Dear editor,

Thank you for the review of our manuscript "*A recombinant protein Schistosoma japonicum-derived molecule (rSj16) attenuates DSS-induced colitis by inhibiting miRNA-217-5p to alleviate apoptosis*". We appreciate the constructive comments from the reviewers, and we are pleased to enclose the revised manuscript and point-by-point responses to the comments. We hope this revision can make our paper acceptable. Revised portion are marked in red in the manuscript.

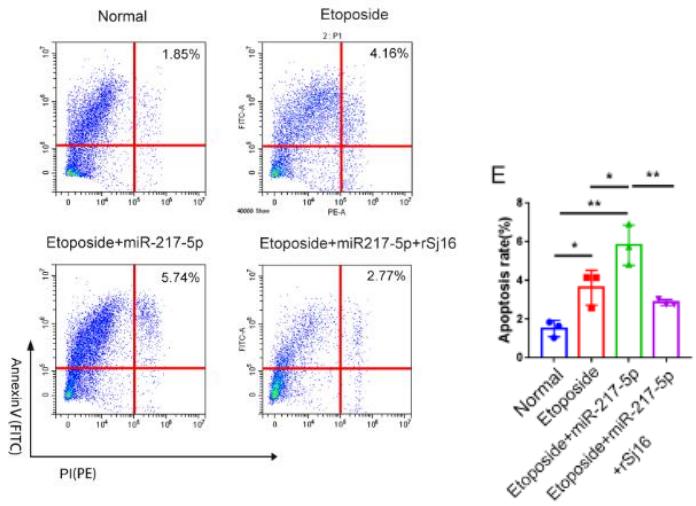
Reviewer #1:

1. Q: The descriptions in Figure 1 do not fully cover all of the items and should be corrected and supplemented.

   A: Thank you for your suggestion. We have added the relevant content in the manuscript.
   As found in our previous study, after DSS administration, the mice lost weight over time. At the same time, the DAI of mice with colitis also increased with time. After treatment with rSj16, body weight loss and DAI were both significantly alleviated in mice with colitis (Figure 1A and B). Colon length was significantly reduced by application of DSS, and was restored after rSj16 treatment (Figure 1C and D). Mean colon macroscopic scores were significantly suppressed in DSS+rSj16 group compared with DSS+PBS group (Figure 1E). Additionally, H&E histopathology results showed that treatment with rSj16 significantly reduced inflammation (Figure 1F). Consistent with this, histopathological scores after treatment with rSj16+DSS were significantly lower than after treatment with DSS+PBS (Figure 1G).

2. Q: In the result section, Fig 4-D doesn't show the flow cytometry results properly, the specific results of flow cytometry should present.

   A: Thank you for your very professional comments. We have revised it in the manuscript and quantified the results based on the previous repeated experiments.
   Flow cytometry results also showed that miR-217-5p could obviously promote MODE-K apoptosis. However, rSj16 could significantly inhibit MODE-K apoptosis induced by Etoposide and miR-217-5p. (Figure 4E, F).



3. Q: The results presented in this article are presented in more detail in the recent study of the authors (rSj16 Protects against DSS-Induced Colitis by Inhibiting the PPAR-α Signaling Pathway), and this issue should be considered and only new results should be presented.

   A: Thanks for your suggestion. In this study, in order to facilitate readers to intuitively understand the effects of rSj16 on DSS-Induced Colitis while introducing rSj16 attenuates colitis by inhibiting miRNA-217-5p to alleviate apoptosis, so we present the results of rSj16 alleviating colitis in this experiment.

4. Q: Some of the results are similar to previous study.

   A: Thank you. Our previous study focused on rSj16 protects against DSS-induced colitis by inhibiting the PPAR-α signaling pathway, then we found that rSj16 is multi-targeted in the treatment of colitis, and miRNA-217-5p also plays role. Therefore, in this study, we mainly focus on rSj16 attenuates colitis by inhibiting miRNA-217-5p to alleviate apoptosis. At the same time, in this study, in order to facilitate readers to intuitively understand the effects of rSj16 on DSS-Induced Colitis while introducing rSj16 attenuates colitis by inhibiting miRNA-217-5p to alleviate apoptosis, so we present the results of rSj16 alleviating colitis in this experiment.

