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OPEN PEER REVIEW

Evaluating the Protective Effect of Rutin Nanoformulation in a Rat Model of Acetic Acid-Induced Ulcerative Colitis

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About this report

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1 Original submission

2 comments · 14 Sep 2024 – 2 Nov 2024

Referee 1 Reviewer

MINOR REVISION 14 Sep 2024

With greetings and respect
We would like to express our gratitude to the research team.
1- In the pathology samples, the cases of damage indicated in the text should be marked with an arrow or a colored star.
2- If another slide photo is available for Figure 9, it should replace the current photo. The current photo is not of good quality.
3- Please specify the magnification at which each figure was studied.

Associate Editor

MAJOR REVISION 2 Nov 2024

Dear Dr. Farzaei
Your manuscript entitled "Evaluating the protective effect of rutin nano-formulation in a rat model of acetic acid-induced ulcerative colitis", which you submitted to the JJNPP, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter. The reviewer(s) have recommended major revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
With regards
Reviewer1:
While the manuscript addresses a relevant research question, its quality is hampered by grammatical errors, insufficient methodological detail, and a lack of clarity in statistical analysis.
1. Can you provide a more detailed description of the microbial analysis methods used, including any controls or standards implemented during the testing of the rutin nanoparticles?
2. What techniques were applied in the characterization of nanoparticles in the article?
3. What criteria were used to select the specific dosages of rutin nanoparticles (100, 150, and 200 mg/kg) for testing? How do you justify the sample size in terms of statistical power and representativeness of the data?
4. The results section presents numerous statistical comparisons. Can you clarify how you determined the significance thresholds for these comparisons?
5. How do the observed changes in biochemical markers (GSH, NO, SOD, TNF-alpha) translate to clinical relevance in the context of ulcerative colitis treatment?
6. What limitations do you acknowledge in your study, and how might they affect the interpretation of your results? Did you consider any potential biases in your findings?
7. How do your findings compare with previous studies on the effects of rutin and other natural compounds in the treatment of inflammatory bowel diseases? Can you elaborate on the implications of your results in the context of public health?
8. Can you provide more insight into the underlying mechanisms through which rutin nanoparticles exert their protective effects in the context of ulcerative colitis?
9. Could you please provide the conclusion section or specify the main findings of the article? This way, I can help you draft a suitable conclusion.
10. Ensure that the context is clear regarding what the numbers represent. For example, specify what the "4" refers to (e.g., mean score of colonic damage) and what the "±0.81" represents (standard deviation). For example (Table 3):
"4.00 ± 0.81" (using two decimal places for consistency).

"Mean ± SD: 4.00 ± 0.81" for clarity.
11. The manuscript contains numerous grammatical and typographical errors. Have you thoroughly proofread the manuscript, and how do you plan to improve the clarity and structure of the manuscript for better readability? Here are some examples of grammatical and typographical errors in the manuscript:
1. Sentence Structure:
• Original: "Although the pathophysiological nature of these two is different, they are comparable in one aspect which they are influenced by the same factors..."
• Suggested Correction: "Although the pathophysiological nature of these two conditions is different, they are comparable in that they are influenced by the same factors..."
2. Punctuation:
• Original: "IBD is a chronic gastrointestinal illness that includes both health conditions named, Crohn's disease and ulcerative colitis."
• Suggested Correction: "IBD is a chronic gastrointestinal illness that includes both conditions: Crohn's disease and ulcerative colitis."
3. Word Choice:
• Original: "Recent researches have suggested that, through underlying mechanisms, oxidative stress is a major factor in the development process of intestinal inflammation."
• Suggested Correction: "Recent research has suggested that oxidative stress is a major factor in the development of intestinal inflammation through various underlying mechanisms."
4. Awkward Phrasing:
• Original: "Rutin has been synthesized into nanoparticles and tested subsequently."
• Suggested Correction: "Rutin has been synthesized into nanoparticles and subsequently tested."
5. Inconsistent Terminology:
• Original: "A medical-grade polyurethane canal measuring 2 mm in outer diameter was placed into the anus for enteral feeding..."
• Suggested Correction: "A medical-grade polyurethane catheter measuring 2 mm in outer diameter was inserted into the anus for enteral feeding..."
6. Misplaced Modifiers:
• Original: "The findings showed that, in comparison to the negative control group, a group of rats given 200 mg/kg of the medication had a significantly higher SOD level."
• Suggested Correction: "The findings showed that a group of rats given 200 mg/kg of the medication had a significantly higher SOD level compared to the negative control group."
7. Grammatical Agreement:
• Original: "The group that was given the medication at a dose of 150 mg/kg showed a relative decrease."
• Suggested Correction: "The group that received the medication at a dose of 150 mg/kg showed a relative decrease."
These examples illustrate the need for careful proofreading to enhance the clarity and professionalism of the manuscript.
Reviewer2:
With greetings and respect
We would like to express our gratitude to the research team.
1- In the pathology samples, the cases of damage indicated in the text should be marked with an arrow or a colored star.
2- If there is another slide photo of Figure 9, it should be replaced. The current photo is not of good quality.
3- Please specify the magnification at which each figure was studied.
4- Why did the authors use both mesalazine and sulfasalazine together?

