Response letter

Dear Editor and Reviewers:

Thank you for offering us an opportunity to improve the quality of our submitted manuscript entitled "Study on the chemical components and therapeutic effect of *Atractylodes japonica* Koidz. ex Kitam against acetic acid-induced gastric ulcer in rats"(ID: 87865). We really appreciated the reviewers' constructive and insightful comments. In this revision, we have addressed all of these comments/suggestions. We hope the revised manuscript has now met the publication standard of your journal. We highlighted all the revisions in yellow colour. Our point-to-point responses to the queries raised by the reviewers are listed.

Reviewer#1

Comment 1: The study's focus on histological slides without detailed explanation of the underlying pathophysiological changes and the absence of an ulcer score calculation or macroscopic images of the stomach mucosa represent significant limitations in comprehensively assessing the therapeutic effects of *A. japonica* on gastric ulcers. Histological data alone may not provide a holistic understanding of ulcer severity, and macroscopic images could offer valuable insights into the extent and appearance of ulcers. Additionally, the lack of an ulcer score limits the quantitative evaluation of treatment outcomes. To enhance the scientific value of this research, the authors should consider incorporating macroscopic observations, providing a clear pathophysiological explanation, and utilizing an established ulcer scoring system to better characterize and quantify the ulcer-healing effects of *A. japonica.*

Response： We are extremely grateful to reviewer for raising this question. We have made additions based on your suggestions, as detailed on page 8-9 and supplementary material.

Comment 2: The absence of a bioavailability study to assess the active

ingredients of *A. japonica* is a notable gap in this research. Understanding the bioavailability of these compounds is crucial as it directly influences their efficacy and potential clinical applications. Bioavailability studies help determine how much of the administered substance reaches the bloodstream and target tissues, impacting its therapeutic effects. Without this data, it's challenging to ascertain the practical significance and dosage requirements for *A. japonica* as a treatment for gastric ulcers. To enhance this study's scientific value and practical relevance, the authors should consider conducting bioavailability experiments to provide a more comprehensive assessment of the herb's therapeutic potential.

Response：Thank you for reviewer's suggestion. Traditional herbal medicine has most probably a longer history than mankind, there's something unique about treating disease. However, since TCM is multi-target and multi-pathway regulation, its therapeutic potential is attributed to the complex interaction and combination of its multiple components, which means that the overall effect of these components represents the curative effect, and the bioavailability of some compounds cannot replace the overall situation. Moreover, this part of the content does not belong to the scope of this study. This part of the content will be further discussed through the study of drug in vivo into blood and other studies. The specific dosage was also formulated with reference to the relevant preliminary research content and pharmacology books of the research group. The specific reference information is as follows. [DOI: 10.1016/j.biopha.2020.110554 ; DOI : 10.1016/j.jep.2014.10.066 ; DOI:10.1016/j.jep.2021.114026 ; Wei W, Wu XM, Li YJ. Methodology of Pharmacological Experiment (4th edition). Beijing: People's Medical Publishing House. (2010)71:742-74.]

The dosage used in this paper is calculated as follows:

According to the dose conversion relationship between experimental rats and humans, in terms of dose per unit of body weight, the equivalent dose for

rats is 6.25 times that for human. The dose of *A. japonica* was converted according to the 1, 2 and 4 times of the normal dose of human, which were divided into low, middle and high dose groups. The low dose is calculated according to the normal dose of 9 g, the following data was calculated for each rat at low dose.

1. Converting daily dose for adults (60 Kg) to the specific dosage per Kg of body weight and multiplied by the conversion factor,
the specific dosage of rats per Kg body weight per day was obtained.

2. The dosage per kg of rat body weight was converted to the dosage per rat.

3. Each rat was given 2mL of gavage, which was converted to the daily gavage concentration of each rat.

4. The final concentration was calculated by gavage twice a day.

1. $\frac{9g/d}{60Kg}$ *6.25=0.94g/Kg/d

2. 0.94g/1000g/d *200g=0.188g/d

3. 0.188g/2mL=0.094g/mL/d

4. 0.094g/mL/2=0.047g/mL

The middle and high dose groups were calculated and prepared according to the specific multiple relationship.

