatment of inflammation-related diseases. Therefore, the results of the author's study initially indicate the role of pinoresinol on ROS in the process of causing inflammation in macrophages. This is an innovation that is different from other research groups. With these new results, authors will continue to evaluate the effects of pinoresinol on the ROS production mechanism from elements in nicotinamide adenine dinucleotide phosphate NADPH oxidase such as: p47phox, p67phox, p40phox and a GTPase Rac1 or Rac2 and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3).

+ The observations made in Fig 5: why the cells in the pinoresinol treatment group are so different in color. Logically, the amount of ROS should be decreased in all cell populations (the intensity of the color should be changed). The authors should carefully describe/interpret the obtained results.

The authors have added

+ The authors should provide some statements as to why they selected/employed the indicated concentrations of pinoresinol.

The authors have added

Thanking you,

Yours' sincerely,

Trinh Tat Cuong

2 Revision 1

3 comments · 17 Mar 2025 – 17 Mar 2025

Referee 1 Reviewer

MINOR REVISION 17 Mar 2025

Dear Editor
Respected authors must respond to all referee comments accurately and patiently (point-by-point), and indicate in their responses what changes they have made to the text/figures and figure captions, and highlight the line/page of the corrections in the response (and in the main text as well).
If the manuscript is revised successfully, it may be accepted as a short communication, due to its limited data.

Associate Editor

MINOR REVISION 17 Mar 2025

Dear Authors,

We hope this email finds you well. We have received feedback from the reviewers regarding your manuscript entitled "Effects of pinoresinol from Vietnamese Gnetum montanum Markgr inhibits the inflammation in macrophages" submitted to the Journal of Reports in Pharmaceutical Sciences. The reviewers have recommended minor revisions before the manuscript can be reconsidered for publication.
We kindly request that you address each reviewer's comments point-by-point in your revised manuscript. Additionally, please provide a detailed response to each comment, highlighting the changes made. This will facilitate a clear and efficient review process.
Should you have any questions or need further clarification, feel free to reach out to us.

We appreciate your cooperation and look forward to receiving your revised manuscript.

Best regards,

Editorial Team Journal of Reports in Pharmaceutical Sciences

Editor-in-Chief

MINOR REVISION 17 Mar 2025

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We kindly request that you address each reviewer's comments point-by-point in your revised manuscript. Additionally, please provide a detailed response to each comment, highlighting the changes made. This will facilitate a clear and efficient review process.
Should you have any questions or need further clarification, feel free to reach out to us.

We appreciate your cooperation and look forward to receiving your revised manuscript.

Best regards,

Editorial Team Journal of Reports in Pharmaceutical Sciences

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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

On behalf of the author group, I would like to thank the comments and authors edited the manuscript in accordance with the comments.

Reviewer 1:

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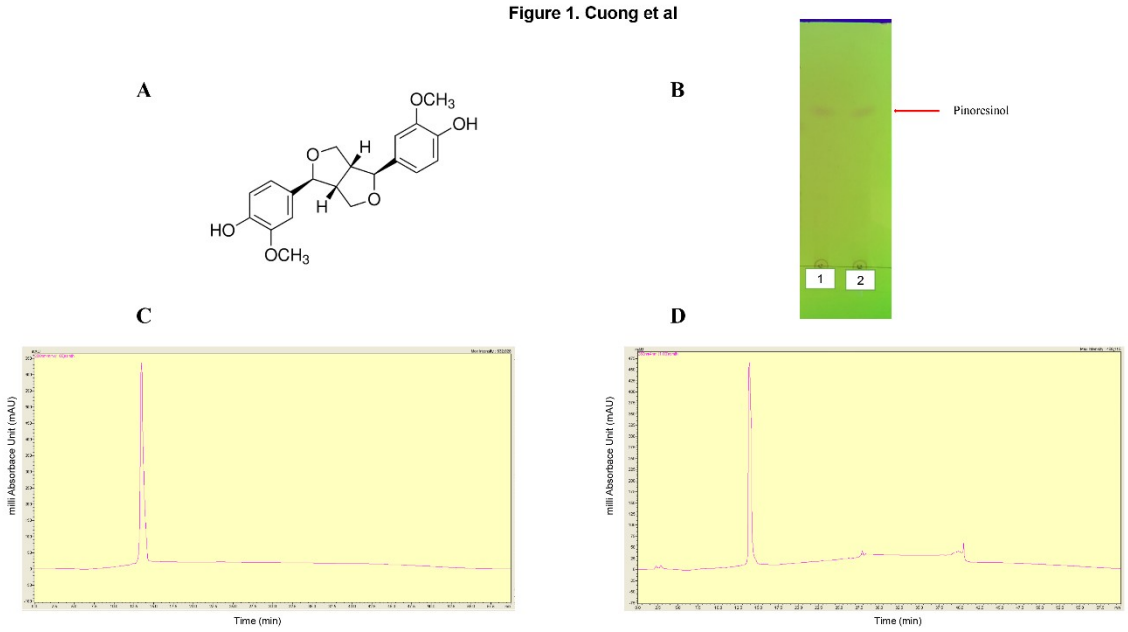
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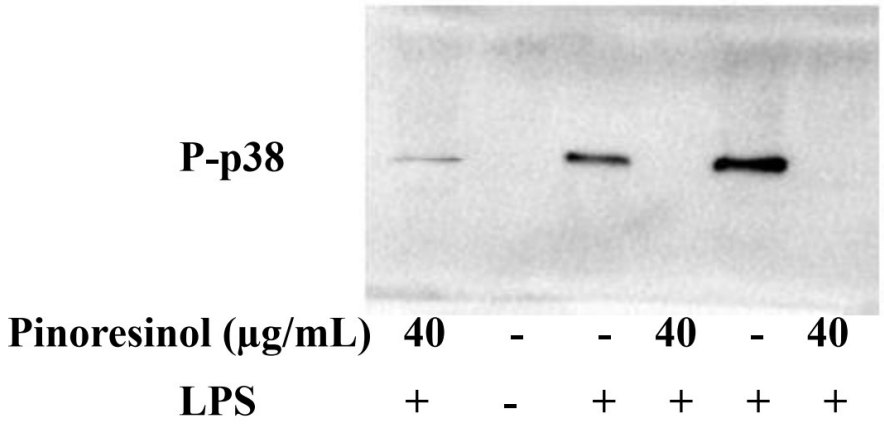
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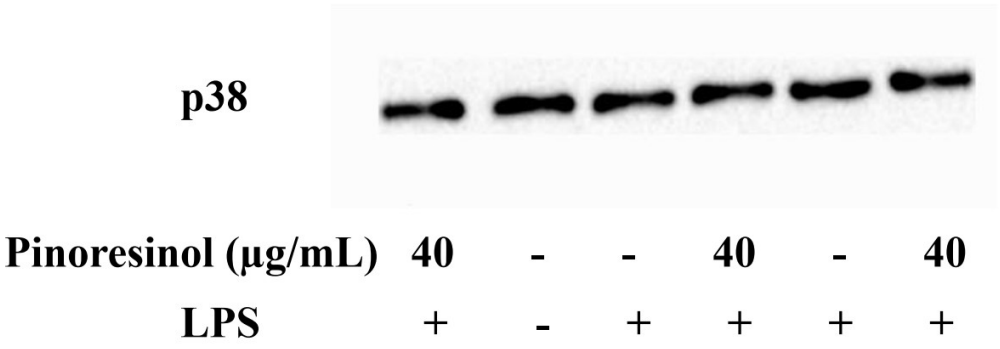
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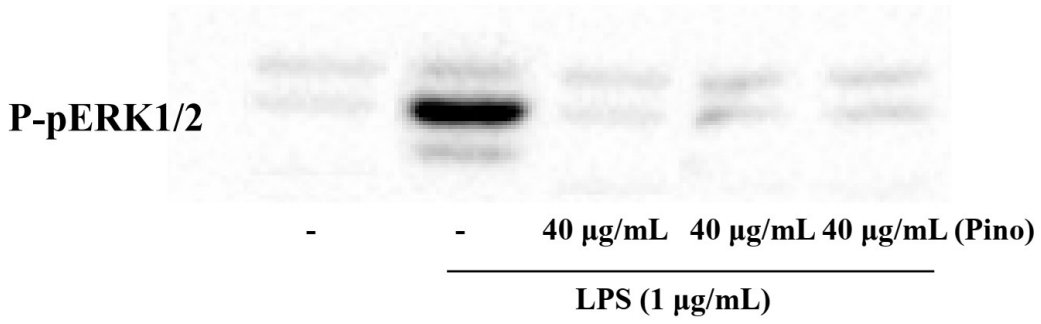
The author group has added

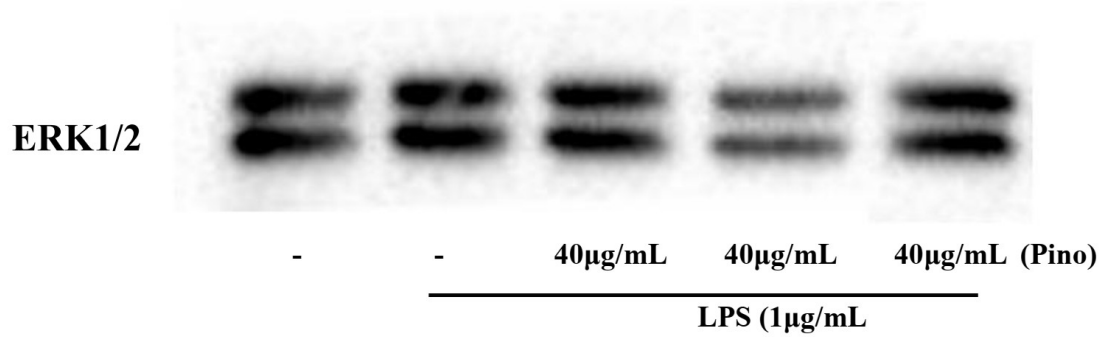
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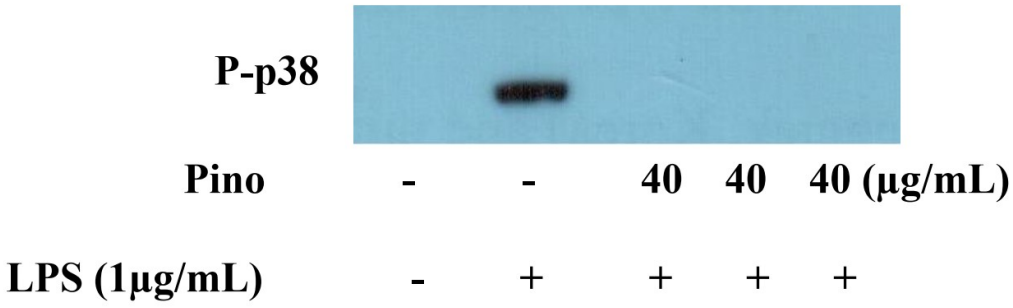