Reviewer #2:

1. Q: Abstract: Please describe briefly the material and methods used in the study, specify the in-vitro and in-vivo animal study.

   A: Thank you for your advice. We have reformatted the abstract in the manuscript.

   Background: Inflammatory bowel disease (IBD) affects millions of people worldwide and has emerged as a growing problem in industrialized nations. The lack of therapeutic

targets has limited the treatment of inflammatory bowel disease (IBD). Studies found that parasitic nematode infections can ameliorate clinical and experimental colitis. Our previous study found that rSj16, a 16-kDa secreted protein of *Schistosoma japonicum* produced by *Escherichia coli*, has protective effects on dextran sulfate sodium (DSS)-induced colitis in mice. Apoptosis is an important factor in the pathogenesis of colitis. However, it is not clear whether the effect of rSj16 on colitis is related to apoptosis.

Aim: To investigate whether the protective effects of rSj16 on colitis is related to apoptosis and its mechanism

Methods: In-vivo, colitis was induced by DSS. The severity of colitis was assessed. WB was used to detect the changes of apoptosis-related genes in colon tissues. Q-PCR was used to detect the changes of miRNA-217-5p and HNF1B. In-vitro, WB was used to detect the changes of apoptosis-related genes in intestinal epithelial cells. TUNNEL staining and flow cytometry were used to detect cell apoptosis.

Results: rSj16 attenuates clinical activity in DSS-induced colitis mice. TUNNEL staining and WB results showed that apoptosis was increased in colon tissue after treatment with DSS, and the apoptosis of colon tissue was significantly reduced after treatment with rSj16. Compared with normal mice, the expression of miR-217-5p was increased in colon tissue of DSS-induced colitis mice. In addition, the miR-217-5p target gene HNF1B was decreased after administration of DSS. After treatment with rSj16, the expression of miR-217-5p was decreased and the expression of HNF1B was increased compared with the DSS-treated group. When Etoposide was used in combination with miR-217-5p mimic on MODE-K cells, the expression of cleaved-Caspase-3 Bax was increased, and Bcl-2 was decreased compared with only Etoposide treatment, and the expression of HNF1B was significantly reduced, suggesting that miR-217-5p acts as a pro-apoptotic in colon epithelial cells and down-regulates the target gene HNF1B. After rSj16 administration in MODE-k cells, miR-217-5p expression was significantly decreased, HNF1B expression was increased, and apoptosis was reduced.

Conclusion: The protective effects of rSj16 on colitis is related to apoptosis and miRNA-217-5p may be a further target for therapeutic intervention against IBD.

Keywords: Schistosoma japonicum, rSj16, IBD, apoptosis, miRNA-217-5p.

2. Q: Introduction: Please point briefly on the points of the study. Remove the last paragraph of the introduction and merge it to the discussion (conclusion part).

A: Thank you very much for your professional advice. We have changed the introduction according to your suggestion, see the part highlighted in red.
In this study, we investigate whether the protective effects of rSj16 on colitis is related to apoptosis and its mechanism. miRNA may function through regulating the expression of

encoding genes in IBD. We explore the relationship between rSj16, miR-217-5p and IBD, providing theoretical support for the clinical application of rSj16 in the treatment of IBD.

3. Q: Materials and Methods: Animals and Ethics: please specify how many mice was used and how they were assigned to the groups, in detail. Were the mice treated with certain treatments, food and water? if so, please clarify.

   A: Thanks for your suggestion, we have added relevant information in the Materials and Methods.
   Recombinant protein (rSj16) was expressed and purified as described previously [8]. A total of 15 mice were randomly assigned to three groups. Acute colitis was induced by administering water with 3% (wt/vol) DSS (36–50 kDa; MP Biomedicals, Illkirch, France) to mice over a period of 7 days. The control mice (n=5) received drinking water. Over the same period, rSj16 was administered to the colitis mice (n=5) via intraperitoneal (i.p.) injection (100 μg per mouse) on each day from 1 to 7. Control groups (n=5) received the same volume of vehicle (phosphate buffered saline; PBS) over the same time frame. The mice were fed standard mouse chow.