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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.

Subject: Manuscript Revision Required

Manuscript Title: Evaluating the protective effect of rutin nano-formulation in a rat model of acetic acid-induced ulcerative colitis

Manuscript ID:154573

The authors of this manuscript express their sincere gratitude to the reviewers for their critical evaluation of our work. They have responded to the recommendations sent by the referees on December 2024 and have made changes in the article's text.

All modifications incorporated in the manuscript are highlighted in Yellow font. Below is a “point-by-point” response to the reviewers’ comments.

These measures have led to a significant improvement in the quality of this version.

Reviewer Comments:

Reviewer 1:

✓ **Comment 1:**In the pathology samples, the cases of damage indicated in the text should be marked with an arrow or a colored star.

Response:

It's done

✓ **Comment 2:** If there is another slide photo of Figure 9, it should be replaced. The current photo is not of good quality.

Response:

It's done; we hope this Figure provides appropriate quality.

✓ **Comment 3:** Please specify the magnification of each figure with which magnification it was studied.

Response:

It's done

Reviewer 2:

✓ **Comment 1:** Can you provide a more detailed description of the microbial analysis methods used, including any controls or standards implemented during the testing of the rutin nanoparticles?

Response:

Thank you for your valuable feedback on our manuscript. We appreciate the opportunity to clarify our methods concerning microbial analysis, even though it was not the primary focus of our current study. But in our previous study, the following explanations were investigated, which we have avoided retelling and just referenced (*Gravandi MM, Pourmanouchehri Z, Behbood L, Fakhri S, Mohammadi-Noori E, Zhaleh M, Shirvani S, Kiani A, Farzaei MH. Rutin-loaded chitosan nanoparticles alleviated Freund's adjuvant induced rheumatoid arthritis via modulating oxidative stress and inflammatory parameters in Wistar rats. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024 Jul;397(7):4771-90.*). However, as per your comment, we explain the following text briefly in sections 2.3 and 2.4 **(**2.3 Nanoparticle Characterization and 2.4 Cytotoxicity and Biocompatibility Assessment**),** and in addition, we add this information briefly to the end of the discussion section (highlighted in yellow)

Our study utilized rutin-loaded chitosan nanoparticles (R-CNPs) prepared through the ionic gelation method, which ensured optimal size and charge for biological interactions. Detailed characterization using SEM and FTIR confirmed their stability and integrity, which is essential for consistent interaction with biological systems.

While the direct microbial analysis wasn't conducted, the in vivo results in our rat model—specifically improvements in oxidative stress and inflammatory markers—indirectly suggest that our treatment did not disrupt the microbial balance. These physiological benefits help substantiate that the gut environment was not adversely affected.

We employed cytotoxicity assays (MTT assay) to confirm the biocompatibility of R-CNPs, ensuring they are safe for biological environments and indirectly suggesting minimal impact on gut microbiota.

Extensive biochemical and histopathological evaluations reflected significant therapeutic outcomes without adverse reactions that could imply microbial imbalance. This indirectly supports the microbial safety of our nanoparticle formulation.