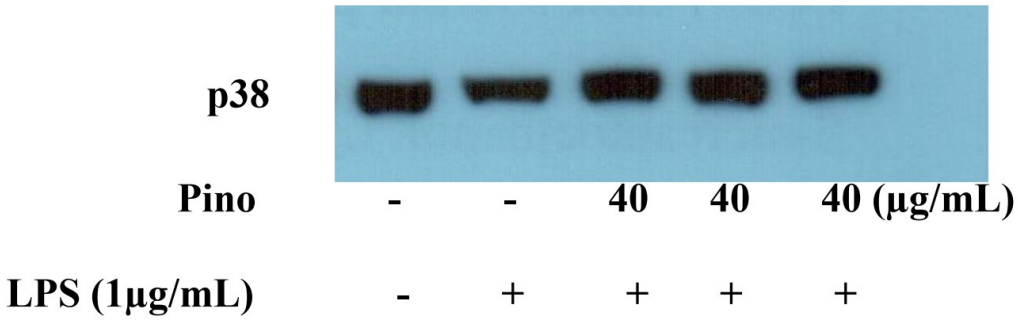




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add more: Figure 6

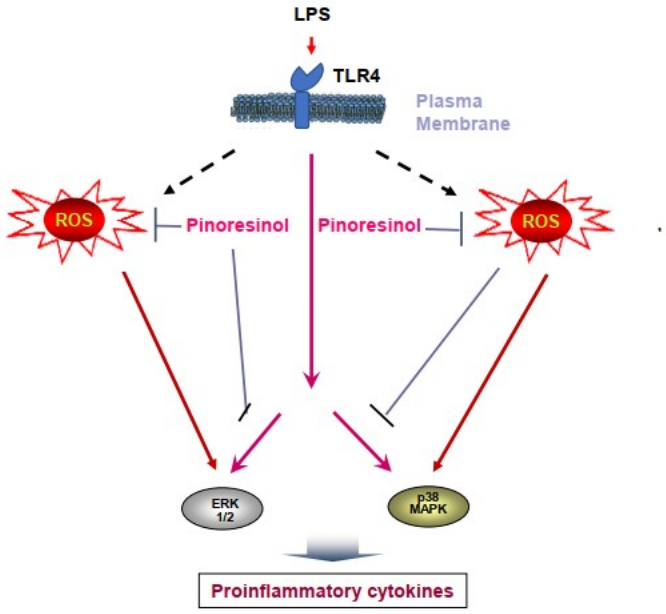


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The authors have added (page 17of 36; line number: 123-127

+ The authors should provide some statements as to why they selected/employed the indicated concentrations of pinoresinol.

The authors have added (page 17 of 36; Line 112-114)

Reviewer Assistant Comments:

Peer Review Using CONSORT Checklist

1. **Title and Abstract**
    - **Question**: Identification as a randomized trial in the title.
    - **Peer Review**: The abstract does not identify the study as a randomized trial in the title. It focuses on the anti-inflammatory effects of pinoresinol.

2. **Abstract**
    - **Question**: Structured summary of trial design, methods, results, and conclusions.
    - **Peer Review**: The abstract provides a structured summary of the study's background, objectives, methods, results, and conclusions. However, it does not specify a trial design, indicating it may not be a randomized trial.
3. **Introduction**
    - **Question**: Scientific background and explanation of rationale.
    - **Peer Review**: The abstract provides a scientific background and rationale for the study, highlighting the need for further research on pinoresinol's anti-inflammatory activity.
4. **Methods**
    - **Question**: Description of trial design (such as parallel, factorial), including allocation ratio.
    - **Peer Review**: The abstract does not describe a trial design or allocation ratio, suggesting it may not be a randomized trial.
5. **Participants**
    - **Question**: Eligibility criteria for participants.
    - **Peer Review**: The abstract does not mention eligibility criteria for participants, indicating a lack of detail typical for randomized trials.
6. **Interventions**
    - **Question**: The experimental and control interventions for each group with sufficient details to allow replication.
    - **Peer Review**: The abstract describes the intervention (pinoresinol) but does not mention a control group, which is essential for a randomized trial.
7. **Outcomes**
    - **Question**: Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed.
    - **Peer Review**: The abstract specifies outcomes such as the decrease in proinflammatory cytokines and ROS inhibition but lacks detail on how and when these were assessed.
8. **Results**
    - **Question**: For each primary and secondary outcome, results for each group and the estimated effect size and its precision (such as 95% confidence interval).
    - **Peer Review**: The abstract provides results indicating significant decreases in cytokines and ROS but does not provide effect sizes or confidence intervals.

Categorization of Comments

Major Comments:

- The study does not identify itself as a randomized trial, which is a significant requirement for using the CONSORT checklist.
- Lack of detail on trial design, participant eligibility, and control interventions suggests it may not be a randomized trial.

Minor Comments:

- The abstract provides a good summary of the background, objectives, and results but lacks specific methodological details typical of a randomized trial.
- Absence of effect sizes and confidence intervals in the results section.

Given these observations, it seems the study may not fit the criteria for a randomized trial, and thus, the CONSORT checklist may not be entirely applicable. The study might be more aligned with an experimental design without randomization.

1. **Title and Abstract**

o Question: Identification as a randomized trial in the title.

o Peer Review: The abstract does not identify the study as a randomized trial in the title. It focuses on the anti-inflammatory effects of pinoresinol.

+ Since this article focuses on the inflammatory effects of pinoresinol, the subjects were pre-selected and therefore not a randomized trial.

2. Abstract

o Question: Structured summary of trial design, methods, results, and conclusions.

o Peer Review: The abstract provides a structured summary of the study's background, objectives, methods, results, and conclusions. However, it does not specify a trial design, indicating it may not be a randomized trial.

+ As this article focuses on a specific subject, pinoresinol, the trial design of the randomized trial is not mentioned.

3. Introduction

o Question: Scientific background and explanation of rationale.

o Peer Review: The abstract provides a scientific background and rationale for the study, highlighting the need for further research on pinoresinol's anti-inflammatory activity.

