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Research article

Effects of Chinese herbal diet on hematopoiesis, immunity, and intestines of mice exposed to different doses of radiation

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ABSTRACT

Radiotherapy causes a series of side effects in patients with malignant tumors. Polygonati Rhizoma, Achyranthis Bidentatae Radix, and Epimedii Folium are all traditional Chinese herbs with varieties of functions such as anti-radiation and immune regulation. In this study, the above three herbs were used as a herbal diet to study their effects on the hematopoietic, immune, and intestinal systems of mice exposed to three doses of radiation. Our study showed that the diet had no radiation-protective effect on the hematopoietic and immune systems. However, at the radiation dose of 4 Gy and 8 Gy, the diet showed an obvious radiation-protective effect on intestinal crypts. At the dose of 8 Gy, we also found that the Chinese herbal diet had an anti-radiation effect on reducing the loss of the inhibitory nNOS$^+$ neurons in the intestine. That provides a new diet for relieving the symptoms of hyperperistalsis and diarrhea in patients after radiotherapy.

1. Introduction

Radiotherapy is often applied to treat patients with malignant tumors, such as malignant lymphoma [43], nasopharyngeal carcinoma [4], non-small cell lung cancer [7], breast cancer [17], cutaneous malignancies [42], etc. The tumors with high radiosensitivity usually receive a more satisfying therapeutic effect than the low-radiosensitive ones. However, after radiation exposure, patients are often accompanied by complex clinical complications such as myelotoxicity and gastrointestinal toxicity, collectively known as acute radiation syndrome (ARS; [21].

Due to the high radiosensitivity of the hematopoietic system, blood cell counts can be affected even in asymptomatic patients [10]. Total body irradiation (TBI) causes decreases in all three types of hemocytes. White blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs) count sharply decreased by nearly 97%, 30%, and 59% in the irradiated mice compared to those of unirradiated control mice at 7 days post-TBI [33]. Radiation can damage the immune system by locally or systematically killing immune cells. In general, lymphocytes are more radiosensitive than macrophages, dendritic cells (DC), or natural killer cells [15].

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The intestine is also one of the most sensitive organs to radiation [39]. Exposed to a high dose of radiation, gastrointestinal system will be impaired, causing multiple clinical symptoms such as anorexia, nausea, diarrhea, dehydration, and systemic infection [11]. Those adverse reactions seriously affect the quality of life and may even endanger life. Although the acute symptoms of radiation enteritis are mainly caused by mucosal inflammation and degradation, some of these symptoms can be attributed to the effect of radiation on intestinal movement. Ryoo's study found that the migrating motor complex (MMC), originating from the enteric nervous system (ENS) in small intestine movement, presents a process of frequency increase, disappearance, and recovery after radiation. This change may be related to nitric oxide related neurotransmitter changes [35]. NO is a non-adrenergic non-cholinergic (NANC) neurotransmitter. The main source of NO in the gastrointestinal tract is neuronal nitric oxide synthase (nNOS), which is expressed in inhibitory intermuscular neurons and can relax smooth muscle and regulate physiological tension [13, 28].

Polygonati Rhizoma (Huang Jing in Chinese), Achyranthis Bidentatae Radix (ABR, Niu Xi in Chinese), and Epimedii Folium (Yin Yanghuo in Chinese) are all traditional Chinese herbs with various functions and are allowed to be used as health food. Polygonati Rhizoma contains polysaccharide and saponin components. Polygonatum sibiricum polysaccharides (PSP) have various pharmacological effects and biological activities such as anti-oxidation, anti-aging and anti-fatigue effects, enhancing immunity, anti-bacterial and anti-inflammatory effects, anti-osteoporosis, and liver protection [5]. PSP can improve Parkinson's behavior because it not only promotes the proliferation of dopaminergic neurons but also protects them from oxidative stress damage [19]. ABR contains peptides, polysaccharides, and triterpenoids. Achyranthes bidentata polypeptides k (ABPPk), one of the ABR's active components, can significantly protect dopaminergic neurons from neurotoxin-induced apoptosis [31]. Epimedii Folium mainly contains flavonoids, alkaloids, volatile oils, and other components. Modern pharmacological research and clinical practice have shown that Epimedium and its active components have a wide range of pharmacological effects, especially on hormone regulation, anti-osteoporosis, immune function regulation, anti-oxidation, anti-atherosclerosis, anti-depression, anti-tumor and anti-aging activities [27]. Recent studies have also shown that Epimedium extracts can play a neuroprotective role after radiation against ionizing radiation-induced impairment of neurogenesis [22]. Chinese herbs are of great treasure and may provide us with a rich source of innovative ways to deal with health problems. At present, there is no good recipe for radiation-induced multisystem damage. Therefore, based on the above three kinds of Chinese herbs approved for health food, we intend to study their effects on the hematopoietic and immune system, intestine, and ENS, which may provide a new healthcare diet for patients receiving clinical radiotherapy with poor immune function and severe gastrointestinal symptoms.