4. Q: Statistical Analysis: Please provide the dataset of Mean and SD for each group.

   A: Thanks for your suggestion, we have provided the dataset of Mean and SD for each group:

	Body weight loss on day 7 (%) (means ±SD)	**DAI on day 7 (means ±SD)**	**Colon length (means ±SD)**	**Macroscopic scores (means ±SD)**	**Histopathological scores (means ±SD)**	**n**
Water+PBS	125.30 ±6.30	0.00 ±0.00	9.70 ±0.56	0.00 ±0.00	0.00 ±0.00	5
DSS+PBS	89.11 ±8.02	5.20 ±0.45	6.58 ±0.48	7.8 ±1.30	13.00 ±2.55	5
DSS+rSj16	106.00 ±5.97	1.40 ±0.89	8.12 ±0.35	3.20 ±0.84	4.4 ±1.14	5

5. Q: Discussion: Please add the discussion, especially regarding the result of the study and the possible correlations. Please point out the limitations of your study.

   A: Thank you for your detailed advice, we have modified the discussion part, see the part marked in red for details in the manuscript.

   The specific regulatory mechanism between miR-217-5p and apoptosis needs to be further studied. In our study, miRNA miR-217-5p was expressed at a high level in IBD mice colon tissues, and was decreased significantly following treatment with rSj16. After

inducing MODE-K apoptosis, miR-217-5p expression was significantly increased, after rSj16 treatment, miR-217-5p expression was significantly reduced. Therefore, we hypothesized that miR-217-5p is involved in the protective effects of rSj16 on colitis. Bcl-2, caspase-3, and Bax play key roles in cell apoptosis. Caspase-3 is a marker of apoptosis because its activity is required for major apoptosis-related morphological and biochemical events, and its activation and function are regulated by the Bcl-2 family of proteins, among other molecules. In the present study, after overexpression of miR-217-5p in MODE-K cells, cleaved caspase-3 and Bax expression were increased, but Bcl-2 was reduced, suggesting that miR-217-5p plays a pro-apoptotic role in MODE-K cells. After rSj16 treatment, the miR-217-5p, cleaved caspase-3 and Bax expression were decreased, but Bcl-2 was increased, indicating that rSj16 could reduce the apoptosis. Results showed that miR-217-5p aggravated MODE-K cell apoptosis and rSj16 could significantly inhibit the apoptosis by inhibiting miRNA-217-5p expression.

As for the limitations of the study, because rSj16 affects the progress of the disease through multiple pathways, we only explore one of them, suggesting that miR-217-5p/HNF1B axis could be used as a potential target for the treatment of enteritis. In addition, rSj16 may attenuate IBD through other pathways which we didn't make a comprehensive exposition, but it is still worth exploring. Next, we will conduct a more comprehensive study on the treatment of IBD with rSj16, to provide more possibilities for the development of enteritis drugs.

6. Q: References: Please provide according to the guidelines for authors.
   A: Thank you for your detailed advice, we have revised it in the manuscript according to the guidelines for authors.

Reviewer #3:

1. Q: Many standard procedures used in this study as given in materials and methods were not referenced. Please give more details about the nature of the miRNA mimic (for non-specialists).

   A: Thank you for your professional advice. We have modified it in the manuscript.

   Cells were incubated in a serum-free medium for starvation overnight, then stimulated with miRNA mimic (Assay ID: MIMAT0000679) or mimic control (50 nM, Ruibo, Guangzhou, China) using RNAi MAX (Invitrogen, USA). MiRNA mimics are miRNAs that mimic endogenous miRNAs and can be synthesized by chemical synthesis to enhance the function of endogenous miRNAs.

2. Q: Introduction: Add references and literature elements about miR-217-5p and HNF1B in the gut, the relationship with IBD, the relationship with apoptosis to the second paragraph from the bottom of introduction, which could show the necessity and importance of detecting the level of miR-217-5p in your studies.

A: Thank you for your suggestion. We have quoted references in the paper to make the paper clearer and highlight the necessity and importance of detecting the level of miR-217-5p.