We acknowledge the importance of directly analyzing microbial interactions and propose to include such assessments in future studies to deepen our understanding of the role of the microbiome in modulating the therapeutic effects of R-CNPs.

We hope this explanation addresses your concerns. We are grateful for your input, which will inform future research directions to explore the impact of our formulations on microbial health fully.

✓**Comment 2:** What techniques were applied in the characterization of nanoparticles in the article?

Response:

As I mentioned in the previous answer to your comment, in our last study, the characterization of nanoparticles was investigated, which we have avoided retelling these results and just referenced (*Gravandi MM, Pourmanouchehri Z, Behbood L, Fakhri S, Mohammadi-Noori E, Zhaleh M, Shirvani S, Kiani A, Farzaei MH. Rutin-loaded chitosan nanoparticles alleviated Freund's adjuvant induced rheumatoid arthritis via modulating oxidative stress and inflammatory parameters in Wistar rats. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024 Jul;397(7):4771-90.*). However, per your comment, we explain the following text briefly in sections 2.3 and 2.4 **(**2.3 Nanoparticle characterization and 2.4 Cytotoxicity and biocompatibility sssessment**),** highlighted in yellow.

✓**Comment 3:** What criteria were used to select the specific dosages of rutin nanoparticles (100, 150, and 200 mg/kg) for testing? How do you justify the sample size in terms of statistical power and representativeness of the data?

Response:

The specific dosages of rutin nanoparticles (100, 150, and 200 mg/kg) were selected based on previous studies and preliminary experiments to determine effective doses for anti-inflammatory and antioxidant effects. The selected dosages were chosen to cover a range of concentrations that could exhibit therapeutic efficacy without causing toxicity. These dosages allow for the evaluation of dose-dependent responses and the identification of the optimal dosage for therapeutic benefits.

The specific dosages of rutin nanoparticles (100, 150, and 200 mg/kg) were selected based on the following:

1. Previous studies on the therapeutic effects of rutin and its nanoparticles informed the initial selection of doses. Studies have indicated that rutin exhibits significant pharmacological effects at these concentrations.

- Example Reference: Naseri M, Delfani H, Mirzaeei S, Bahrami G, Farzaei MH, Pour PM. Protective Effect of Nanoparticles of Oleoresin of Pistacia atlantica var. mutica against Acetic Acid-Induced Ulcerative Colitis in Rats. Traditional and Integrative Medicine. 2022 Jun 28.
- Zhao X, Chen X, Yue C. Rutin ameliorates inflammation and oxidative stress in ulcerative colitis by inhibiting NLRP3 inflammasome signaling pathway. Cell Biochemistry and Biophysics. 2024 Aug 13:1-2.

2. Initial trials identified these doses as effective and non-toxic, providing a strong basis for the main study.
3. These doses allow for evaluating therapeutic efficacy and dose-dependent responses.

The sample size was determined through statistical power analysis to ensure adequate power (80% at a 5% significance level), balancing reliability and ethical considerations. Thirty adult male Wistar rats were divided into six groups, aligning with ethical guidelines and study feasibility.

In this study, thirty adult male Wistar rats were divided into six groups, each comprising five. This sample size was selected to balance statistical power, ethical considerations, and the feasibility of conducting the experiments within available resources.

By adhering to these criteria, we ensured that the selected dosages of rutin nanoparticles and the sample size were scientifically and ethically justified, providing robust and reliable data for our study.

✓Comment 4: The results section presents numerous statistical comparisons. Can you clarify how you determined the significance thresholds for these comparisons?

Response:

We appreciate your comment. In addition to the following explanations, we added a paragraph to the section of 3.2 Biochemical Analyses, with the title 3.2.1. Statistical analysis explanation. As per your comment, we explain the following text briefly in this section (3.2.1), highlighted in yellow. This way, readers understand how the significance thresholds were determined and applied throughout our analysis.

As you mentioned, the results section presents numerous statistical comparisons. We determined the significance thresholds for these comparisons based on commonly accepted statistical standards in biomedical research. Specifically, we used the following p-value thresholds to assess statistical significance:

- For p-value < 0.05, we used a single asterisk (*).
- For p-value < 0.01, we used two asterisks (**).