2. Materials and methods

2.1. Reagents

Polygonati Rhizoma (Anhui Wansheng Chinese Herbal Slices Co., Ltd.), Achyranthis Bidentatae Radix (Shanghai Qingpu Chinese Medicine Tablet Co., Ltd.), Epimedii Folium (Hebei Grain Pharmaceutical Co., Ltd.) were purchased from Shanghai Bailutang Traditional Chinese Medicine Store (Shanghai, China).

Tissue Fixative Solution (G1101-500 ML), Phosphate Buffered Saline (G4202-500 ML), and Phosphate Buffered Saline Powder (G00002-2 L) were purchased from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China). Ethanol absolute (10009218) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Xylene (X820585-500 ml) and Acetic acid (A801295-500 ml) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). TritonX-100 (ZLI-9308), Rabbit SP Detection Kits (Biotin-Streptavidin HRP Detection Systems) (SP-9001), DAB Kits (ZLI-9018) and Hematoxylin Staining Solution (ZLI-9610) were purchased from Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd. (Beijing, China). Citrate Antigen Retrieval Solution was purchased from Beyotime Biotech Inc. (Shanghai, China). The nNOS (C7D7) Rabbit mAb (#4231S) were purchased from Cell Signaling Technology, Inc (Boston, US).

2.2. Animals and treatments

Animal welfare and experimental procedures were carried out under the approval of the Institutional Animal Care and Use Committee of Naval Medical University and in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Male C57BL/6 mice (4 weeks old) were purchased from Shanghai JieSiJie Laboratory Animal Co., Ltd. And were housed under specific pathogen-free conditions (free access to food and water, 23 ± 3 °C, 12 h light/dark cycle). Animals were randomly divided into eight groups ($n = 6$ for each group) and treated with various regimens: (a) Deionized Water Control (DI Ctrl) group; (b) DI 2 Gy group; (c) DI 4 Gy group; (d) DI 8 Gy group; (e) Chinese Herbal Diet Control (CHD Ctrl) group; (f) CHD 2 Gy group; (g) CHD 4 Gy group; (h) CHD 8 Gy group.

The dosage of Chinese herbs in the diet was converted according to the adult dosage and body surface area, and the daily dosage of CHD was determined to be 3.03 g/kg body weight.

Mice of four CHD groups were treated with oral CHD administration once daily for 26 consecutive days and mice of four DI groups were treated with oral DI administration once daily for 26 consecutive days. The radiation source was γ ray ^{60}Co from the Radiation Center (Faculty of Naval Medicine, Navy Medical University). On day 27 after DI or CHD treatments, DI and CHD 2, 4, and 8 Gy groups were exposed to TBI of a single shot dose of 2, 4 and 8 Gy.

On the 9th day after radiation [6,44], the mice were sacrificed. Orbital blood was collected into the anticoagulant tube. The blood routine analysis was carried out by Mindray Automatic Blood Cell Analyzer. The bone marrow nucleated cells were collected from the mouse femur and counted by Countstar software. The thymus and spleen of mice were collected to record organ weight and calculate organ index. Thymus or spleen index = thymus or spleen weight (g)/body weight (g) × 100%. The intestinal tissues of mice were fixed in 4% paraformaldehyde for section staining.

2.3. Histomorphology of small intestine

A 2-cm section of the small intestine of mice was taken from 3 to 4 cm away from the distal end of the Treitz ligament. The contents of the intestine were washed out by Phosphate Buffer Saline (PBS), and then the intestine was fixed in 4% paraformaldehyde. The samples were embedded in paraffin and observed under the microscope after the HE stains.

For evaluation of mucosal damage, villus length (from villus junction to apex) and crypt depth (from villus junction to the base of the intestinal gland) were measured, and the average villus length and crypt depth of each mouse were analyzed.

2.4. Immunohistochemistry

The immunohistochemical staining was consistent with the previous description [23]. After a series of dewaxing and dehydration treatments, fixed tissue sections were quenched with 3% peroxide for 10 min. Then, the slices were incubated with primary antibody nNOS at 4 °C overnight. The next day, the slices were washed with PBS three times, followed by incubation with the biotinylated goat antirabbit antibody diluted with a secondary antidilution buffer for 20 min. The cell nuclei were stained with hematoxylin, and the sections were sealed with neutral resin.

The Immunochemical staining images of intestinal nNOS^+ cells were then obtained using an optical microscope. Four 400X visual fields were randomly selected for each sample, and the nNOS^+ cells in each visual field were counted.

2.5. Statistics

All of the data were presented as mean ± standard error of the mean (SEM). Excel 2010 was used for data summary, and GraphPad Prism 9 was used for statistical analysis and chart drawing. P-value <0.05 was considered to be statistically significant. Unpaired T-test was used for comparison between two groups. One-way ANOVA was used for comparison among multiple groups, and Tukey's multiple comparisons test was used for the post hoc test.

3. Results

3.1. Effect of Chinese herbal diet on body weight of irradiated mice

Our study indicates that there was no statistical difference in body weight between the irradiated mice and unirradiated mice whether treated with DI (Fig. 1A) or CHD (Fig. 1B). At the same radiation dose, no statistical difference in body weight was found between the mice of the DI and CHD groups.

