

Manuscript NO.: 55303

Title: New approach of medicinal herbs and sulfasalazine mixture on ulcerative colitis induced by DSS

Thank you for your comments regarding our manuscript. We have studied the reports of the editor and reviewers carefully, and have revised the manuscript accordingly (the revised parts are red color). The responses to the comments are as follows:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: Comments on the manuscript : “New approach of medicinal herbs and sulfasalazine mixture on ulcerative colitis induced by DSS”
Inflammatory bowel disease (IBD) has emerged as a major public health problem worldwide. Treatment with commonly used medications such as sulfasalazine revealed a number of side effects. Several mixtures of herbal medicines have been screened as candidates for alleviating these disorders. In this study, the authors compared the anti-inflammatory action of sulfasalazine alone or in combination with herbal medicine for ulcerative colitis in mouse in which colitis was induced with dextran sodium sulfate (DSS). For that, they studied several parameters (ROS serum, anatomy and histology of the colon, expression of several proteins, and especially apoptotic and anti-apoptotic) in normal mice, mice with induced colitis, mice treated with sulfasalazine alone and in combination with Bupleuri radix and Citri unshiu pericarpium mixture. The works is useful with several experiments and a lot of results. Nevertheless, I think this manuscript needs to be improved before to consider it to be published.

Minor : Line 139 : write “Ethanol” instead “EtOH” I changed “EtOH” for “Ethanol”. (line 139)

RESULTS : SCPB attenuated the progression of DSS-induced colitis in mice Line 223 : the authors wrote about the “histologic alterations”. Specify what were the main histological characteristics observed in DSS-induced colitis mice and in mice treated according to the treatment. I added the texts associated with “histologic alterations”. (line 223-224)

SCPB inhibited apoptosis in DSS-induced colitis mice Line 274 : The authors wrote : “The DSS control group showed the disease-characteristic apoptosis features of inflamed colon epithelial cells, supported by an observable increase in Bax and caspase-3 activities (Figure 6).” Nothing is written about Bcl2. It seems there are no effects on this anti-apoptotic protein. Please, specify this point. I referred to about Bcl2 (Line 276-277)

DISCUSSION Line 300 : The authors wrote : “the DSS-induced mice showed significantly decreased final body weight and both the sulfasalazine and SCPB treatments curtailed the body weight loss and the shortening of the colon length. ” In the results, there were no statistically significant results about this last point. I added at Line 301-302 as follows.

Herein, each groups showed the improvement effect like 30sulfa (1.96%), 60sulfa (5.36%), and SCPB (4.79%) on the colon length compared to the DSS control mice although it was not significant.

Line 355 : “and suppression of anti-apoptotic proteins, namely Bcl-2,...”.I did not see any suppression of this protein, and in the results, I haven't seen anything about it. This point deserves to be shown in the results and discussed in the discussion. I added at Line 358-360 as follows.

However, Bcl-2 just showed a tendency to increase without a significance. Maybe it judged that other anti-apoptotic protein such as survivin must have been is involved in apoptosis.

Line 361 : There is no mention of the schematic hypothesis in Figure 7. Present this hypothesis here.

I added at Line 361-362 as follows.

As a result, the administration of SCPB to mice treated with DSS ameliorated acute inflammation and apoptosis in the colon, as shown in Figure 7.

And I added new sentence at Line 365-366 as follows.

Nevertheless, the underlying mechanism of SCPB is still ambiguous and further profound researches are needed.

FIGURES Figure 1 : A : specify the time between the two measurements; B : Specify here what are the "vehicles", 30 sulfa, 60 sulfa, SCPB, as in the other figures (you have to wait for Figure 2 to have the explanations)) ; C : To specify the main characteristics of the histological sections: what were the notable differences between the different treatments. Figure 2 : A : Specify the unit of measurement; B : specify what is the first figure (Western Blot, I guess?) and the others (quantification). Figure 6, line 758 : Bax instead Bsc? Figure 7 : I did not find the call of this figure in the text. The possible mechanism needs to be emphasized in the conclusion such as an hypothesis. I checked all figures and revised from page 18 to 24.

Thank you very much.

Yours sincerely,

Mi-Rae Shin and Prof. Seong-Soo Roh