Dear editors and reviewers,

Thank you very much for your comments and professional advice. These opinions help to improve academic rigor and research novelty of our article. Based on your suggestion and request, we have made corrected modifications and highlighted revised contents in yellow on the revised manuscript. Furthermore, we would like to show the details as follows:

**Reviewer #1:** This is a nice and complex study, which describes new opportunity to manage constipation with hydrogen rich water (HRW) using the animal model (mice with constipation induced by loperamide). Manuscript is well-organized and effects of HRW were described and discussed in details. However, authors are advised to exclude the part of the manuscript which described study of SIRT1 in humans, as the role of SIRT1 in constipation in humans is well known and discussed in numerous publications, therefore this part of the manuscript does not contain any new information, and may be excluded.

**The author's reply:** Thank you very much for the reviewer's feedback. The section describing SIRT1 research in humans has been removed from the article. Instead, the latest cutting-edge research findings on oxidative stress and SIRT1 in intestinal diseases have been added to the discussion:

Oxidative stress refers to the accumulation of free oxygen radicals to levels that exceed the ability of the antioxidant defense system, leading to oxidative damage to cells and tissues[1]. Oxidative stress can increase intestinal mucosal inflammation, destroy the intestinal barrier, reduce the mucus layer thickness, and affect intestinal peristalsis and water absorption[2]. Oxidative stress can also affect the function of the intestinal nervous system, inhibit the contraction of intestinal smooth muscles and the activity of ganglion cells, interfere with intestinal nerve conduction, slow intestinal peristalsis, and promote constipation[3].

SIRT1, a member of the NAD⁺-dependent deacetylase family, has been extensively explored as a potential therapeutic target to attenuate oxidative stress and inflammation-induced disorders[4]. The deficiency of SIRT1
markedly increases ROS and inflammatory reactions[5], while modulation of the SIRT1/NF-kappaB pathway can suppress NLRP3 inflammasome and oxidative stress[6]. Increasing evidence has suggested that SIRT1 play a role in intestinal disease[7]. SIRT1 can regulate intestinal inflammation in aging mice by altering the gut microbiota[8], and achieve mitochondrial homeostasis through the NAD+-SIRT1 pathway to protect the intestinal barrier function in patients with severe malnourishment[9]. There are also reports emphasizing the dual role of SIRT1[10]. SIRT1 inhibitors can alleviate radiation-induced intestinal stem cell death, promote crypt recovery, and improve the survival rate of mice[11]. In this study, low SIRT1 expression was observed in the colons of constipated rats. Following intervention with HRW, the expression level of SIRT1 was upregulated. EX527, a SIRT1 inhibitor, also verified that HRW alleviates oxidative stress and improves constipation symptoms by regulating the SIRT1/Nrf2/HO-1 signaling pathway.

REFERENCES


Thank you very much for your attention and time. Look forward to hearing from you.

Yours sincerely,
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