3.2. Effect of Chinese herbal diet on blood routine examination and bone marrow nucleated cells count in irradiated mice

3.2.1. The counts of WBCs RBCs and PLTs decreased after different doses of radiation, but the Chinese herbal diet did not reverse the decline

The WBC counts of mice were significantly decreased after radiation, and there was a dose dependence. The higher the radiation dose, the more severe the drop in WBC count. The same trend was observed in both DI and CHD groups. The WBC counts of the DI 2 Gy, DI 4 Gy, and DI 8 Gy groups were lower than that of the DI Ctrl group ($p < 0.001, p < 0.0001, p < 0.0001$). The mice of the CHD 2 Gy, CHD 4 Gy, and CHD 8 Gy groups had fewer WBCs compared with the CHD Ctrl group ($p < 0.0001, p < 0.0001, p < 0.0001$). However,

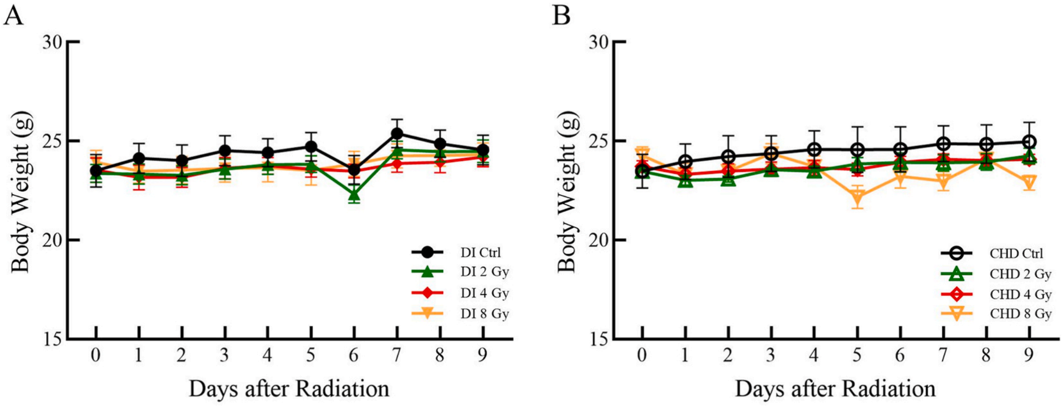

Fig. 1. The average body weight of mice in DI and CHD groups from pre-radiation to 9 days after radiation. A: DI Groups. B: CHD groups.

no difference was observed between the CHD and the DI groups when exposed to the same radiation dose (Fig. 2A).

The RBCs in DI 8 Gy group were less than those in the DI Ctrl group ($p < 0.05$). The RBC counts of the CHD 4 Gy and CHD 8 Gy groups decreased after radiation, which was statistically different from those of the CHD Ctrl group ($p < 0.01$, $p < 0.0001$). As with WBCs, no statistical difference in RBC count was observed between the CHD groups and the DI groups at the same radiation dose (Fig. 2B).

Similar to the WBC, the PLT counts of the DI groups and CHD groups decreased after radiation. The DI 2 Gy group, DI 4 Gy group, and DI 8 Gy group showed a statistically significant difference compared with the DI Ctrl group ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$) in PLT count. CHD 2 Gy, CHD 4 Gy, and CHD 8 Gy groups showed significant differences compared with the CHD Ctrl group ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$). As with WBC and RBC, no difference in PLT count was observed between the CHD and DI groups at the same radiation dose (Fig. 2C).

3.2.2. Chinese herbal diet had little effect on bone marrow nucleated cells count after radiation

As shown in Fig. 2D, the bone marrow nucleated cell count of mice in DI groups showed a slight trend of gradual decline with the increase in radiation dose, but no statistical difference was observed. No significant difference was observed between DI groups and CHD groups at the same radiation dose.

3.3. Effects of Chinese herbal diet on the immune organs of irradiated mice

The weight of the thymus in three DI irradiated groups decreased compared with the DI Ctrl group, and the difference was statistically significant ($p < 0.05$). At the radiation dose of 4 Gy and 8 Gy, the thymus index of DI irradiated groups significantly decreased compared with the control group ($p < 0.05$). Similarly, the thymus weight and thymus index of mice in the CHD groups also decreased at the radiation dose of 4 Gy and 8 Gy, with statistical significance ($p < 0.05$, $p < 0.01$). At the same radiation dose, no difference was found between the DI and CHD groups in thymus weight or thymus index (Fig. 3A, B).

