ANSWERING REVIEWERS

Qctober 15, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 513-review.doc).

Title: Moxibustion inhibits interleukin-12 and tumor necrosis factor alpha and modulates intestinal flora in rat with ulcerative colitis

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 513

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
(1) MATERIALS AND METHODS; Treatment; "on acupoints ST25 [21,22] and RN6"
Please briefly explain ST25 and RN6 because a majority of readers of this journal do not know these place points and their significance.
Response: RN6 is located on ventral midline 3.5 cun above the symphysis pubis and 1.5 cun below the navel. RN6 is a point of the conception meridian, and it strengthens original qi and improves immune function. ST25 is located on a horizontal line 2 cun laterally to the midline and 5 cun above the symphysis pubis. ST25 is the mu point of the large intestine meridian of hand Yangming and regulates the function of the intestine and stomach.
Corresponding contents have been added to the method part of the manuscript.

(2) RESULTS: HPM inhibits tissue damage in colonic tissues of UC rats: "the general morphological score of the colonic tissue in the model group" and HPM inhibits immunopathology in colonic tissues of UC rats: "evaluated the extent of immunopathology by looking for inflammatory cell tissue infiltration by H&E staining"
Please explain the details and differences of two histological scoring systems, the general morphological scoring and immunopathological scoring.
Response: According to references 25 and 26, the general morphological scoring was obtained by the macroscopic observation, the immunopathological scoring was obtained by H&E staining.
Table 1 General morphological scoring

General morphological manifestation		score
Colon adhesion	No adhesion	0
	Mild adhesion	1
	Severe adhesion	2
Ulcer and inflammation	No ulcer and no inflammation	0
	Local congestion, no ulcer	1

	1 ulcer without congestion or bowel wall thickening	2
	1 ulcer with inflammation	3
	>2 ulcer and inflammation	4
	>2 ulcer and/or inflammation area > 1 cm	5
	>2 ulcer and/or inflammation area > 2 cm, one more damage, plus 1	6-8

Table 2 Immunopathological scoring

Immunopathological manifestation		score
Ulcer	No ulcer	0
	Ulcer area <3 cm	1
	Ulcer area >3 cm	2
Inflammation	No inflammation	0
	Mild inflammation	1
	Severe inflammation	2
Granuloma	No granuloma	0
	Granuloma	1
Lesion depth	No lesion	0
	Submucosa	1
	Muscular layer	2
	Serosa layer	3
Fibrosis	No fibrosis	0
	Mild fibrosis	1
	Severe fibrosis	2

Corresponding contents have been added to the table and figure part of the manuscript.

(3) The authors should discuss the relationship between the level of IL-12 /TNF-α expression and the modulation of bacterial flora.

Response: Additionally, the intestinal flora and their products have been found to trigger cytokine expression, such as induce TNF-α in macrophage and epithelial cell systems of inflammatory bowel disease[52]. In addition to restoring beneficial intestinal flora, probiotics may enhance host protective immunity such as down-regulation of pro-inflammatory cytokines, IL-12 and TNF-α in colitis [53,54]. Rifaximin administration decreased the protein and mRNA levels of IL-12 and TNF-α, and caused a significant reduction of colon bacterial translocation towards mesenteric lymph nodes, in colon of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice[55]. The results of this study showed

that HPM can modulate intestinal flora towards a more normal flora and inhibit the expression of TNF-α and IL-12 in UC rats. These indicated that there may be a relationship between the release of IL-12 /TNF-α and the modulation of bacterial flora.