+ This article focuses on the in-depth study of the anti-inflammatory mechanism of pinoresinol

4. Methods

o Question: Description of trial design (such as parallel, factorial), including allocation ratio.

o Peer Review: The abstract does not describe a trial design or allocation ratio, suggesting it may not be a randomized trial.

+ This article is not a randomized trial, so the experimental design or the supplementary ratio is not described in the experimental description

5. Participants

o Question: Eligibility criteria for participants.

o Peer Review: The abstract does not mention eligibility criteria for participants, indicating a lack of detail typical for randomized trials.

+ Since the article does not mention randomized trials, the abstract does not mention eligibility criteria for participants.

6. Interventions

o Question: The experimental and control interventions for each group with sufficient details to allow replication.

o Peer Review: The abstract describes the intervention (pinoresinol) but does not mention a control group, which is essential for a randomized trial.

+ The comparisons of differences in the presence of pinoresinol with controls have been added to the results in Figures 2, 3, 4, and 5 (page 17 of 36, line number: 106-107, 111-112; 126).

7. Outcomes

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

o Peer Review: The abstract specifies outcomes such as the decrease in proinflammatory cytokines and ROS inhibition but lacks detail on how and when these were assessed.

+ The pro-inflammatory cytokine reduction and ROS inhibition outcomes were added to the results section of the article (page 17 of 36; line number: 111-112; 126)

8. Results

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

o Peer Review: The abstract provides results indicating significant decreases in cytokines and ROS but does not provide effect sizes or confidence intervals.

+ A summary of significant cytokine and ROS reductions along with confidence intervals has been added to the results section (page 17 of 36; line number: 111-112; 126)

Major Comments:

• *The study does not identify itself as a randomized trial, which is a significant requirement for using the CONSORT checklist.*

• *Lack of detail on trial design, participant eligibility, and control interventions suggests it may not be a randomized trial.*

+ This article focuses on the inflammatory effects of pinoresinol, the subjects were pre-selected and therefore not a randomized trial

Minor Comments:

• The abstract provides a good summary of the background, objectives, and results but lacks specific methodological details typical of a randomized trial.

+ As this article demonstrated the anti-inflammatory potential of pinoresinol, methodological details relevant to the randomised trial are not included.

•Absence of effect sizes and confidence intervals in the results section.

+ *This has been added to the results section of the article.*

Thanking you,

Yours’ sincerely,

TTC

3 Revision 2

3 comments · 9 Apr 2025 – 10 Apr 2025

Referee 1 Reviewer

MINOR REVISION 9 Apr 2025

Minor Revision for acceptance. The authors are cordially required to add some paragraphs to the manuscript, similar to those they wrote/provided in response to all the questions/comments.

Associate Editor

MINOR REVISION 10 Apr 2025

Dear Author
The manuscript needs minor revision.
Best regards,
JRPS office

Editor-in-Chief

MINOR REVISION 10 Apr 2025

Dear Author
The manuscript needs minor revision.
Best regards,
JRPS office

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.

On behalf of the author group, I would like to thank the comments and authors edited the manuscript in accordance with the comments.

Reviewer 1:

Dear Editor

Review of: 156394

The respected authors have tried to show the anti-inflammatory activity of pinoresinol on the Raw 264.7 cell. Pinoresinol appears to has the strongest antiinflammatory properties (https://pubmed.ncbi.nlm.nih.gov/22955517/).

The authors are required to provide more clarified data on the identity and purity of the pinoresinol. In this regard, the quality of all sections of Figure 1 is unacceptable.

According to the TLC (figure 1b) the purity of the compound is questionable.

Authors must discuss the retention times, mention the employed equipment, and show the X-Y axes labels on Fig 1C/D.

All the Figure captions are unfortunately uninformative and should be provided as comprehensive and informative so that the reader realizes the massage of the figure caption, independent of the main text.

The provided western blotting data should be done in triplicates. Please show/claim/declare this in the supplementary data. Also, please provide raw data of figure 4 with no excision/cropping.

Complete disappearance of p-P38 under the effect of pinoresinol in Figure 4 is so strange.

The mechanism of action of the compound should be discussed and authors are required to show that using a schematic presentation.

Why have the authors selected the mentioned parameters (p-ERK/p-P38) and why not other mediators of inflammation?

The biggest concern of this reviewer is about work innovation/novelty. Another group has researched this issue more deeply.

https://www.researchgate.net/publication/377492430_Pinoresinol_targets_NF-kB_alongside_STAT3_pathway_to_attenuate_IL-6-induced_inflammation

Pinoresinol targets NF-κB alongside STAT3 pathway to attenuate IL-6-induced inflammation

The observations made in Fig 5: why the cells in the pinoresinol treatment group are so different in color. Logically, the amount of ROS should be decreased in all cell populations (the intensity of the color should be changed). The authors should carefully describe/interpret the obtained results.

The authors should provide some statements as to why they selected/employed the indicated concentrations of pinoresinol.

