

# ANSWERING REVIEWERS

June 20, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 19553-revised manuscript.doc).

**Title:** Kefir Treatment Ameliorates Dextran Sulfate Sodium-Induced Colitis in Rats

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

**ESPS Manuscript NO:** 19553

The manuscript has been improved according to the suggestions of editor and reviewer:

1- Format has been updated. Revision has been made according to the suggestions of the editor and marked as yellow font in the text.

2 - Revision has been made according to the suggestions of the reviewer and answers indicated below.

(1) There is not a spesific probiotic available for IBD treatment. As we discussed, there are some probiotic species in the literature, which shown beneficial effects on IBD, however, there are a little studies about kefir. So we aimed to investigate beneficial effects of kefir on IBD treatment in animal model.

(2) Five mililiter dose was determined with our own experience in our previous studies and according to literature. There is not any study about how does this relate to levels attainable in the human diet. We didn't investigate this condition in our study.

(3) We can't provide additional data. However, especially NF-kB could provide an additional contribution to our results, as pointed out by the reviewer.

(4) We didn't provide any background introduction about HT29 and LS174T cell lines. Because, these cell lines are mainly related to tumourigenicity.

(5) DSS can be cause inflammation entire colon when given by orogastric route. Whe used mid-colon for the analysys, because we observed more inflammation in this area.

(6) We investigated the therapeutic effects of preventive approach. This also was a preliminary study.

(7) Methods for the biochemical analyses are presented below and has been added to the article, and marked as blue font.

- MPO concentration was determined with the *enzyme-linked immunsorbent assay* (ELISA) kit (Immunodiagnostic AG-K6631-061109, Germany), based on the informations in homogenized supernatants, provided by the manufacturer. Its level expressed as ng/mg-protein.

- TNF-alpha and IL-10 concentrations was also determined with ELISA method (ASSAYPRO LLC St. Charles, MO; BIOSOURCE, Invitrogen Immunoassay kit, California, USA) and levels expressed as pikogram/mg-protein.

- MDA was determined by HPLC (ThermoFinnigan Spectra High Performance Liquid Chromatograph with Diode Array Detector San Jose, California, USA). Alkaline supernatants, which obtained from colonic tissue, were incubated in a water bath for 30 minutes at 60 °C (The hydrolysis of protein-bound MDA). After the



cooling of examples, 30% perchlorate was added to provide precipitation of proteins. Mixture was centrifuged at 2800xg. 250 µL of supernatant was transferred to eppendorf tubes and then 25 µL dinitrophenyl hydrazine (DNPH, 5mM) was added. 50 µL of samples that are left in the dark for 30 minutes, was injected in to the HPLC system. Results were presented as mmol/g-protein.

- For Western blotting analyses, 80 µg protein for each samples was separated by SDS-PAGE and was transferred to nitrocellulose membrane. The membrane was probed with anti-iNOS antibody (final dilution 1:1000) and then was incubated with horseradish peroxidase-conjugated anti-mouse antibody (final dilution 1:5000) at room temperature. Immunoreaction was visualized with an enhanced chemiluminescence system.

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

Sincerely yours,



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