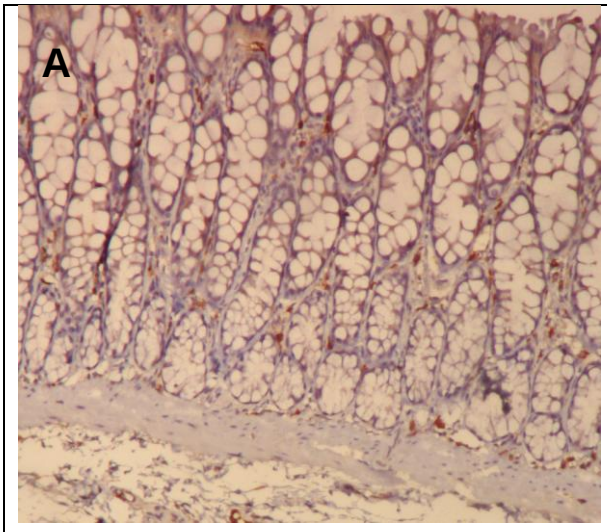
52. Klapproth JM, Sasaki M. Bacterial induction of proinflammatory cytokines in inflammatory bowel disease. Inflamm Bowel Dis 2010;16(12):2173-2179. PMID: 20848533 DOI: 10.1002/ibd.21332
53. Chen CC, Kong MS, Lai MW, Chao HC, Chang KW, Chen SY, Huang YC, Chiu CH, Li WC,Lin PY, Chen CJ, Li TY. Probiotics have clinical, microbiologic, and immunologic efficacy in acute infectious diarrhea. Pediatr Infect Dis J 2010;29(2):135-138. PMID: 20135748 DOI: 10.1097/INF.0b013e3181b530bf
54. Nanda Kumar NS, Balamurugan R, Jayakanthan K, Pulimood A, Pugazhendhi S,Ramakrishna BS. Probiotic administration alters the gut flora and attenuates colitis in mice administered dextran sodium sulfate. J Gastroenterol Hepatol 2008;23(12):1834-1839. PMID: 19120873 DOI: 10.1111/j.1440-1746.2008.05723.x
55. Fiorucci S, Distrutti E, Mencarelli A, Barbanti M, Palazzini E, Morelli A. Inhibition of intestinal bacterial translocation with rifaximin modulates lamina propria monocytic cells reactivity and protects against inflammation in a rodent model of colitis. Digestion 2002;66(4):246-56. PMID: 12592101 DOI: 10.1159/000068362

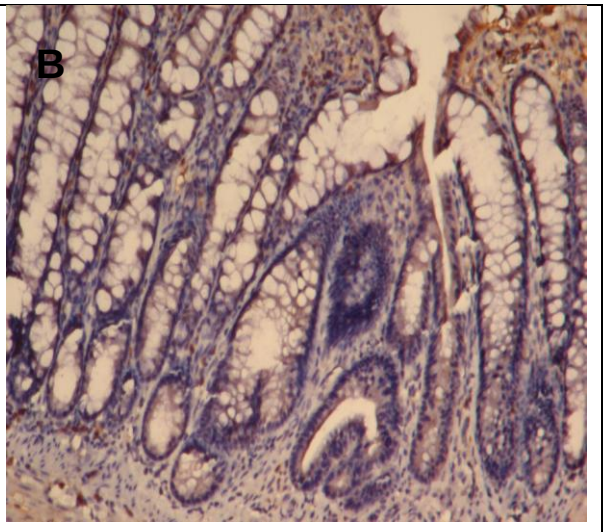
Corresponding contents have been added to the discussion and reference parts of the manuscript.

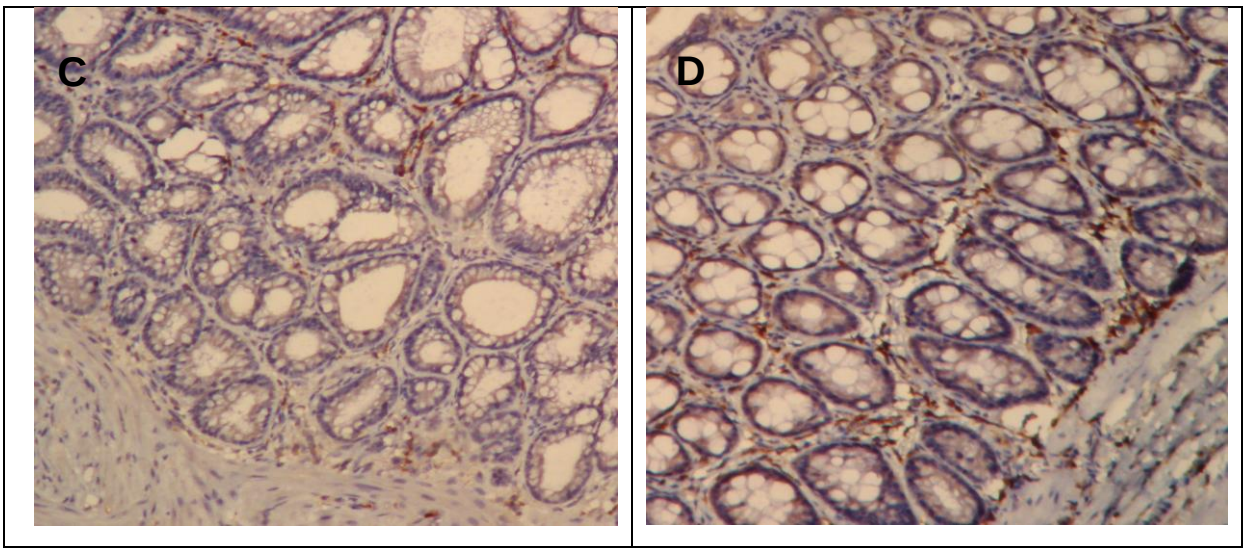
(4) Figures 5a and 5b: The authors should provide some photographs at the higher magnification, because the readers cannot recognize any positive cells in these photographs at the lower magnifications.

