Dear Editor:

We are pleased to resubmit for publication in *World Journal of Hepatology* the revised version of manuscript **Number ID:** 03648011" Chronic Exposure to Ethanol Causes Steatosis and Inflammation in Zebrafish Liver".

We appreciated the constructive criticisms of the reviewers. We are grateful for their valuable time and useful contribution. We hope we have properly addressed each of their concerns as outlined below

1. **What was the rationale for the ethanol dose selection?**

R: The dose was selected according to the effect observed by Schneider et al. in zebrafish liver after exposure to 0.5% of ethanol ("Effects of Lactobacillus rhamnosus GG on hepatic and serum lipid profiles in zebrafish exposed to ethanol", Zebrafish Journal, 2014). This paper related an increased liver damage between two and four weeks of ethanol exposure. We hypothesized that early inflammatory and ultrastructural alterations happen in liver during the development of hepatic steatosis.

We added this rationale in the manuscript highlighted in yellow (Section Material and Methods, *Animal care and use statement)*

2. **What was the rationale for using 104 fish?**

R: For histological analyzes we calculated the number of animals considering the cited study of Schneider et al. For these evaluations we utilized 10 animals/group/time; n=40.

For PCR methods we calculated the number of animals based in the paper of Craig, Hogstrand *et al.*, 2009 (Gene expression endpoints following chronic waterborne copper exposure in a genomic model organism, the zebrafish, Danio rerio. Physiological Genomics 40(1): 23-33). For these analyzes we utilized 15 animals/group/time; n=60.

The objective of ultrastructural evaluation was to describe liver earliest alterations; therefore we utilized 2 animals for control group and 2 for ethanol. We understood that for description purposes, this number of animals was sufficient. Yao and cowokers utilized just two animals for similar purpose (Yao, Y., Lin, J., Yang, P., Chen, Q., Chu,

X., Gao, C. and Hu, J. (2012), Fine Structure, Enzyme Histochemistry, and Immunohistochemistry of Liver in Zebrafish. Anat Rec, 295: 567–576).

3. **How these fish were tested for the entire experiment? A detailed information is required. With these revisions the manuscript may be accepted.**

R: We are sorry that this part was not clear in the original manuscript. We included in the text more detailed information which are highlighted in yellow, as follows:

Wild-type, adult zebrafish (*Danio rerio*), male and female, were purchased from a commercial distributor (Fish Flower, Porto Alegre, RS). The animals were of heterogeneous wild type stock from the standard short-fin phenotype and were housed for 2 weeks before the experiments in order to acclimate to the laboratory facility. The animal protocol was designed to minimize pain or discomfort to the animals. Fish were maintained in aerated water at 28 ± 2 ℃, 6.8 – 7 pH, on a 12/12 light/dark photoperiod cycle (lights on at 7:00 am). Biochemical parameters and quality of the water were monitored regularly: pH, presence of nitrates and nitrites, oxygen and ammonia levels. The animals were fed twice a day with fish food until satiety. Experiments were performed using a total of 104 animals. All fish used in this study were healthy and free of any signs of disease.

After acclimation period, the fish were randomly allocated into experimental tanks, density of 1 fish per liter of water. The following groups were performed (n=52/group): Control (C) and Ethanol (E). E group received 0.5% (v/v) of ethanol (Merck KGaA, Germany) directly added into water; tank water was changed every two days and the ethanol replaced.[15] This ethanol dose was chosen due to the liver damage observed by Schneider and coworkers in zebrafish exposed to 0.5% of ethanol. [15] The tank water of C group was also changed in same days of E group. At 2 and 4 weeks, fish were euthanized by hypothermal shock [16] and livers were completely removed for molecular and histological analysis.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.