- For p-value < 0.001, we used three asterisks (***).

Our study made multiple comparisons across different groups, including the negative control, sham, and various treatment groups. To ensure clarity, the following symbols were employed to indicate statistical significance relative to specific comparisons:

- * indicates significance compared to the fictitious group.
- # indicates significance compared to the negative control group.
- @ indicates significance compared to the positive control group.
- $ indicates significance compared to the group that received a 100 mg/kg dose.
- & indicates significance compared to the group that received a 150 mg/kg dose.

For example, in the Glutathione Assay (GSH)**,** as shown in Figure 2**:**

- The negative control group's GSH level was significantly lower than the sham group's (p-value < 0.001).
- The group of rats given 100 mg/kg of the medication showed significantly higher GSH levels compared to the negative control group (p-value < 0.001), and it was also different from the sham group (p-value < 0.05).

Similarly, for the Nitrite Assay (NO)**,** as indicated in Figure 3**:**

- The negative control group's nitric oxide level was significantly higher than that of the sham group (p-value < 0.01).
- The positive control group's NO level was lower than the negative control group (p-value < 0.001).

These significance levels were determined using one-way ANOVA followed by a post hoc Newman-Keuls multiple comparison test, ensuring robust statistical analysis. This approach allows us to confidently determine and report differences among the groups, providing a clear understanding of the treatments' effects.

By adhering to these statistical standards, we ensured that our results were both rigorous and interpretable, supporting the conclusions drawn from our study.

✓ **Comment 5:** How do the observed changes in biochemical markers (GSH, NO, SOD, TNF-alpha) translate to clinical relevance in the context of ulcerative colitis treatment?

Response:

The observed changes in biochemical markers (GSH, NO, SOD, and TNF-alpha) have significant clinical relevance in the context of ulcerative colitis (UC) treatment.

1. Glutathione (GSH): Higher GSH levels indicate improved antioxidant defenses, which help reduce oxidative stress and prevent tissue damage in UC patients. This enhances mucosal integrity and overall colonic health, potentially leading to better disease management and symptom relief.

Reference:

- Dziąbowska-Grabias, Katarzyna, et al. "Antioxidant therapy in inflammatory bowel diseases." *Antioxidants* 10.3 (2021): 412. This review discusses the potential of antioxidant therapy, including glutathione, in managing inflammatory bowel diseases like UC.

- Lamb, Christopher Andrew, et al. "British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults." *Gut* 68.Suppl 3 (2019): s1-s106. These guidelines highlight the importance of managing oxidative stress and inflammation in UC treatment.
- Raine, Tim, et al. "ECCO guidelines on therapeutics in ulcerative colitis: medical treatment." *Journal of Crohn's and Colitis* 16.1 (2022): 2-17. These guidelines summarize recommended medical treatments for UC, including using antioxidants to manage oxidative stress.

2. **Nitric Oxide (NO)**: Elevated NO levels are associated with inflammation and tissue damage. Lower NO levels suggest reduced inflammation and oxidative stress, aiding mucosal healing and restoring normal tissue function in UC.

Reference:

- Cross, Raymond K., and Keith T. Wilson. "Nitric oxide in inflammatory bowel disease." *Inflammatory bowel diseases* 9.3 (2003): 179-189. This review discusses the role of nitric oxide in the pathophysiology of inflammatory bowel diseases, including UC.
- Kimura, Hiroyuki, et al. "Increased nitric oxide production and inducible nitric oxide synthase activity in colonic mucosa of patients with active ulcerative colitis and Crohn's disease." *Digestive diseases and sciences* 42 (1997): 1047-1054. This study highlights the correlation between NO levels and disease activity in UC patients.

3. **Superoxide Dismutase (SOD)**: Increased SOD activity enhances the antioxidant capacity, protecting against oxidative damage caused by reactive oxygen species. This is

crucial for maintaining tissue health and preventing further damage to the inflamed colon of UC patients.

References:

- Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002; 347(6):417-29. This article provides a comprehensive overview of inflammatory bowel disease, including the role of oxidative stress and antioxidants in disease management.
- Hanauer, Stephen B. "Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities." *Inflammatory bowel diseases* 12.suppl_1 (2006): S3-S9. This review discusses the pathogenesis of inflammatory bowel disease and highlights the importance of managing oxidative stress.