The spleen weight and spleen index decreased gradually with the increase in radiation dose. This trend was shown in both DI and CHD groups. At the dose of 4 Gy and 8 Gy, the spleen weight and index of the DI irradiated groups decreased compared with the DI Ctrl group ($p < 0.001$, $p < 0.0001$). The spleen weight and spleen index of the CHD 2 Gy, 4 Gy, and 8 Gy groups decreased with a statistical

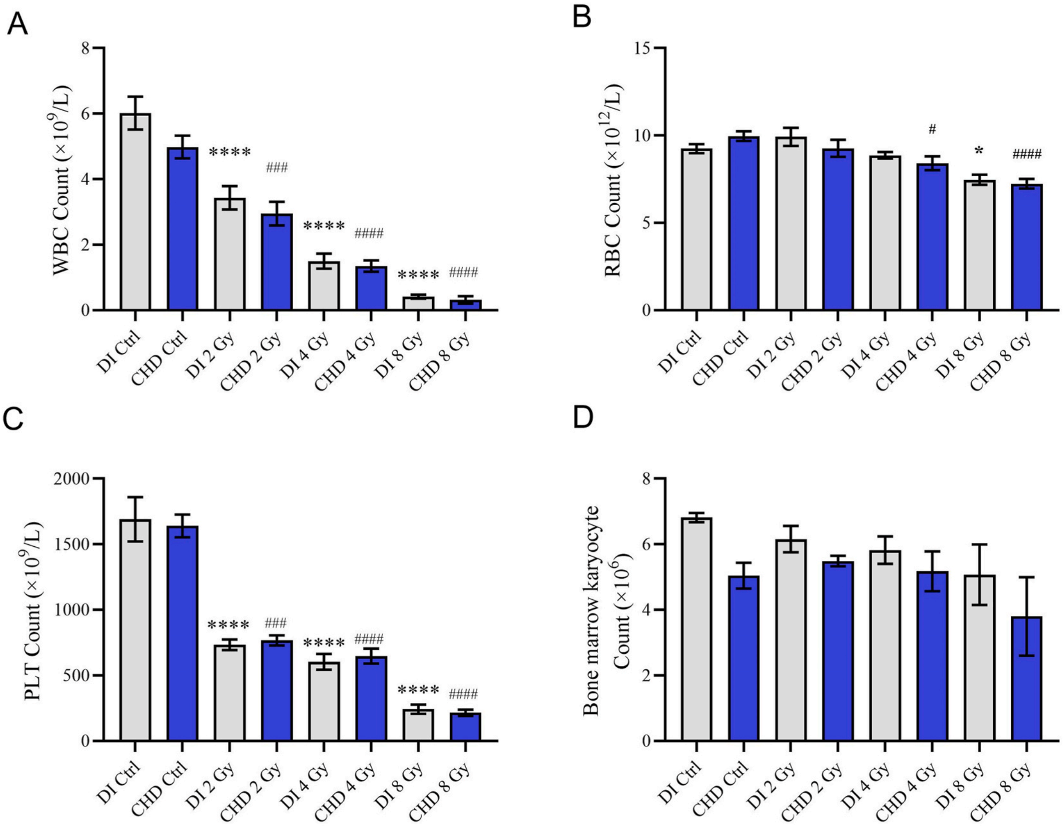

Fig. 2. Effects of radiation on the counts of WBC, RBC, PLT, and bone marrow nucleated cells in DI and CHD groups. A: WBC. B: RBC. C: PLT. D: Bone marrow nucleated cells (n = 3). *$p < 0.05$, ****$p < 0.0001$, VS DI Ctrl group; #$p < 0.05$, ###$p < 0.001$, ####$p < 0.0001$, VS CHD Ctrl group.

