

Title: Herbal cake-partitioned Moxibustion inhibits colonic autophagy in Crohn's Disease via PI3KCs signaling

The authors demonstrate that Herbal cake-partitioned Moxibustion (HM) ameliorated inflammation and colon damage in TNBS-induced rats Crohn's disease model. They show that rats treated with HM have reduced alterations of the colonic mucosa and an inhibition of autophagy by activating PI3K1 / Akt1 / mTOR signaling pathway and inhibiting the VPS34-Beclin-1 protein complex in CD rats.

This is an interesting study with a consistent number of data. However, my major concern regards the morphological method and findings.

### Material and method

It should be indicated what the acronym "CST" stands for

Reply: Thank you for your valuable advices.

Based on your opinion, we have given the full name and abbreviation (Cell Signaling Technology, CST) in the first occurrence of "CST" in the article. (At Chemicals and reagents section)

It should be specified that HM group consists of CD rats treated with moxa cones

Reply: We have added instructions for the HM group in this section based on your comments, indicating that its interventions are based on the CD group.

What does "the gastric perfusion needle was slowly withdrawn from the gastric perfusion needle" mean?

Reply: There is a misrepresentation here, and we have deleted it.

For transmission electron microscopy, it should be indicated the embedding agent used because generally for the dehydration of the samples is used, at increasing concentrations, either ethanol or acetone but not both. In addition, it should be indicated the thickness of the ultrathin sections, the number of samples analyzed with transmission electron microscopy and the brand of the electron microscope used.

Reply: We have supplemented here based on your opinion, clarifying the embedding agent used, the brand of the electron microscope and the thickness of the samples. (Embedding agent: 618 epoxy resin 40.5g, DDSA 36g, DBP 2.7g, DMP-30 0.675ml. Thickness of the samples:80 nm. The brand of the electron microscope: Leica EMUC 7, Germany)

### Results

Figure 2: "ultrastructure" instead of "microstructure" is more appropriate for electron microscope images

Reply: We have replaced "microstructure" with "ultrastructure" based on your comments.

The magnification of the images of Figure 1b should be increased in order to detect morphological changes described in the results. Figure 4: it is difficult from the images to make a comparison

between the morphological alterations of the colonic mucosa in the different groups and to recognize in them the histopathological observations reported in the results. As suggested for Figure 1, the images should be of a higher magnification.

Reply for both Figure 1b and Figure4 comments:

Dear reviewer, thank you very much for your guidance and suggestions.

Since the HE and electron microscope images we used in this study were taken and selected by two pathologists, thus, we did not make changes; However, based on your opinion, we modified the description of the pathological observations in the results section to make it more in line with the pathological characteristics of the pictures selected in the article. In addition, the pictures in the article may be due to the use of related software for zooming, which may affect the resolution and clarity of pictures. Therefore, we uploaded the original pictures this time to facilitate pathological observation..

### **Discussion**

In the discussion, the interesting description of the signaling pathways that regulate autophagy overshadows the findings of the work. So, in my opinion, discussion part should be rewritten in order to highlight the comparison of the results with the other observations.

Reply: Based on your comments, we have rewritten and edited this section to better emphasize the findings of this study. Thanks again.