4. **Tumor Necrosis Factor-alpha (TNF-alpha)**: High TNF-alpha levels are implicated in UC's inflammatory processes. Reduced TNF-alpha levels indicate a strong anti-inflammatory effect, essential for reducing inflammation, promoting mucosal healing, and improving clinical outcomes in UC patients.

References:

- Feuerstein, Joseph D., et al. "AGA clinical practice guidelines on the management of moderate to severe ulcerative colitis." *Gastroenterology* 158.5 (2020): 1450-1461. These guidelines discuss the role of TNF-alpha in UC and the benefits of reducing its levels.
- Cross, Raymond K., and Keith T. Wilson. "Nitric oxide in inflammatory bowel disease." *Inflammatory bowel diseases* 9.3 (2003): 179-189. This review discusses the role of TNF-alpha in the pathophysiology of inflammatory bowel diseases, including UC.
- Jang, Dan-in, et al. "The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics." *International journal of molecular sciences* 22.5 (2021): 2719. This article highlights the involvement of TNF-alpha in the pathogenesis of inflammatory and autoimmune diseases, including UC.

These biochemical changes demonstrate that rutin nanoparticles can modulate key pathways involved in UC pathogenesis, translating to clinical benefits such as reduced inflammation, enhanced antioxidant defenses, and improved mucosal healing. These findings suggest that rutin nanoparticles could be a valuable adjunctive therapy in managing UC, improving patient outcomes and quality of life.

✓ **Comment 6:** What limitations do you acknowledge in your study, and how might they affect the interpretation of your results? Did you consider any potential biases in your findings?

Response:

Thank you for your valuable comment. It should be noted that in evaluating the therapeutic potential of rutin-loaded chitosan nanoparticles for ulcerative colitis, it is crucial to recognize the inherent limitations and potential biases associated with our research methodology. Following, we expand on these limitations and biases. In addition, we add this information briefly to the last of the discussion section (highlighted in yellow).

Although widely accepted, the acetic acid-induced ulcerative colitis model used in this study only partially represents the complex pathophysiological processes found in human inflammatory bowel disease. This may affect how our findings can be generalized to human clinical scenarios.

The treatment and observation periods were relatively short, possibly limiting the ability to observe long-term effects and potential delayed toxicities of the rutin nanoparticles. Future studies should extend these periods to capture a more comprehensive safety and efficacy profile.

Our study exclusively utilized male Wistar rats. Given the potential for sex-based biological differences, this choice could introduce gender-related bias into the results. Including both male and female subjects in future studies could help mitigate this bias and provide a more complete picture of the therapeutic effects.

While efforts were made to control environmental factors such as diet and housing conditions, these elements could still contribute to outcome variability. Stringent controls or detailed documentation of these factors are essential for reducing non-treatment-related influences.

Although we comprehensively characterized the nanoparticles using techniques such as DLS, SEM, and FTIR, the in vivo interactions between the nanoparticles and bioentities like the gut microbiota remain unexplored. Future research should include such analyses to determine unintended effects on gut microbial populations and their implications for human health.

The potential for bias in data interpretation was mitigated by using blinded assessors for tissue evaluation; however, subjective interpretations could still influence results. Employing more standardized scoring systems or automated analysis methods could reduce this bias.

Addressing these limitations, our future research agenda includes expanding the study design to incorporate longer treatment durations and diverse animal models that more closely mimic human disease. Moreover, we plan to conduct sex-specific analyses to explore gender responses to treatment. Incorporating advanced omics technologies and microbial profiling will deepen our understanding of nanoparticle-microbiome interactions and their roles in modulating the therapeutic efficacy of rutin-loaded nanoparticles.

✓ **Comment 7:** How do your findings compare with previous studies on the effects of rutin and other natural compounds in the treatment of inflammatory bowel diseases? Can you elaborate on the implications of your results in the context of public health?