Comment 3: The absence of a detailed investigation into the cellular signaling pathways involved in the therapeutic effects of *A. japonica* on gastric ulcers is a notable limitation of this study. Understanding the specific molecular mechanisms through which *A. japonica* exerts its effects could provide valuable insights for both basic science and potential clinical applications. It would have been beneficial if the authors had explored and elucidated the signaling pathways associated with anti-inflammatory, ulcer-healing, and gastroprotective responses triggered by *A. japonica*. Incorporating cellular

signaling pathway studies could significantly elevate this research's scientific value and depth.

Response： We deeply appreciate the reviewer's suggestion. Your suggestion also inspired us to conduct follow-up studies. At present, our existing pharmacodynamic and metabolomics results have shown that they have good anti-inflammatory effects and ulcer-healing. In addition, according to the existing chemical components, we have also conducted network pharmacology and molecular docking studies to find relevant core targets and related pathways. According to the results of KEGG pathway, EGFR, PI3K-AKT, HIF-1 and other pathways were found to be closely related to GU [doi: 10.1111/1750-3841.16214. ; doi: 10.1002/jcp.28807. ; doi: 10.1097/01.fjc.0000166305.79055.ad.; doi: 10.1016/s0002-9440(10)64420-3.; doi: 10.1016/s0928-4257(01)00046-8.]. In addition, we listed the top 10 intersecting target protein interaction networks (Figure 1), performed molecular docking studies based on the top 5 selected disease targets (Table 1) and part of the core components according to the score values of the screening, and analyzed that the targets and components had better binding effects, all the binding affinity < -5.0 kcal·mol^{-1}, some pharmacodynamic results were also verified in manuscript. The specific results provide a theoretical basis for the follow-up study, the specific results of molecular docking also carried out a visual display, as shown in Figure 2-6.

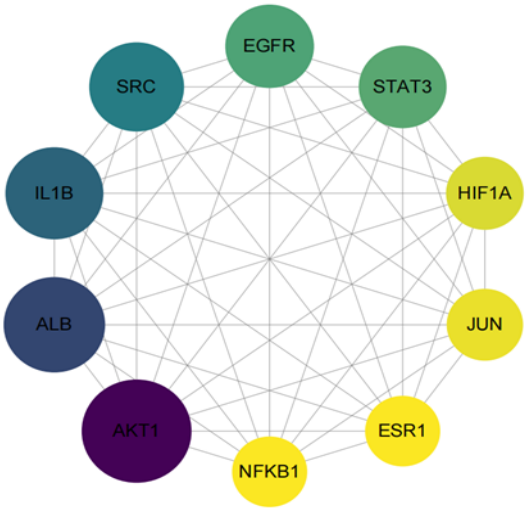
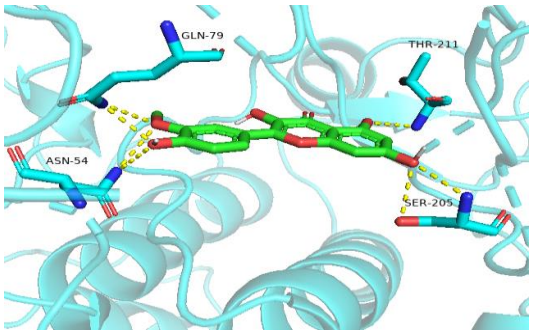


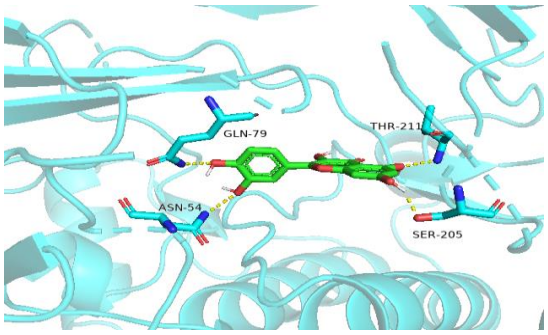
Figure 1. The top 10 intersecting target protein interaction networks