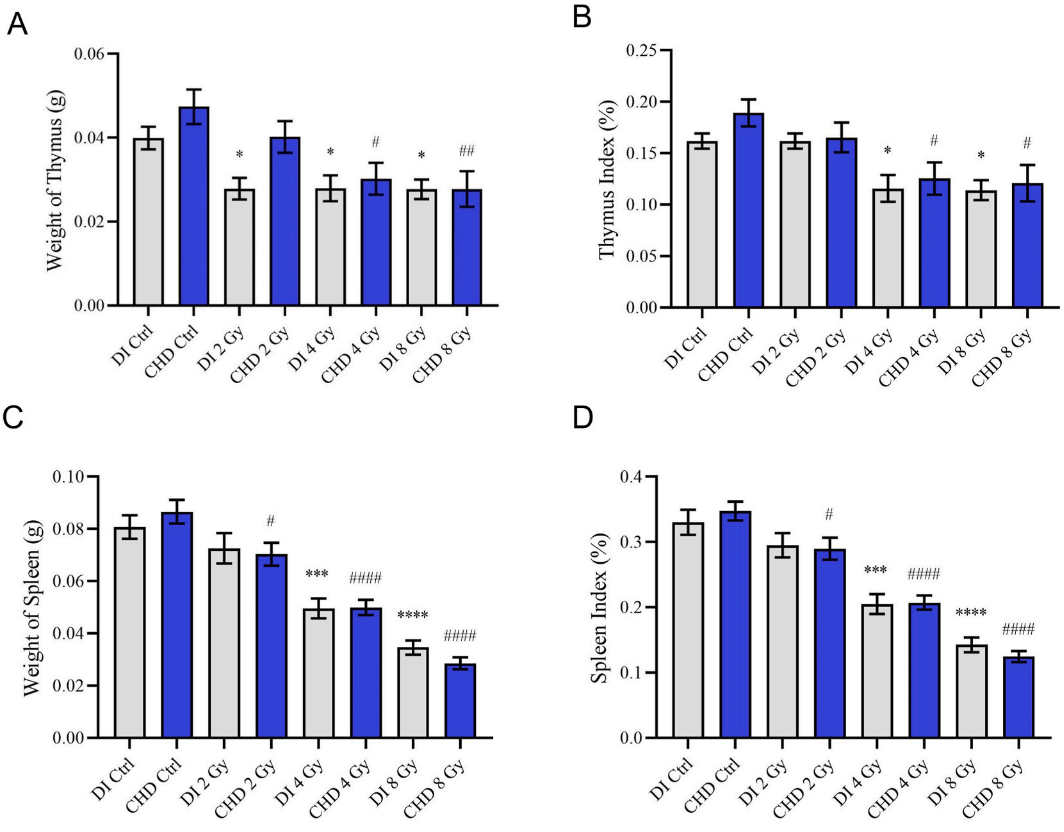

Fig. 3. Effects of radiation on immune organ weight and organ index. A: Thymus weight. B: Thymus index. C: Spleen weight. D: Spleen index. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$, VS DI Ctrl group. #$p < 0.05$, ##$p < 0.01$, ####$p < 0.0001$, VS CHD Ctrl group.

difference compared to the CHD Ctrl group ($p < 0.05$, $p < 0.0001$, $p < 0.0001$). At the same radiation dose, No difference in spleen weight or spleen index was found between CHD and DI groups (Fig. 3C, D).

3.4. Effects of Chinese herbal diet on intestinal histomorphology of irradiated mice

As shown in Fig. 4A, radiation could lead to intestine damage; the higher the dose was, the more serious the injury was. The Chinese herbal diet has a certain anti-radiation effect on the intestines. In HE-stained sections, the small intestine structure was complete in the DI Ctrl and CHD Ctrl groups, the small intestinal mucosa tissue structure was intact, the glands were abundant, and the small intestine villi were developed normally and arranged neatly. Compared with the DI Ctrl group, the small intestinal submucosal space of DI groups with different doses of radiation increased, the intestinal mucosa was separated from the submucosa, the small intestinal villi were sparse, and the villous epithelial cells were shed. At the same radiation dose, the villi damage of the small intestine in the CHD group was slighter than that in the DI group.

The Chinese herbal diet had little effect on the length of villi in the small intestine. At the same radiation dose, the villus length in CHD groups showed no difference from that in DI groups (Fig. 4B).

As shown in Fig. 4C, the crypt depth of the small intestine showed obvious differences at the radiation dose of 8 Gy ($p = 0.07$). The crypt depth of the small intestine in the CHD group was deeper than that in the DI group at the same radiation dose of 4 Gy or 8 Gy ($p < 0.05$).

3.5. Radiation caused ENS injuries, and the number of inhibitory neurons expressing nNOS decreased after radiation. Chinese herbal diet protective effect on the post-radiation damage of neurons

As shown in Fig. 5A, B, the numbers of nNOS$^+$ neurons in the DI 8 Gy group were significantly decreased compared to the DI Ctrl group ($p < 0.05$), while the numbers of nNOS$^+$ are remarkably much more in the CHD 8 Gy group than those in the DI 8 Gy group ($p < 0.05$).