Response:

Our findings are consistent with previous studies demonstrating the beneficial effects of rutin and other natural compounds in treating inflammatory bowel diseases (IBD) such as ulcerative colitis. Rutin has been shown to possess anti-inflammatory and antioxidant properties, which help reduce inflammation and oxidative stress in the intestinal mucosa. Regarding your comment, we added the following explanation briefly to the end of the discussion section (highlighted in yellow).

Comparison with Previous Studies:

1. Rutin**:** Similar to our study, previous research has highlighted rutin's ability to modulate inflammatory pathways and enhance antioxidant defenses. For instance, "Rutin ameliorates inflammation and oxidative stress in ulcerative colitis by inhibiting NLRP3 inflammasome signaling pathway" (Zhao et al., 2024) demonstrated that rutin reduced inflammation and oxidative damage in a colitis model by inhibiting NLRP3 inflammasome signaling. Another study, "Rutin mitigates acetic acid-induced ulcerative colitis: novel coloprotective mechanism" (Sherif et al., 2024), supports our findings by showing rutin's protective effects in a similar model.
2. Other Natural Compounds**:** Compounds like curcumin, quercetin, and resveratrol have also shown promise in treating IBD by modulating inflammatory pathways and oxidative stress. For example, "Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice" (Kwon et al., 2005) highlighted the effectiveness of rutin over quercetin in reducing colonic inflammation. Similarly, "The small intestinal mucosa acts as a rutin reservoir to extend flavonoid anti-inflammatory activity in experimental ileitis and colitis" (Mascaraque et al., 2015) demonstrated rutin's prolonged anti-inflammatory effects.

Our results suggest that rutin-loaded nanoparticles could offer a novel therapeutic approach for IBD, providing several public health benefits (Mascaraque et al., 2015, Habtemariam et al. 2018):

1. Alternative to Conventional Therapies**:** Natural compounds like rutin may offer a safer and more tolerable alternative to conventional IBD treatments, which often have significant side effects.

2. Cost-Effective Treatment: As natural compounds are generally more affordable and accessible, their use in IBD treatment could reduce healthcare costs and improve accessibility, especially in resource-limited settings.
3. Preventative Health: The anti-inflammatory and antioxidant properties of rutin could also be beneficial in preventing IBD flare-ups and maintaining remission, improving patients' quality of life and reducing the burden on healthcare systems.

References:

- Zhao X, Chen X, Yue C. Rutin ameliorates inflammation and oxidative stress in ulcerative colitis by inhibiting NLRP3 inflammasome signaling pathway. Cell Biochemistry and Biophysics. 2024 Aug 13:1-2.
- Sherif IO, Al-Shaalan NH, Awadin WF. Rutin mitigates acetic acid-induced ulcerative colitis: novel coloprotective mechanism. Toxicology Research. 2024 Aug;13(4):tfae108.
- Kwon KH, Murakami A, Tanaka T, Ohigashi H. Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. Biochemical pharmacology. 2005 Feb 1;69(3):395-406.
- Mascaraque C, López-Posadas R, Monte MJ, Romero-Calvo I, Daddaoua A, González M, Martínez-Plata E, Suárez MD, González R, Marín JJ, Zarzuelo A. The small intestinal mucosa acts as a rutin reservoir to extend flavonoid anti-inflammatory activity in experimental ileitis and colitis. Journal of Functional Foods. 2015 Mar 1;13:117-25.
- Mascaraque C, López-Posadas R, Monte MJ, Romero-Calvo I, Daddaoua A, González M, et al. The small intestinal mucosa acts as a rutin reservoir to extend flavonoid anti-inflammatory activity in experimental ileitis and colitis. Journal of Functional Foods. 2015;13:117-25.
- Habtemariam S, Belai A. Natural therapies of the inflammatory bowel disease: the case of rutin and its aglycone, quercetin. Mini reviews in medicinal chemistry. 2018;18(3):234-43.

✓ **Comment 8:** Can you provide more insight into the underlying mechanisms through which rutin nanoparticles exert their protective effects in the context of ulcerative colitis?