Table 1 Key targets information

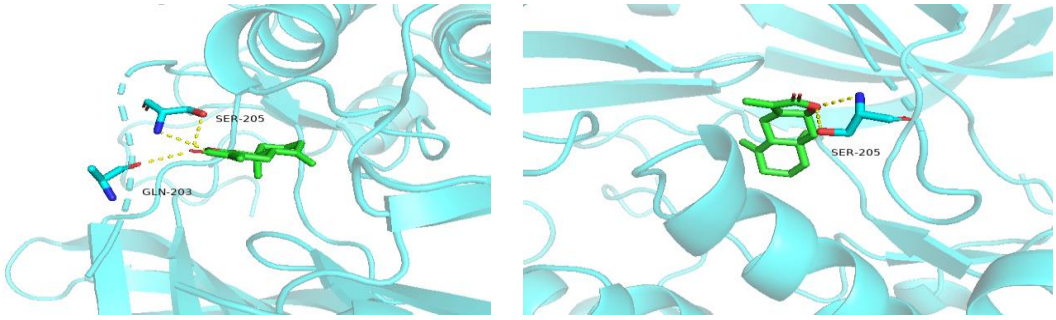
No.	Name	PDB ID	Resolution	Degree
1	AKT1	4EJN	2.19 Å	498
2	ALB	4L8U	2.01 Å	460
3	IL-1β	5R85	1.44 Å	444
4	SRC	1O4A	1.50 Å	430
5	EGFR	3POZ	1.50 Å	402



(A)

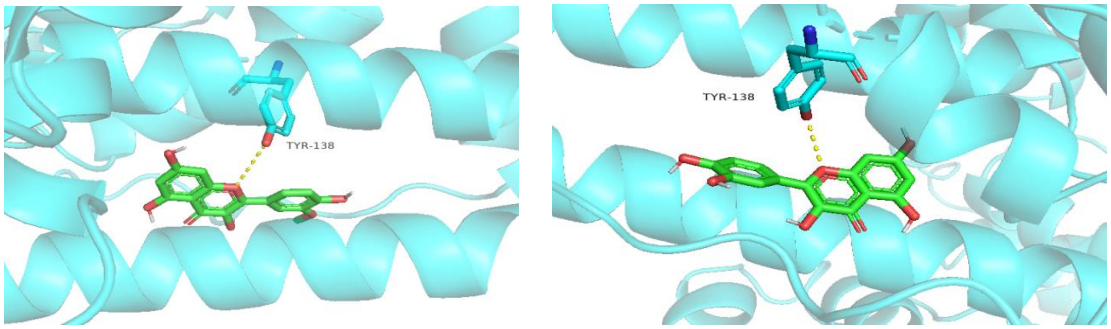


(B)

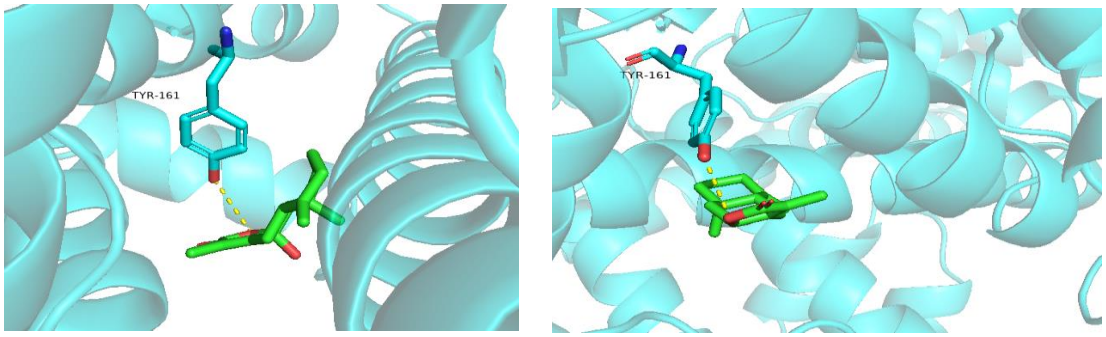


(C) (D)

Figure 2 The binding pattern of AKT1 to Isorhamnetin (A) , Quercetin (B) , Atractylenolide I (C) and Atractylenolide II (D)

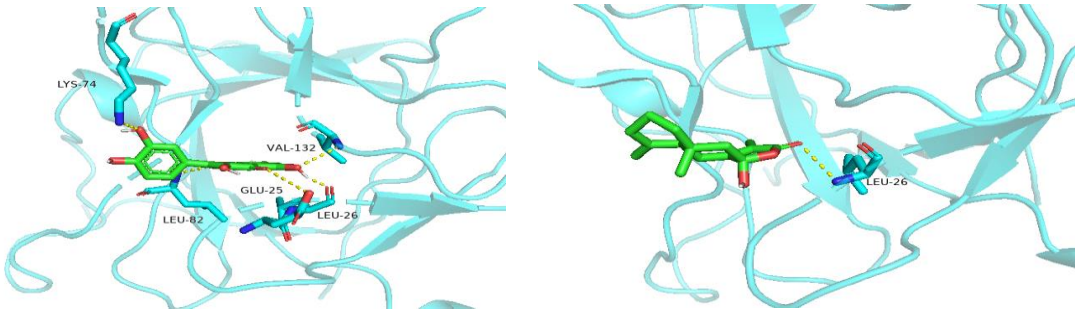


(A) (B)