Ref.

https://onlinelibrary.wiley.com/doi/full/10.1002/hsr2.998

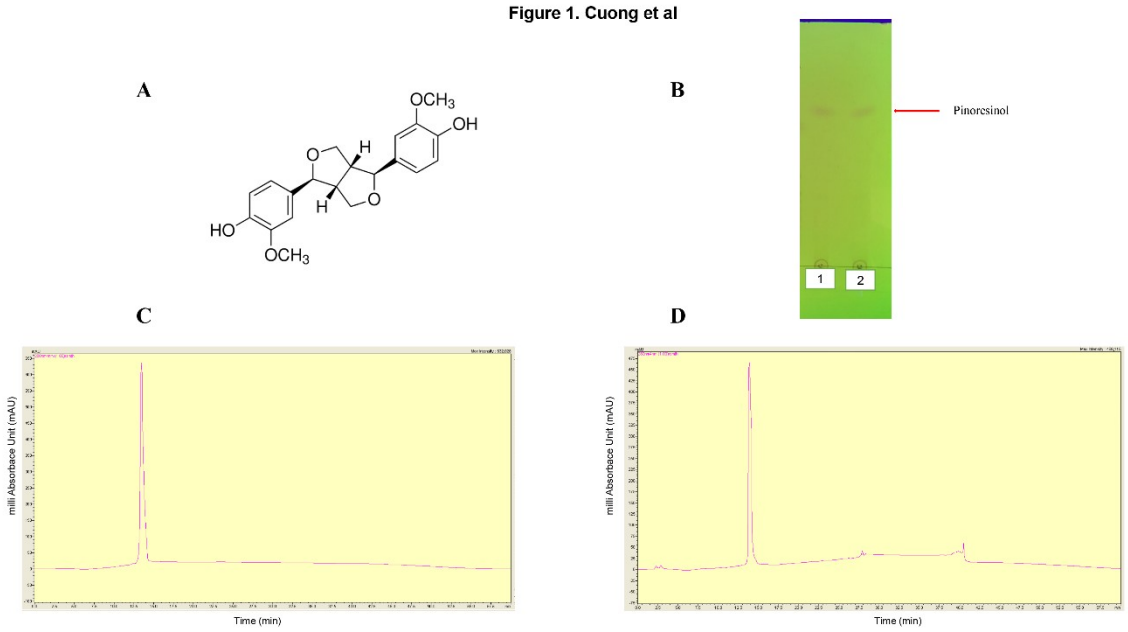
https://pubmed.ncbi.nlm.nih.gov/20600612/

https://applbiolchem.springeropen.com/articles/10.3839/jksabc.2011.027

https://onlinelibrary.wiley.com/doi/10.1155/2020/8704146#:~:text=Isoorientin%20Inhibits%20Inflammation%20in%20Macrophages,of%20Inflammation%20%2D%20Wiley%20Online%20Library

+ The authors are required to provide more clarified data on the identity and purity of the pinoresinol. In this regard, the quality of all sections of Figure 1 is unacceptable. According to the TLC (figure 1b) the purity of the compound is questionable.

Authors changed the TLC result again:



+ Authors must discuss the retention times, mention the employed equipment:

The purified pinoresinol was compatible with standard pinoresinol in both shape and retention time (Figures 1C, 1D). The HPLC results using Shimadzu 20A system indicated that the retention time of pinoresinol extracted from Gnetum montanum is same with standard (13.10 min) and same previously published results [20]. (**page 17 of 37; Line number: 101-104**)

[20] Goels, T., Eichenauer, E., Langeder, J., Hoeller, F., Sykora, C., Tahir, A., ... & Glasl, S. (2020). Norway spruce balm: Phytochemical composition and ability to enhance re-epithelialization in vitro. Planta medica, 86(15), 1080-1088.

+Authors must show the X-Y axes labels on Fig 1C/D.

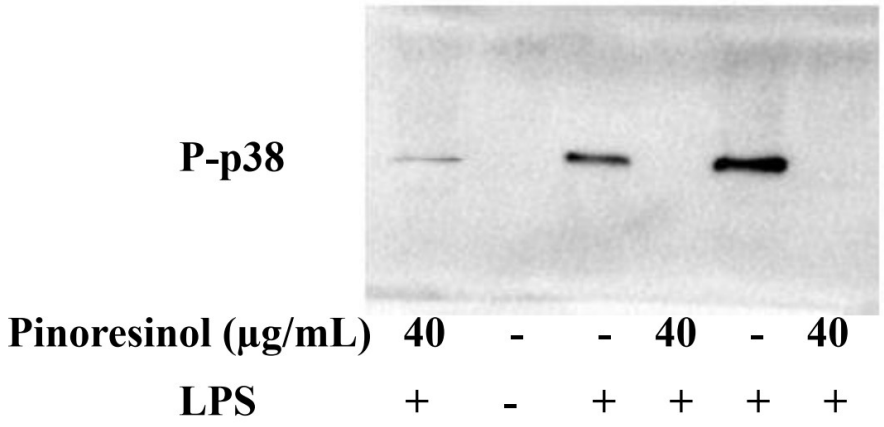
The X-Y axes labels were added on Fig 1C/D.

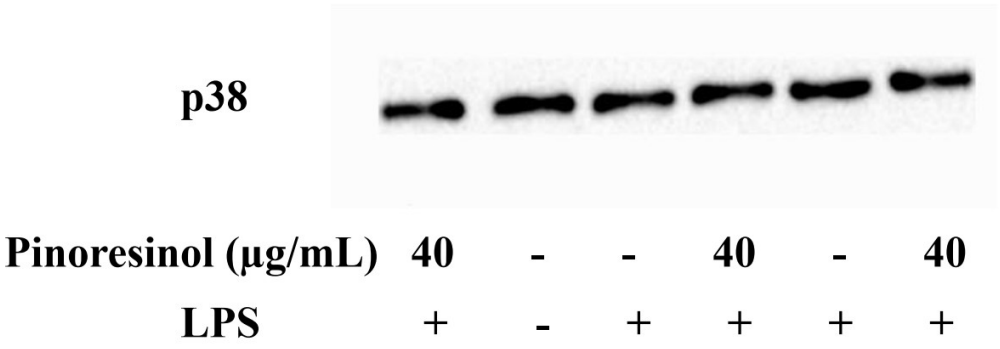
+ All the Figure captions are unfortunately uninformative and should be provided as comprehensive and informative so that the reader realizes the massage of the figure caption, independent of the main text.