Response:

Rutin nanoparticles exert their protective effects in ulcerative colitis (UC) through several underlying mechanisms, offering significant therapeutic benefits:

1. Reduction of Colonic Damage**:** The macroscopic evaluation demonstrated that treatment with rutin-loaded nanoparticles significantly reduced colonic damage dose-dependently. This suggests that rutin nanoparticles contribute to preserving colonic integrity, likely

through their anti-inflammatory and antioxidant properties. Higher doses displayed the most pronounced protective effects, reducing damage as measured by severity scores.

2. Enhancement of Antioxidant Defense **(**Zhao et al., 2024**)**:
    - Glutathione (GSH) Levels**:** Rutin nanoparticles significantly increased GSH levels. Glutathione neutralizes reactive oxygen species (ROS), contributing to UC oxidative stress. By boosting GSH, rutin nanoparticles counteract oxidative damage to colonic tissues.
    - Catalase and SOD Activity**:** Increases in catalase and superoxide dismutase (SOD) activities imply that rutin enhances the enzymatic defense against hydrogen peroxide and superoxide radicals, respectively. This reinforces the antioxidant shield, minimizing tissue damage from oxidative stress.
3. Modulation of Inflammatory Pathways**:**
    - Rutin has been shown to inhibit the NLRP3 inflammasome, a key component of the inflammatory response in UC. By suppressing the activation of this inflammasome, rutin reduces the production of pro-inflammatory cytokines such as IL-1β, IL-6, and IL-18, thereby mitigating inflammation (Zhao et al., 2024).
    - Rutin modulates various signaling pathways involved in inflammation, such as the NF-κB pathway. By inhibiting the activation of NF-κB, rutin reduces the expression of pro-inflammatory genes and mediators, leading to decreased inflammation (Sherif et al., 2024 & Zhao et al., 2024).
    - Treatment with rutin nanoparticles significantly reduced NO levels, often elevated in intestinal inflammatory conditions. By lowering NO, the nanoparticles likely help reduce oxidative stress and inhibit pathways that lead to further tissue damage.
    - TNF-α is a key pro-inflammatory cytokine in UC. The reduced levels of TNF-α observed in treated groups indicate that rutin nanoparticles effectively suppress inflammatory signaling, aiding in the mitigation of mucosal inflammation and subsequent damage.
4. Protection of Intestinal Barrier**:** Rutin helps to maintain the integrity of the intestinal barrier by reducing the production of matrix-degrading enzymes like matrix metalloproteinases (MMPs). This prevents the breakdown of the mucosal lining and reduces the risk of ulceration (Khare et al. 2020).
5. Histological Improvements**:** Microscopic evaluations revealed better-preserved colonic tissue and reduced inflammatory cell infiltration, indicating healing and regenerative processes in groups treated with rutin nanoparticles. The nanoformulation appears to prevent excessive immune cell infiltration and preserve tissue structure.
6. Regulation of Gut Microbiota**:** Emerging research suggests that rutin may also impact the gut microbiota, promoting a healthier microbial balance that can reduce inflammation and improve gut health (Roy et al. 2024).

These mechanisms collectively contribute to the protective effects of rutin nanoparticles in UC, offering a promising therapeutic approach for managing this chronic inflammatory condition. We rewrite the discussion section by adding these mechanisms briefly (highlighted in yellow).

References:

- Zhao X, Chen X, Yue C. Rutin ameliorates inflammation and oxidative stress in ulcerative colitis by inhibiting NLRP3 inflammasome signaling pathway. Cell Biochemistry and Biophysics. 2024:1-12.
- Sherif IO, Al-Shaalan NH, Awadin WF. Rutin mitigates acetic acid-induced ulcerative colitis: novel coloprotective mechanism. Toxicology Research. 2024;13(4):tfae108.
- Khare T, Palakurthi SS, Shah BM, Palakurthi S, Khare S. Natural product-based nanomedicine in treatment of inflammatory bowel disease. International journal of molecular sciences. 2020 May 31;21(11):3956.
- Roy, D., Mukherjee, D., Ghosh, M., Adhikari, D., Rangra, N. K., & Singh, A. (2024). Rutin's Efficacy as a Potent Flavonoid in the Management of Colitis.

✓ **Comment 9:** Could you please provide the conclusion section or specify the main findings of the article? This way, I can help you draft a suitable conclusion.