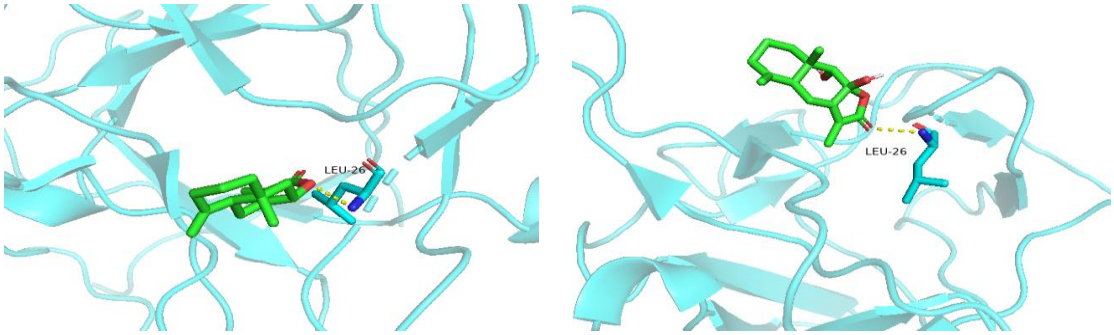


(C) (D)

Figure 3 The binding pattern of ALB to Isorhamnetin (A) , Quercetin (B) , Atractylenolide II (C) and Atractylenolide III (D)

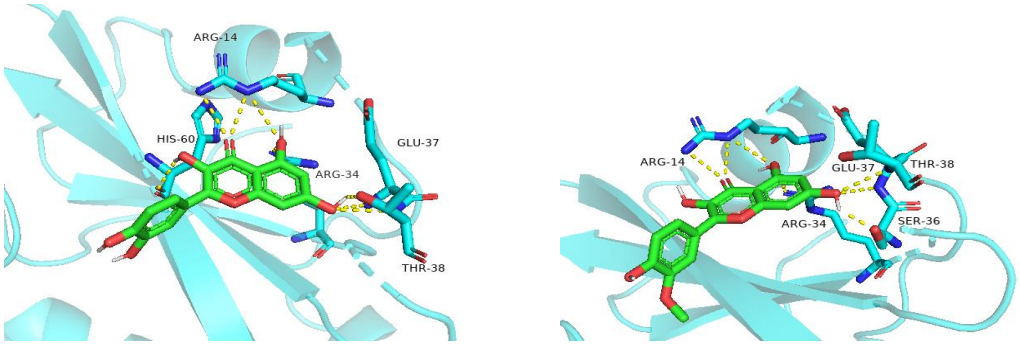


(A) (B)

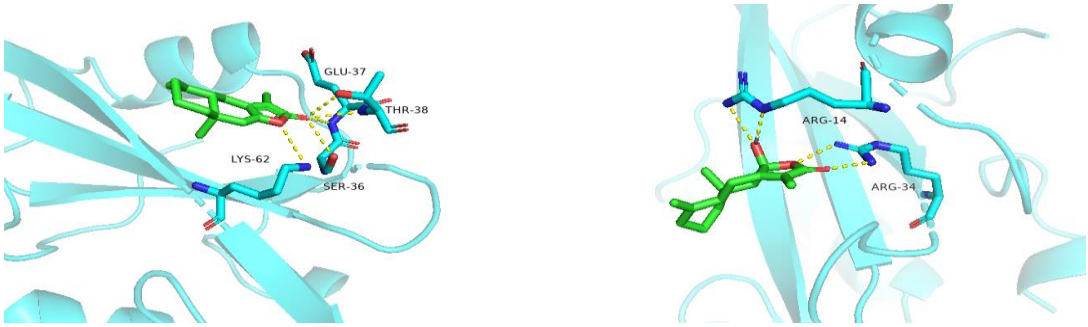


(C) (D)