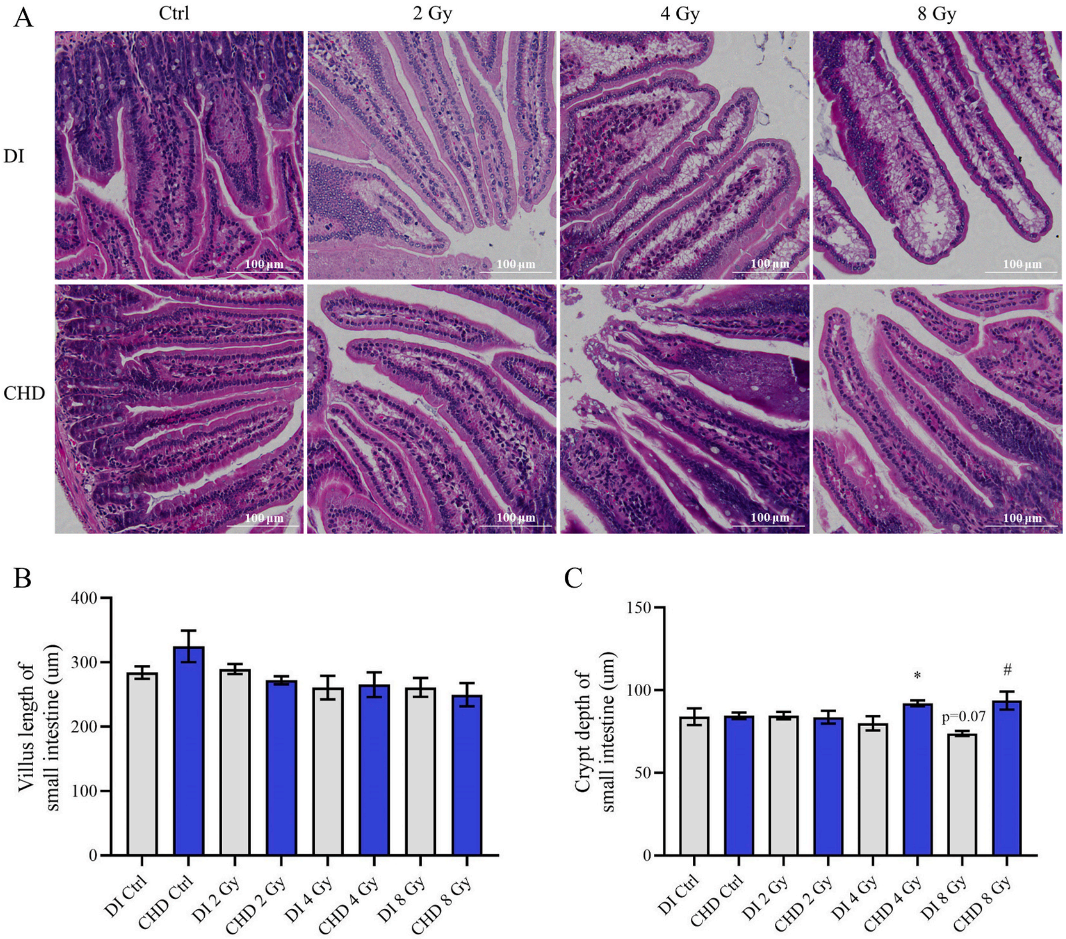

Fig. 4. Effect of radiation on small intestine histomorphology of mice in DI and CHD groups. A: HE staining of small intestinal (400X). B: Villus length of the small intestine. C: Crypt depth of the small intestine. *$p < 0.05$, VS DI 4 Gy group. #$p < 0.05$, VS DI 8 Gy group. $P = 0.07$, VS DI Ctrl group.

4. Discussion

DNA molecules are the most important targets of the biological effects of ionizing radiation. Radiation's direct or indirect effects may lead to base damage and single-strand or double-strand breaks. Double-strand breaks are considered the most serious DNA damage because the chromatin breaks and the cells may not be able to successfully repair. The incidence of DNA damage, especially double-strand breaks, will increase with radiation exposure and will lead to a higher risk of cell death [10]. As a result, patients receiving radiotherapy have to bear the side effects of various systems brought by radiotherapy based on their original low immunity. However, there is no good recipe for the multi-system damage caused by radiation. In this study, we studied the effects of three kinds of Chinese herbal formulations (Polygonati Rhizoma, Achyranthis Bidentatae Radix, and Epimedii Folium) on the hematopoietic, immune, and intestine and ENS of mice exposed to different doses of ionizing radiation, which may provide new ideas and methods for the development of healthcare diet for patients receiving clinical radiotherapy with poor immune function and gastrointestinal symptoms. The counts of peripheral blood cells can directly reflect the state of the body's immune system, and the thymus is also an important immune organ in the body. The spleen is the largest peripheral lymphatic organ, containing a quarter of the body's lymphocytes and mediating innate and adaptive immune responses [2, 14]. He et al. found that pretreatment with Polygonatum sibiricum ethanol 30 (PSE30) and Polygonatum sibiricum ethanol 75 (PSE75) on the immunosuppressed mice significantly increased the number of leukocytes and lymphocytes, and this effect was dose-dependent [16]. Our study found that leukocytes and platelets in mice decreased significantly after exposure to different radiation doses. The higher the radiation dose, the more obvious the decrease. RBC also decreased at the high dose of radiation, indicating that the immune and hematopoietic functions of mice were impaired. Similar findings were also found in the thymus and spleen of irradiated mice. This is consistent with the results of other researchers [1, 3, 8, 20, 25, 38, 49]. However, we did not see the Chinese herbal diet show a radiation protective effect in WBC, RBC, PLT, thymus, or spleen. We speculate that the content of active ingredients in the herbal decoction is too low to reach an effective dose to protect hematopoietic and immune functions. It is also possible that the oral treatment period is not long enough. As the effect of traditional Chinese herbal

