Point-by-point responses

Reviewer #1

Dear Author, I found your manuscript well documented and interesting. The performance of methodology and the statistical analysis of your results are very well established. You analyzed many biomarkers in the serum as well as in the liver tissue, so as, to support your basic hypothesis that HRW can prevent progression of steatosis, liver damage and fibrosis. I believe that your research will be a useful guidance for the treatment of NASH in humans also. Concerning the limitations of your study, that you are mentioning, I agree that it should be better to have measured des-acyl ghrelin, as well as, leptin levels in the serum to enhance your results, but your effort still remains reliable.

Answer: We appreciate your suggestions about our current work, and we will investigate the detail mechanisms including des-acyl ghrelin and leptin in the future.

Reviewer #2

We appreciate your suggestions about our current work, and we have revised our manuscript based on your comments. All the changes are marked in blue font. We also created new Figure 3 and new Figure 6 for better illustration. The manuscript has been versed by a professional, native English speaker in a well-organized and more concise manner.

Q1. The authors assessed the levels of some hepatic antioxidant enzymes, all of which increased in HRW+ groups. Since HRW can directly scavenged ROS (hydrogen peroxide), the hepatic ROS level was lower in EtOH group, why the expression of these antioxidant enzymes were be up-regulated in the liver? Whether the roles of these antioxidant enzymes were more important than HRW or not in the protection of EtOH-induced early fatty liver? PRDXs, a crucial antioxidant enzyme family, which can also directly scavenged ROS (hydrogen peroxide), should be assessed in the present study.

Answer:

We had according to the reviewer's comment. We modified the sentences in Discussion section: "A number of studies reported that H_2 reduces oxidative stress not only directly but also indirectly, by regulating anti-oxidative signal transduction, including nuclear factor erythroid 2-related factor 2 (Nrf-2) and sirtuin 1 (Sirt1)[14, 18, 20, 35]. Antioxidants regulated by Nrf-2 via an ARE-driven mechanism are including SOD, GSH-PX, CAT, heme oxygenase-1 (HO-1), peroxiredoxins (PRDXs), etc[48]." **(page 25, line 15-20)**

Q2. Hepatic steatosis, fibrosis and cirrhosis can ultimately lead to hepatocellular carcinoma, and the incidence in male is much more than that in female. So, why only female mice were employed in this study?

Answer: Females have an increased susceptibility to alcoholic liver diseases compared with males. This is why we used female mice in this study. We had mentioned it in Introduction section: "Among humans and rodents, females are more susceptible to ALD, even if they consume less

alcohol as compared with males." (**page 6, line 8-10**)

Q3. Besides Liquid diets, were the mice given some other diets? Single Liquid diet during a long time is unnatural, which maybe affect the secretion, digestion and absorption in gastrointestinal tract.

Answer:

Mice were not given any other diets. We add the sentences in Materials and methods section: "This nutritional diet (containing 41.4 g/L casein, 0.5 g/L L-cystine, 0.3 g/L DL-methionine, 8.5 g/L corn oil, 28.4 g/L olive oil, 2.7 g/L safflower oil, 115.2 g/L maltose dextrin, 10 g/L cellulose, , 8.75 g/L mineral mix, 2.5 g/L vitamin mix, 0.53 g/L choline bitartrate, and 3 g/L xanthan gum) allowed for the prolonged exposure of EtOH in a rodent model and allowed for modification to calories provided by EtOH[2, 3, 5, 33, 34]". (**page 10, line 14-20**)

Q4. Although the biomarkers of liver injury, ALT and AST, were provided in this study, but the levels of ALT and/or AST were not always coincide with the severity of liver damage. Therefore, the changes in histopathology are more important in this study, and significant enhanced resolution pictures should be provided.

Answer:

According to your valuable comments, we have added a new Figure 3.

Q5. ROSs also play diverse roles in many signaling, however, HRW can directly scavenged ROS in the whole body, and it should be considerable that whether HRW have some side effects to other organs, such as kidney, or not. The histological pictures of main organs should be provided.

Answer:

Numerous studies have revealed that hydrogen has no known side effects to organs (*Medical gas research* 2015; **5**: 12, reference 18 in the revised manuscript). We had mentioned this point in Introduction section: "Thus, HRW could be used in preventive and clinical applications as a safe and effective antioxidant with minimal side effects[17-21, 23, 28-31]." (**page 8, line 5-6**)

According 28 day toxicity in rat, histopathological examination of tissues including heart, lungs, liver, kidneys, spleen, adrenals, testes, ovaries, thyroid glands, pituitary gland, thymus, prostate, uterus, salivary glands, seminal vesicles and brain from the control and HRW group revealed no treatment-related differences [*Toxicology and industrial health* 2010; **26**(4): 203-216, reference 28 in the revised manuscript]. Besides, we did not observe any gross abnormalities of the kidneys at necropsy in our experiments.

Q6. The protect roles of alone HRW showed not better than silymarin, and only HRW combined with silymarin showed significant beneficial roles protecting from EtOH-induced early fatty liver. Were the mice from alone silymarin and HRW combined with silymarin groups given equivalent dose of silymarin? If so, properly increased dose of silymarin maybe produce the same results as to HRW+ silymarin.

Answer:

The mice from alone silymarin and HRW combined with silymarin groups were given equivalent dose of silymarin. We modified the sentences in Materials and methods section: "(5) EtOH + silymarin (200 mg/kg) + HRW group—mice receiving an EtOH diet and gavaged with silymarin and HRW." (**page 10, line 25**)

Silymarin breaks down extremely quickly when taken orally. Pervious clinical study have reported that patients with hepatitis C received up to 700 mg of silymarin, about 10 times more silymarin than they would get from the recommended dose, didn't seem to have any effect on the liver function [*Journal of clinical pharmacology* 2010; **50**(4): 434-449]. Therefore, properly increased dose of silymarin does not produce the same results as to HRW plus silymarin. In addition, the protect roles of HRW alone showed better than silymarin in some biomarkers in protecting from EtOH-induced early fatty liver, such as acyl ghrelin, IL-22, CAT, etc.