MicroRNAs are critical post-transcriptional regulators of gene expression and key mediators of pathophysiology of inflammatory bowel disease (IBD). However, the molecular basis of IEC apoptosis in the pathogenesis of IBD remains unclear. Studies have shown that miRNAs play an important role in IBD. For example, miR-301a promotes intestinal mucosal inflammation by inducing IL-17A and TNF-α in IBD. MiR-31 is increased in colon tissues of patients with IBD, reduces inflammatory signaling and promotes colon regeneration. Myeloid-derived miR-223 limits intestinal inflammation by constraining the nlrp3 inflammasome. Upregulation of miR-665 promotes apoptosis and colitis in inflammatory bowel disease by repressing the endoplasmic reticulum stress components XBP1 and ORMDL3. In Shamran's study, miR-217 may induce Sirt-1 and provide protection against intestinal inflammation. The hepatocyte nuclear factor (HNF) superfamily of transcription factors is essential for the development and maintenance of a variety of humans and mice tissues, and is further classified into four families, HNF1, FOXA, HNF4, and ONECUT, based on their functional domains. In the gut, HNFs are expressed in IECs, which regulate a variety of physiological functions, including differentiation, barrier function, and metabolism. Hepatic nuclear factor-4α (HNF4α) mRNA level was also downregulated in mouse model of ileitis (SAMP) compared with control mice. Hepatocyte nuclear factor-1beta (HNF1B) is the most important liver-specific transcription factor, with responsibility for sequence-specific DNA binding. HNF1B is reportedly a target of miR-217, with a role in circ-TTBK2- and miR-217-mediated modulation of malignant glioma progression.

3. Q: In many places it was suggests apoptosis of colon rather than the cells which is very confusing to read.

   A: Thank you for your correction. We have corrected this in the paper. At the beginning, we detected colon cell apoptosis in the tissue and conducted cell experiments. We have corrected the colon apoptosis to colon cell apoptosis, as shown in red letters.

4. Q: The sequences of primers are not complete. For example, mRQ3' Primer (Takara, Kyoto, Japan) - the complete sequence should be given.

   A: Thank you for reminding, we've been proactive in trying to solve this problem. The reverse transcription and qPCR kits in this study were purchased from Takara company. After contacting with the manufacturer, they indicated that the primer sequence was not provided outside. In other studies, we only provided the primer name and manufacturer (Wang et al., 2020).

Wang, L., Liao, Y., Yang, R., Yu, Z., Zhang, L., Zhu, Z., Wu, X., Shen, J., Liu, J., Xu, L.,

Wu, Z., & Sun, X. (2020). Sja-miR-71a in Schistosome egg-derived extracellular vesicles suppresses liver fibrosis caused by schistosomiasis via targeting semaphorin 4D. Journal of extracellular vesicles, 9(1), 1785738. doi.org/10.1080/20013078.2020.1785738

5. Q: Some parts of results, such as macroscopic assessment, histologic analysis and clinical scoring, were not discussed to testify and convince the reader of the conclusion. The author should restate the all results in the discussion section to make the article clearer.

   A: Thank you for your suggestions, we have been described in detail in the discussion section of the manuscript.

   We have found that Sj16 (a 16-kDa secreted protein of Schistosoma japonicum) has protective effects on DSS-induced mouse colitis. Body weight loss was alleviated in mice with colitis after treatment with rSj16. DAI (evaluated based on weight loss, diarrhea, and bleeding) also alleviated in colitis mice after treatment with rSj16. The results of colon length, mean colon macroscopic scores (assessed by hyperemia, wall thickening, ulceration, inflammation extension, and damage), H&E, and histopathological scores (based on extent of inflammation, neutrophil and lympho-histiocyte infiltration, crypt damage, crypt abscess formation, sub-mucosal edema, goblet cell loss, and reactive epithelial hyperplasia displayed) indicate that rSj16 protects against acute DSS-induced colitis.

6. Q: All the “Sj” in rSj16 should be italic.

   A: Thank you for your suggestions. rSj16 in this manuscript refers to the abbreviation for recombinant protein in the text and does not stand for gene or schistosomiasis, so we don't need to use italics.

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We hope our manuscript can be accepted after revision. We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Sincerely yours

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