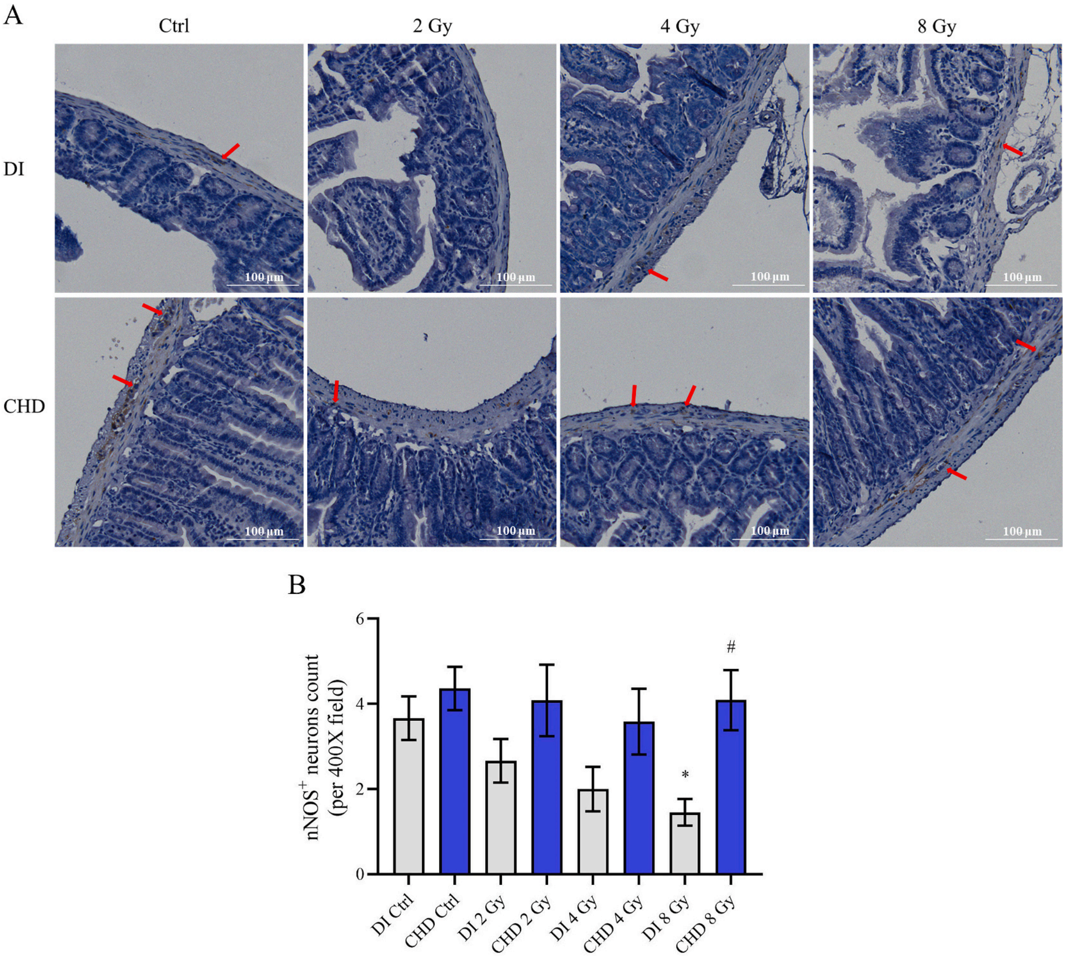

Fig. 5. Effects of radiation on nNOS + neurons in the small intestine in DI and CHD groups. A: nNOS immunohistochemical staining of the small intestine (400X). B: nNOS$^+$ neurons count in the small intestine. *$p < 0.05$, VS DI Ctrl group. #$p < 0.05$, VS DI 8 Gy group.

decoction is quite slow, it needs a long time of administration to show the radiation protection effect. This suggested that we could use the active ingredient monomer of the traditional Chinese herbs or extend the administration time in the later experiments to explore the effects of this diet on the hematopoietic and immune systems of irradiated mice.

The intestine consists of the proliferative crypt containing intestinal stem cells and the villi containing differentiated specialized cell types. The intestine is covered by a single layer of epithelial cells that are renewed every 4–5 days [37]. The maintenance of intestinal structural integrity is essential for intestinal absorption and defense function. After radiation, varying degrees of villi dullness and fusion, attenuation and hypertrophy of villi epithelial cells, and severe crypt loss may occur, leading to the destruction of epithelial homeostasis and epithelial integrity [9]. In addition to the ability of the intestinal epithelium to continuously renew itself, the intestine shows remarkable intestinal renewal and repair capacity after damage [29,36]. Luo's study found that the number of Bacteroides decreased and the abundance of Lactobacillus increased after PSP treatment in mice, indicating that PSP can regulate intestinal probiotics and protect intestinal health [26]. Icariin and its phosphorylated derivatives reduce intestinal epithelial barrier damage caused by enterotoxin Escherichia coli by regulating p38 MAPK in vitro and in vivo [46]. In our study, we did not find the radiation to affect the villus length in both DI or CHD groups, which might be due to the intestinal villi having been repaired on the 9th day since the radiation when the mice were sacrificed. This was similar to Wang's research, in which mucosal villus height of non-human primates was dramatically decreased at days 4 and 7 after a single uniform dose of 6.7 or 7.4 Gy TBI, and then recovered at day 12 after the radiation [40]. The depth of the intestinal crypt represents the rate of autopoiesis. The deeper the crypt, the more active the cell proliferation, which leads to the proliferation and differentiation of the intestinal stem cells. Liu's research proved a positive correlation between crypt depth and stem cell survival after radiation in mice [24]. Intestinal stem cells are closely related to the repair of the intestinal tract after radiation. Our study find that the crypt depth of the DI 8 Gy group decreased compared with the DI Ctrl group ($p = 0.07$). At the radiation dose of 4 Gy and 8 Gy, the crypt depth of the CHD groups was deeper than that of the DI groups, indicating that the Chinese herbal diet could maintain intestinal damage repair ability under higher radiation conditions. Garg et al. found that the vitamin E analog gamma-tocotrienol (GT3) significantly deepened the crypt depth of naïve rhesus macaques at day 7 after a 12 Gy dosage radiation, suggesting that more stem cells survived and proliferated after TBI [12], which was consistent with our research. Also, the mode of radiation may be one of the influencing factors. Our study chose the mode of TBI, which might be less specific to the intestine than total abdominal radiation.