Figure 4 The binding pattern of IL-1β to Quercetin (A) , Atractylenolide III (B) , Atractylenolide II (C) and Atractylenolide V (D)

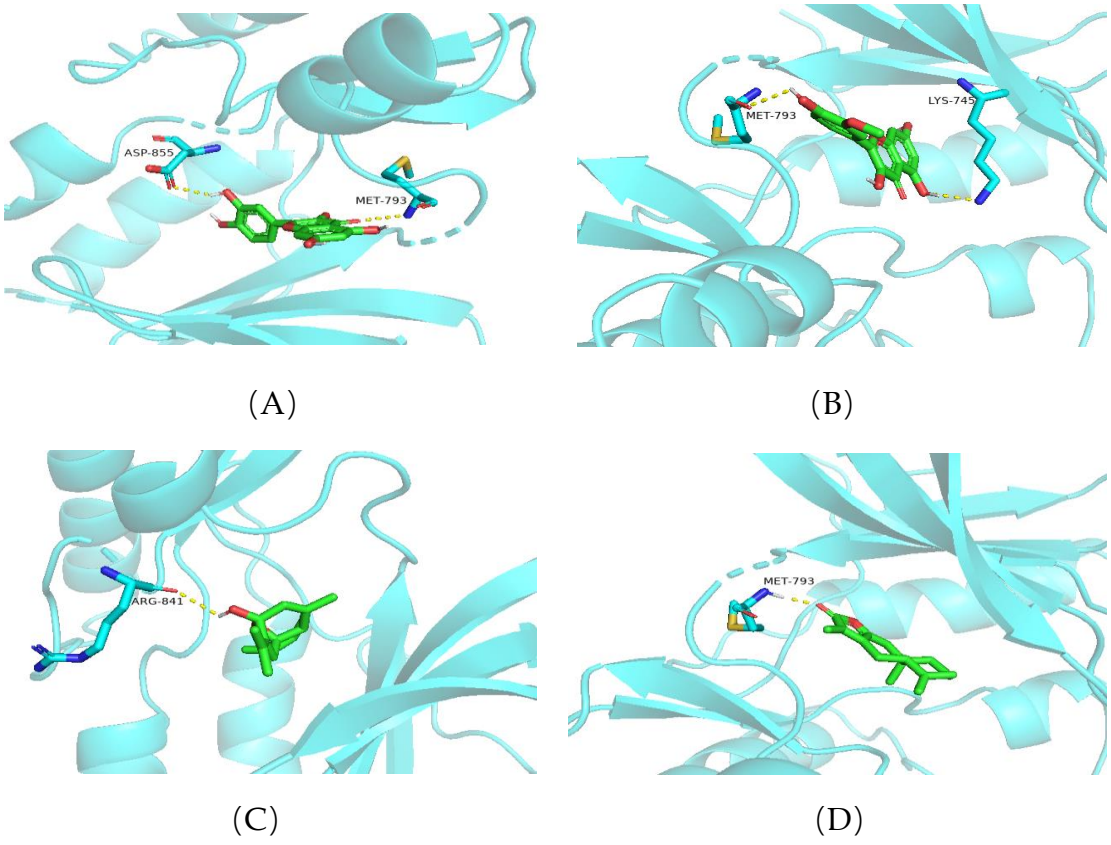


(A) (B)



(C) (D)

Figure 5 The binding pattern of SRC to Quercetin (A) , Isorhamnetin (B) , Atractylenolide I (C) and Atractylenolide III (D)



(A) (B)

(C) (D)

Figure 6 The binding pattern of EGFR to Quercetin (A) , Isorhamnetin (B) , Curcumenol (C) and Atractylenolide I (D)

Reviewer#2

Comment 1: Discussion of the role of the identified metabolites should not be as clear-cut as the authors have, since in different situations metabolites can have opposite effects. This dualism of metabolites should be reflected in the discussion to increase objectivity.

Response： We deeply appreciate the reviewer's suggestion. According to the reviewer's comment, our results are more complete and objective. We have added a more detailed interpretation in discussion section on page 16-17.

Supplementary notes

According to the specific requirements of the magazine, part of the content was added and modified.

1. Such as adding running title, co-corresponding authors, core tip on page 1,2

and 4.

2. Re-searching the subject words and standardize the writing of key words on page 4.
3. Changing the English expression forty-eight into roman numerals on page 11.