Response:

Thank you for your comment. As you said, we added the section of conclusion at the end of the article (highlighted in yellow).

Comment 10: Ensure that the context is clear regarding what the numbers represent. For example, specify what the "4" refers to (e.g., mean score of colonic damage) and what the "±0.81" represents (standard deviation). For example (Table 3):

"4.00 ± 0.81" (using two decimal places for consistency).

"Mean ± SD: 4.00 ± 0.81" for clarity.

Response:

It's done and it was corrected inside the text.

✓**Comment 11**: The manuscript contains numerous grammatical and typographical errors. Have you conducted a thorough proofreading, and how do you plan to improve the clarity and structure of the manuscript for better readability? Here are some examples of grammatical and typographical errors in the manuscript:

1. Sentence Structure:

• Original: "Although the pathophysiological nature of these two is different, they are comparable in one aspect which they are influenced by the same factors..."

• Suggested Correction: "Although the pathophysiological nature of these two conditions is different, they are comparable in that they are influenced by the same factors..."

2. Punctuation:

• Original: "IBD is a chronic gastrointestinal illness that includes both health conditions named, Crohn's disease and ulcerative colitis."

• Suggested Correction: "IBD is a chronic gastrointestinal illness that includes both conditions: Crohn's disease and ulcerative colitis."

3. Word Choice:

• Original: "Recent researches have suggested that, through underlying mechanisms, oxidative stress is a major factor in the development process of intestinal inflammation."

• Suggested Correction: "Recent research has suggested that oxidative stress is a major factor in the development of intestinal inflammation through various underlying mechanisms."

4. Awkward Phrasing:

• Original: "Rutin has been synthesized into nanoparticles and tested subsequently."

• Suggested Correction: "Rutin has been synthesized into nanoparticles and subsequently tested."

5. Inconsistent Terminology:

• Original: "A medical-grade polyurethane canal measuring 2 mm in outer diameter was placed into the anus for enteral feeding..."

• Suggested Correction: "A medical-grade polyurethane catheter measuring 2 mm in outer diameter was inserted into the anus for enteral feeding..."

6. Misplaced Modifiers:

• Original: "The findings showed that, in comparison to the negative control group, a group of rats given 200 mg/kg of the medication had a significantly higher SOD level."

• Suggested Correction: "The findings showed that a group of rats given 200 mg/kg of the medication had a significantly higher SOD level compared to the negative control group."

7. Grammatical Agreement:

• Original: "The group that was given the medication at a dose of 150 mg/kg showed a relative decrease."

• Suggested Correction: "The group that received the medication at a dose of 150 mg/kg showed a relative decrease."

These examples illustrate the need for careful proofreading to enhance the clarity and professionalism of the manuscript.

Response:

We appreciate your comment and valuable suggestion. As per your comment, the whole text was revised, and grammatical and typographical errors were corrected to improve the clarity and structure of the manuscript.

2 Revision 1

1 comment · 25 Dec 2024

Referee 1 Reviewer

MINOR REVISION 25 Dec 2024

Hello
With utmost respect and gratitude to the respected research team,
The responsible author and respected colleagues in this research project are requested to indicate the types of damage noted in the histopathology images, such as necrosis and infiltration of inflammatory cells, using arrows in different colors, and to present the grading of the damage and recovery based on the development of lesions and tissue damage, according to the table.

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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.

Reviewer 1:
Hello
With utmost respect and gratitude to the respected research team;
The responsible author and respected colleagues in this research project are requested to indicate in the histopathology images the types of damage that have been noted, such as necrosis and infiltration of inflammatory cells, etc., with arrows in different colors, and to present the grading of the damage and its recovery according to the creation of lesions and tissue damage according to the table.

Response: The arrows was added to the images.

Associate Editor's Comments:

Associate Editor 1:
we kindly request that you indicate the types of damage noted in the histopathology images—such as necrosis and infiltration of inflammatory cells—using arrows in different colors. Additionally, please provide a grading of the damage and its recovery, correlating these findings with the creation of lesions and tissue damage as outlined in the accompanying table.

Response: The arrows and grade was provided for microscopic assessment.

3 Revision 2

0 comments

No public comments in this round.