The small intestine epithelium is highly sensitive to radiation and is the main target in abdominal and pelvic radiation therapy. Radiation can lead to the radiation-induced gastrointestinal syndrome. Diarrhea is one of the general symptoms of the syndrome, which is the major limiting factor for radiation dose in rectal cancer and other abdominal malignancies [34]. Patients with diarrhea are often accompanied by hyperperistalsis, and the ENS is involved in the regulation of intestinal movement. The $nNOS^+$ neurons are the main inhibitory motor neurons in the gastrointestinal tract, which release the neurotransmitter NO, relax the gastrointestinal tract's smooth muscle, and slow intestinal movement. One study found that the number of $nNOS^+$ neurons in the major pelvic ganglia (MPG) decreased at both 2 and 10 weeks after radiation [32]. A study by Yi et al., found that the number of NOS^+ neurons in the longitudinal muscle and myenteric plexus (LMMP) decreased significantly after stress, and increased significantly after treatment with patchouli alcohol (PA) in diarrhea-predominant irritable bowel syndrome (IBS-D) rats model. The mechanism may be that PA up-regulated the transcription and protein expression of the myosin Va gene to promote myosin Va transport in $nNOS^+$ nerve vesicles [48]. Our study also showed that after 8 Gy radiation, the number of $nNOS^+$ neurons was significantly decreased compared with the control group ($p < 0.05$). The number of $nNOS^+$ neurons in mice was reversed when the Chinese herbal diet was given a certain time before radiation, and the diet had a better protective effect on the radiation damage of $nNOS^+$ neurons at the dose of 8 Gy. There are few studies on the mechanism of the Chinese herbal diet on enteric neurons, but there have been several studies on the protective effects of Chinese herbs on the nervous system and intestinal tract. Studies have shown that Epimedium can prevent the loss of new cells or neurons in the brain after radiation, which can be seen as a neuroprotective agent [41]. Icariside II has a protective effect on neurotoxicity induced by methamphetamine (METH), which depends on the activation of the Keap1-Nrf2 pathway [18]. Huang et al. revealed that anti-apoptosis mediated by the Akt/mTOR pathway and Nrf2-regulated antioxidant pathways were involved in the neuroprotective effect of PSP in the mouse model of Parkinson's disease [19]. A study on the intestinal barrier function of Epimedium in piglets found that Epimedium could reduce the incidence of diarrhea, and regulate the NF-kB signaling pathway and intestinal microbiome, which effectively enhances intestinal barrier function [47]. Epimedii Folium extract could significantly attenuate cisplatin-induced intestinal damage by regulating oxidative stress, inflammation, and apoptosis, and the molecular mechanism of action might be mainly related to PI3K/Akt, p53, and NF-κB signaling pathways [45]. Achyranthes bidentata peptides play a neuroprotective role in cultured hippocampal neurons by inhibiting glutamate receptor overactivation and inhibiting Caspase-3-dependent mitochondrial apoptosis pathways [30]. Both Polygonati Rhizoma and Epimedii Folium may play a neuroprotective role through the Nrf2 pathway. The NF-kB and Akt-mTOR signaling pathways may also be one of the underlying mechanisms. So, it seems that Chinese herbs have huge potential on reducing radiation damage, and the diet may play a certain beneficial role on patients suffering both from the disease itself and the radiation therapy.

This study has several limitations. We didn't carry on the survival rate experiment in our study, because we referred to other research that 8 Gy radiation did not affect the survival of C57BL/6 mice in 30 days. We will perform the survival rate experiment of 30 days or longer time. For evaluating the post-radiation intestinal injury and repair more comprehensively, We should study the intestine at multiple time points after 9 days. The mechanism of the Chinese herbal diet on the ENS, especially on the nNOS + neurons, is still not clear in this study. Many studies have shown that radiation causes intestinal microbial changes, resulting in intestinal damage. One possibility is that the changes in nNOS + neurons after radiation are mediated by the gut microbiota. We will conduct further research on the interaction between the Chinese herbs, gut microbiota, and ENS in the future.

5. Conclusion

Our study showed that the Chinese herbal diet composed of Polygonati Rhizoma, Achyranthis Bidentatae Radix, and Epimedii Folium had no radiation protective effect on the immune and hematopoietic system of mice, but showed an obvious protective effect on intestinal crypts, and an anti-radiation effect on reducing the loss of the inhibitory nNOS + neurons in the intestine. This research provides a new diet for relieving the symptoms of hyperperistalsis and diarrhea in patients after radiotherapy.

Author contribution statement

Tianyu Zheng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiaohui Shi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Shuang Nie: Analyzed and interpreted the data.

Lifeng Yin; Jian Zhu: Performed the experiments.

Enda Yu; Hui Shen: Contributed reagents, materials, analysis tools or data.

Fengfeng Mo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The figure of Graphical Abstract was created with Biorender.com.

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