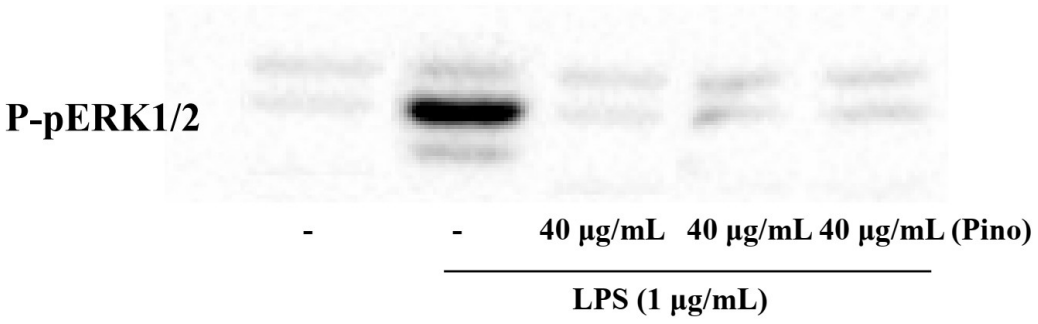
The author group has added

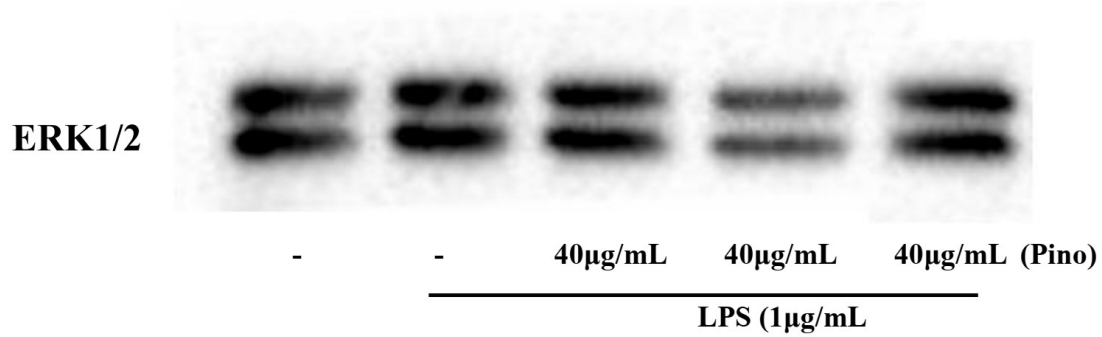
+The provided western blotting data should be done in triplicates. Please show/claim/declare this in the supplementary data. Also, please provide raw data of figure 4 with no excision/cropping.

As following: the western blot results of p-p38 and p-ERK1/2 for 3 times of the concentration of 40 µg/mL for Pinoresinol (supplementary data)



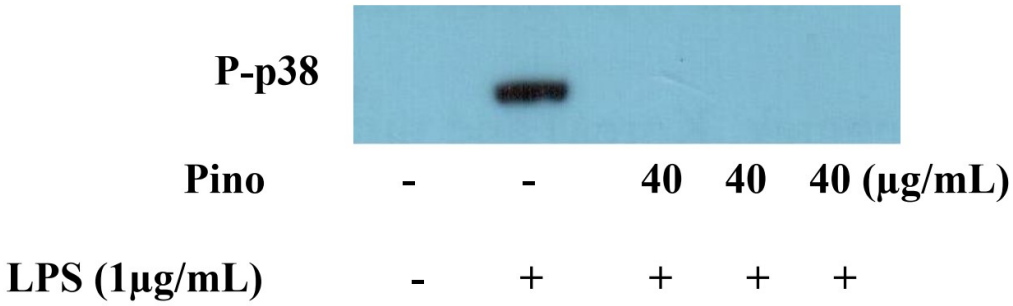


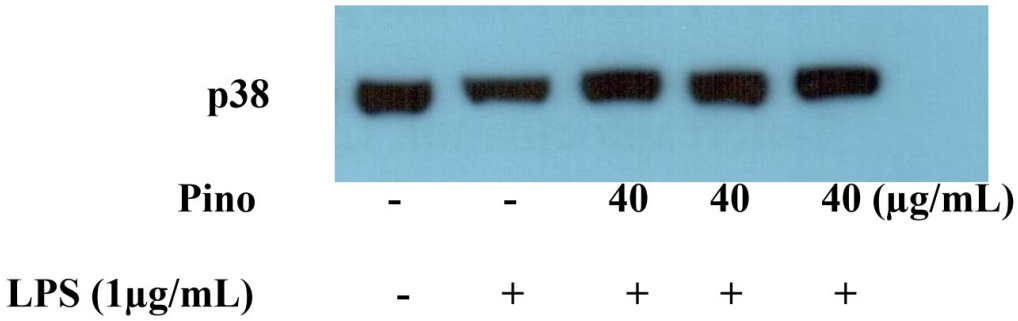




+ Complete disappearance of p-P38 under the effect of pinoresinol in Figure 4 is so strange.