Response: We have provided Figures 5a and 5b at higher magnification(× 400).

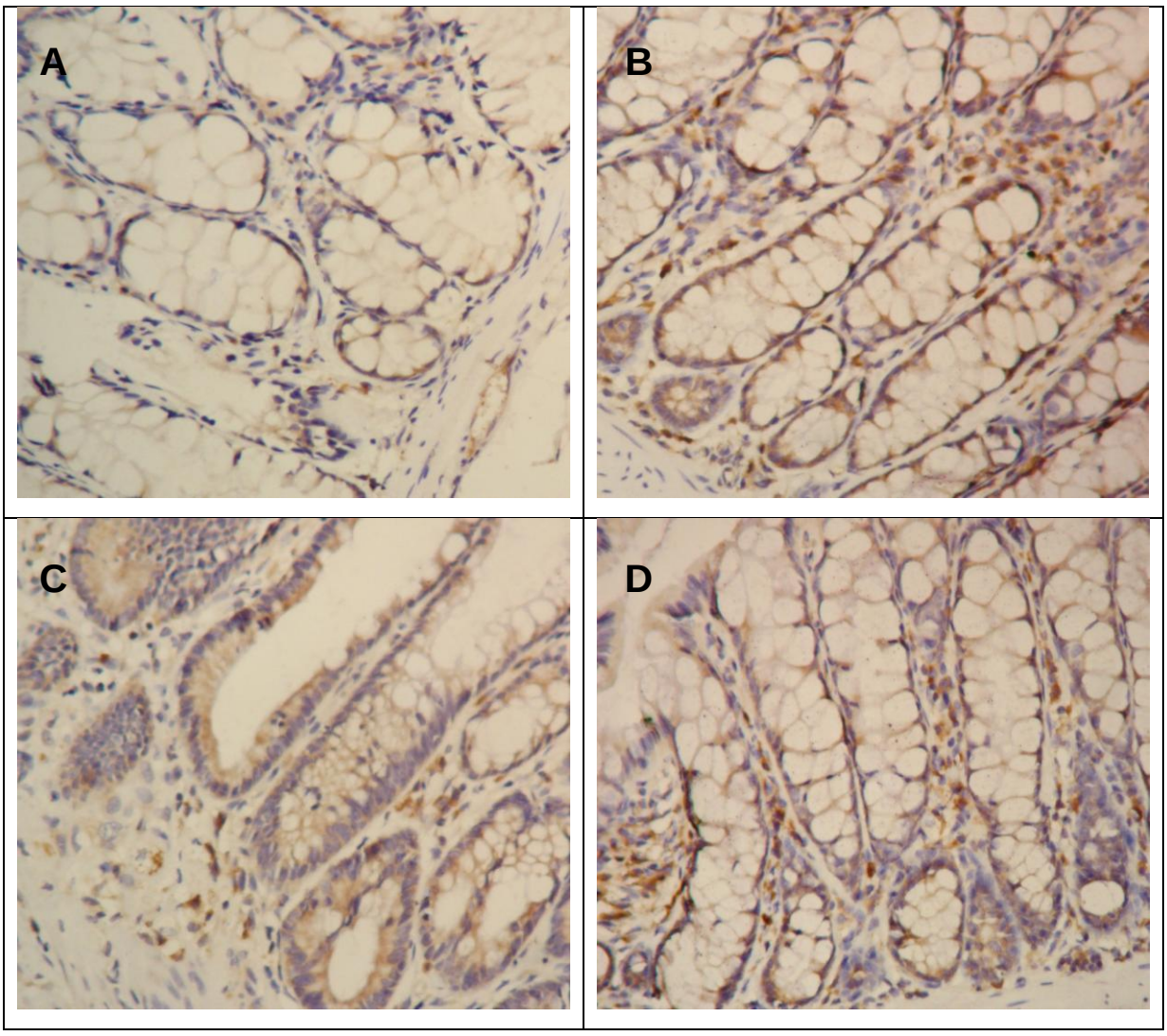
Figures 5a:







Figures 5b:



Corresponding contents have been added to the Figures part of the manuscript.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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