

December 9, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6518-edited.doc).

**Title:** HEPCIDIN EXPRESSION IN COLON DURING TNBS-INDUCED COLITIS IN RATS.

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 6518

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

1. All alterations were in red color.
2. Format has been updated (abstract, figure legends, figures in ppt, etc)
3. Revision has been made according to the suggestions of the reviewer.

Reviewer 00503587:

- (1) English revision was provided by AJE Service again. The certificate key number is: 1609-B862-4E4F-372A-D781.
- (2) A histological analysis was included in manuscript. The HE slides and microscopic analysis it was already done.
- (3) The discussion section was improved with the inclusion of literature reports about hepcidin in serum of IBD patients.

Reviewer 00012328:

- (1) We only demonstrated iron deposits but unfortunately we don't have the anti-ferroportin antibody.
- (2) We carried out an assay for transferrin saturation and data were included in Table 3.
- (3) The ages of experimental animals were included in Material and Methods Section.
- (4) It was included in the legends of Figures the number of animals used.
- (5) The Figure 2 was modified as suggested.
- (6) The discussion was altered in lane 8 as suggested.

Reviewer 02746983:

Colitis induced by administration of TNBS/alcohol is a suitable and well accepted model for intestinal inflammation studies (1087 articles published in PubMed database; 36 articles published in *World Journal of Gastroenterology*). Reactivated colitis induced by TNBS in experimental animals appears to be a very convenient model to study the pathogenesis of IBD, mainly Crohn's disease, because of the ability to induce disease and remission periods and also to promote alterations in mesenteric and perinodal adipose, and lymph node activation with a predominant response Th-1 (for references see Clemente et al., 2012; Acedo et al., 2011; Gambero et al., 2007). Serum alterations (i.e. TNF-alpha) as well as renal alterations and bone loss were described by other research groups for example. In other words, there is an important involvement of immune system and systemic alterations in this model.

- (1) The ages of experimental animals were included in Material and Methods Section.
- (2) Reference about TNBS experimental model was included in Introduction Section.

- (3) Human HT-29 cell line is commonly used in inflammation and absorption studies. A reference was included.
- (4) The number of rats used was corrected in Table 1.
- (5) Alcohol is not only a vehicle in TNBS-induced colitis. It disrupts the mucus barrier to absorption of TNBS. The control group is carried out with saline by several research groups.
- (6) Hematological parameters were measured in ABX Pentra 120, a **hematology** analyzer (Horiba Medical, Horiba Instruments Brazil, Jundiai, SP, Brazil). It was included in Material and Methods Section.
- (7) MPO activity is a marker of neutrophil infiltration. This information was included in Discussion section.
- (8) The sentence was altered for "as described for colon samples" in Material and Methods section.
- (9) The HT-29 experimental conditions (doses and periods) were based on a literature reference with AGS gastric cell line and hepcidin expression. The reference was included in Material and Methods section.
- (10) For a better iron status, transferrin saturation levels were included.

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

Sincerely yours,



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