With this comment, authors re-evaluated the western blot of P-p38 on film for the following results:





+ The mechanism of action of the compound should be discussed and authors are required to show that using a schematic presentation.

add more: Figure 6

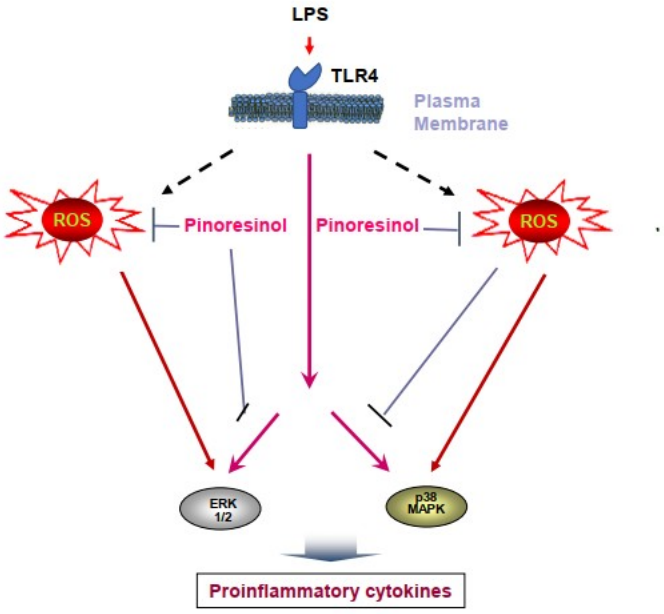


Figure 6. Schematic diagram of the anti-inflammatory and ROS mechanism of pinoresinol in macrophage cells.
LPS induces MAPK and ROS through Toll-like receptor (TLR)4. Pre-treatment with pinoresinol effectively abrogates the phosphorylation of p38, ERK1/2, and ROS production. **(page 21 of 37; Line number: 181-182).**

+ Why have the authors selected the mentioned parameters (p-ERK/p-P38) and why not other mediators of inflammation?

The p38 MAPK pathway is identified as a major regulator of the pro-inflammatory cytokine production of myeloid cells. Many anti-inflammatory drugs target this factor for evaluation on cellular, preclinical and clinical models of inflammatory disease.
Extracellular signal-associated kinases 1 and 2 (ERK1/2) are the final component of the mitogen-activated protein kinase phosphorylated layer (MAPK), an integral module in a variety of different signaling pathways to shape cell behavior and fate. ERK1/2 plays a role in the mechanism of action of immune receptors for inflammatory gene expression in response to infection and cell or tissue damage. In particular, the Toll receptor activates ERK1/2 which leads to dysfunction in the inflammatory response and is also a potential treatment for inflammatory diseases.
Currently, the inflammatory inhibition pathway of pinoresinol through NF-κB has been demonstrated by several studies. However, the effect of this substance on ROS during inflammation has not been published. From the above reasons, the authors chose p38 and ERK1/2 to research.

The biggest concern of this reviewer is about work innovation/novelty. Another group has researched this issue more deeply.

Reactive oxygen species (ROS) are signaling molecules that play an important role in the progression of inflammatory disorders. Enhancement of ROS production by leukocytes at the site of inflammation causes endothelial dysfunction and tissue damage. The past and current research in the field of inflammation, with a particular emphasis on oxidative stress-mediated signaling mechanisms that are associated with inflammation and tissue damage, which have great potential to find inflammatory active substances to aid in the treatment of inflammation-related diseases. Therefore, the results of the author's study initially indicate the role of pinoresinol on ROS in the process of causing inflammation in macrophages. This is an innovation that is different from other research groups. With these new results, authors will continue to evaluate the effects of pinoresinol on the ROS production mechanism from elements in nicotinamide adenine dinucleotide phosphate NADPH oxidase such as: p47phox, p67phox, p40phox and a GTPase Rac1 or Rac2 and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3).

+ The observations made in Fig 5: why the cells in the pinoresinol treatment group are so different in color. Logically, the amount of ROS should be decreased in all cell populations (the intensity of the color should be changed). The authors should carefully describe/interpret the obtained results.

The authors have added (page 18 of 37; line number: 122-127)

+ The authors should provide some statements as to why they selected/employed the indicated concentrations of pinoresinol.

The authors have added (page 18 of 37; Line 112-114)

Reviewer Assistant Comments:

Peer Review Using CONSORT Checklist

1. **Title and Abstract**
    - **Question**: Identification as a randomized trial in the title.
    - **Peer Review**: The abstract does not identify the study as a randomized trial in the title. It focuses on the anti-inflammatory effects of pinoresinol.

2. **Abstract**
    - **Question**: Structured summary of trial design, methods, results, and conclusions.
    - **Peer Review**: The abstract provides a structured summary of the study's background, objectives, methods, results, and conclusions. However, it does not specify a trial design, indicating it may not be a randomized trial.
3. **Introduction**
    - **Question**: Scientific background and explanation of rationale.
    - **Peer Review**: The abstract provides a scientific background and rationale for the study, highlighting the need for further research on pinoresinol's anti-inflammatory activity.
4. **Methods**
    - **Question**: Description of trial design (such as parallel, factorial), including allocation ratio.
    - **Peer Review**: The abstract does not describe a trial design or allocation ratio, suggesting it may not be a randomized trial.
5. **Participants**
    - **Question**: Eligibility criteria for participants.
    - **Peer Review**: The abstract does not mention eligibility criteria for participants, indicating a lack of detail typical for randomized trials.
6. **Interventions**
    - **Question**: The experimental and control interventions for each group with sufficient details to allow replication.
    - **Peer Review**: The abstract describes the intervention (pinoresinol) but does not mention a control group, which is essential for a randomized trial.
7. **Outcomes**
    - **Question**: Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed.
    - **Peer Review**: The abstract specifies outcomes such as the decrease in proinflammatory cytokines and ROS inhibition but lacks detail on how and when these were assessed.
8. **Results**
    - **Question**: For each primary and secondary outcome, results for each group and the estimated effect size and its precision (such as 95% confidence interval).
    - **Peer Review**: The abstract provides results indicating significant decreases in cytokines and ROS but does not provide effect sizes or confidence intervals.

Categorization of Comments

Major Comments:

- The study does not identify itself as a randomized trial, which is a significant requirement for using the CONSORT checklist.
- Lack of detail on trial design, participant eligibility, and control interventions suggests it may not be a randomized trial.

Minor Comments:

- The abstract provides a good summary of the background, objectives, and results but lacks specific methodological details typical of a randomized trial.
- Absence of effect sizes and confidence intervals in the results section.

Given these observations, it seems the study may not fit the criteria for a randomized trial, and thus, the CONSORT checklist may not be entirely applicable. The study might be more aligned with an experimental design without randomization.

1. **Title and Abstract**

o Question: Identification as a randomized trial in the title.

o Peer Review: The abstract does not identify the study as a randomized trial in the title. It focuses on the anti-inflammatory effects of pinoresinol.

+ Since this article focuses on the inflammatory effects of pinoresinol, the subjects were pre-selected and therefore not a randomized trial.

2. Abstract

o Question: Structured summary of trial design, methods, results, and conclusions.

o Peer Review: The abstract provides a structured summary of the study's background, objectives, methods, results, and conclusions. However, it does not specify a trial design, indicating it may not be a randomized trial.

+ As this article focuses on a specific subject, pinoresinol, the trial design of the randomized trial is not mentioned.

3. Introduction

o Question: Scientific background and explanation of rationale.

o Peer Review: The abstract provides a scientific background and rationale for the study, highlighting the need for further research on pinoresinol's anti-inflammatory activity.

+ This article focuses on the in-depth study of the anti-inflammatory mechanism of pinoresinol

4. Methods

o Question: Description of trial design (such as parallel, factorial), including allocation ratio.

o Peer Review: The abstract does not describe a trial design or allocation ratio, suggesting it may not be a randomized trial.

+ This article is not a randomized trial, so the experimental design or the supplementary ratio is not described in the experimental description

5. Participants

o Question: Eligibility criteria for participants.

o Peer Review: The abstract does not mention eligibility criteria for participants, indicating a lack of detail typical for randomized trials.

+ Since the article does not mention randomized trials, the abstract does not mention eligibility criteria for participants.

6. Interventions

o Question: The experimental and control interventions for each group with sufficient details to allow replication.

o Peer Review: The abstract describes the intervention (pinoresinol) but does not mention a control group, which is essential for a randomized trial.

+ The comparisons of differences in the presence of pinoresinol with controls have been added to the results in Figures 3, and 5 (page 18 of 37; line number: 111-112; 126-127).

7. Outcomes

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

o Peer Review: The abstract specifies outcomes such as the decrease in proinflammatory cytokines and ROS inhibition but lacks detail on how and when these were assessed.

+ The pro-inflammatory cytokine reduction and ROS inhibition outcomes were added to the results section of the article (page 18 of 37; line number: 111-112; 126-127)

8. Results

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

o Peer Review: The abstract provides results indicating significant decreases in cytokines and ROS but does not provide effect sizes or confidence intervals.

+ A summary of significant cytokine and ROS reductions along with confidence intervals has been added to the results section (page 18 of 37; line number: 111-112; 126-127)

Major Comments:

• *The study does not identify itself as a randomized trial, which is a significant requirement for using the CONSORT checklist.*

• *Lack of detail on trial design, participant eligibility, and control interventions suggests it may not be a randomized trial.*

+ This article focuses on the inflammatory effects of pinoresinol, the subjects were pre-selected and therefore not a randomized trial

Minor Comments:

• The abstract provides a good summary of the background, objectives, and results but lacks specific methodological details typical of a randomized trial.

+ As this article demonstrated the anti-inflammatory potential of pinoresinol, methodological details relevant to the randomised trial are not included.

•Absence of effect sizes and confidence intervals in the results section.

+ This has been added to the results section of the article.

Reviewers' Comments:

Reviewer 1:

Minor Revision for acceptance. Authors are cordially required to add some paragraphs into the manuscript, similar to what they wrote/provided in response to the all questions/comments.

+ Why have the authors selected the mentioned parameters (p-ERK/p-P38) and why not other mediators of inflammation?

- The p38 MAPK pathway is identified as a major regulator of the pro-inflammatory cytokine production of myeloid cells. Many anti-inflammatory drugs target this factor for evaluation on cellular, preclinical and clinical models of inflammatory disease. Extracellular signal-associated kinases 1 and 2 (ERK1/2) are the final component of the mitogen-activated protein kinase phosphorylated layer (MAPK), an integral module in a variety of different signaling pathways to shape cell behavior and fate. ERK1/2 plays a role in the mechanism of action of immune receptors for inflammatory gene expression in response to infection and cell or tissue damage. In particular, the Toll receptor activates ERK1/2 which leads to dysfunction in the inflammatory response and is also a potential treatment for inflammatory diseases. **(page 19 of 37; line number: 144-153)**

+ The biggest concern of this reviewer is about work innovation/novelty. Another group has researched this issue more deeply.

- Enhancement of ROS production by leukocytes at the site of inflammation causes endothelial dysfunction and tissue damage. The past and current research in the field of inflammation, with a particular emphasis on oxidative stress-mediated signaling mechanisms that are associated with inflammation and tissue damage, which have great potential to find inflammatory active substances to aid in the treatment of inflammation-related diseases. ROS plays an important role in the MAPK signaling pathways [30]. However, the effect of pinoresinol on ROS during inflammation has not been published. **(page 20 of 37; line number: 160-166).**

Thanking you,

Yours’ sincerely,

TTC

4 Revision 3

3 comments · 22 Apr 2025 – 22 Apr 2025

Referee 1 Reviewer

ACCEPT 22 Apr 2025

Dear Editor
In my opinion, the manuscript is now ready to be published.

Associate Editor

ACCEPT 22 Apr 2025

Dear Authors
The manuscript was accepted.
Best regards,
EIC

Editor-in-Chief

ACCEPT 22 Apr 2025

Dear Authors
The manuscript was accepted.
Best regards,
EIC