

# Protective Effects and Mechanisms of Huangqi Decoction Against Radiation-Induced Bystander Effects

## Efectos Protectores y Mecanismos de la Decocción de Huangqi Contra los Efectos Secundarios Inducidos por la Radiación

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**SUMMARY:** The <sup>12</sup>C<sup>6+</sup> heavy ion beam irradiation can cause bystander effects. The inflammatory cytokines, endocrine hormones and apoptotic proteins may be involved in <sup>12</sup>C<sup>6+</sup> irradiation-induced bystander effects. This study characterized the protective effects and mechanisms of Huangqi decoction (HQD) against <sup>12</sup>C<sup>6+</sup> radiation induced bystander effects. Wistar rats were randomly divided into control, <sup>12</sup>C<sup>6+</sup> heavy ion irradiation model, and high-dose/medium-dose/low-dose HQD groups. HE staining assessed the pathological changes of brain and kidney. Peripheral blood chemical indicators as well as inflammatory factors and endocrine hormones were detected. Apoptosis was measured with TUNEL. Proliferating cell nuclear antigen (PCNA) expression was determined with real-time PCR and Western blot. Irradiation induced pathological damage to the brain and kidney tissues. After irradiation, the numbers of white blood cells (WBC) and monocyte, and the expression of interleukin (IL)-2, corticotropin-releasing hormone (CRH) and PCNA decreased. The damage was accompanied by increased expression of IL-1 $\beta$ , IL-6, corticosterone (CORT) and adrenocorticotrophic hormone (ACTH) as well as increased neuronal apoptosis. These effects were indicative of radiation-induced bystander effects. Administration of HQD attenuated the pathological damage to brain and kidney tissues, and increased the numbers of WBC, neutrophils, lymphocyte and monocytes, as well as the expression of IL-2, CRH and PCNA. It also decreased the expression of IL-1 $\beta$ , IL-6, CORT and ACTH as well as neuronal apoptosis. HQD exhibits protective effects against <sup>12</sup>C<sup>6+</sup> radiation-induced bystander effects. The underlying mechanism may involve the promotion of the production of peripheral blood cells, inhibition of inflammatory factors and apoptosis, and regulation of endocrine hormones.

**KEY WORDS:** Huangqi decoction; <sup>12</sup>C<sup>6+</sup> irradiation; Inflammatory factors; Endocrine hormone; Apoptosis.

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## INTRODUCTION

Unlike X-ray and gamma-ray radiation, heavy ion radiation has high transmission line density and characteristic Bragg peaks, and their energy release is characterized by exponential increases or decreases with different depths (Blakely & Kronenberg, 1998). In recent years, it has been widely used for the treatment of cancers. Radiation can induce damages such as sister chromatid exchange,

micronucleus formation, and DNA damages in cancers. However, there are radiation bystander effects in adjacent tissues and other non-irradiated organs. Radiation bystander effect refers to that the irradiated tissues or cells transmit the radiation damaging effects to the non-irradiated tissues or cells. During radiotherapy for head and neck tumors, radiation-induced bystander effects have also been

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extensively investigated. For example, it is reported that 2 Gy irradiation on glioma cells induced significantly increased BRCA1 and phospho-Chk-1 foci formation in T98G bystander cells, supporting the hypothesis of an S-phase dependent bystander DNA damage response involving gH2AX, BRCA1 and FANCD2 (Burdak-Rothkamm *et al.*, 2015). It is also found that the yield of bystander signals was higher in Wistar rats harboring C6 gliomas than in tumor-free rats (Fernandez-Palomo *et al.*, 2013). It is further revealed that the bystander effect induced proteome may confer anti-tumorigenic properties that are based on ROS-induced apoptosis (Smith *et al.*, 2013). DNA damage and apoptosis may be the main mechanisms of radiation-induced bystander effects in head and neck tumors. It is critical to develop safe, less toxic and efficacious agents that protect against irradiation-induced bystander effects.

The Jing Fang Huangqi decoction (HQD) is mainly composed of *Astragali Radix*, *Rehmanniae Radix*, *Ophiopogonis Radix*, *Ginseng Radix et Rhizoma*, *Fructus Lycii* and *Fructus Schisandrae Chinensis*. Modern pharmacological studies have indicated that the medicinal value of HQD is prominent, mainly including liver-protective effect, anti-inflammation, anti-irradiation, heat-clearing and detoxifying effects (Zhao *et al.*, 2021). However, the protective effects and the specific molecular mechanisms of HQD against irradiation-induced bystander effects are less studied.

Therefore, in this study, we investigated the protective effects and underlying mechanisms of HQD on heavy ion radiation induced bystander effects in  $^{12}\text{C}^{6+}$ -irradiated rats. The effects of HQD on pathological changes, blood cells, inflammatory factors, hormones, apoptosis and proliferating cell nuclear antigen (PCNA) were evaluated. Our findings may provide preliminary evidence on the benefits of HQD in protecting against heavy ion radiation induced bystander effects.

## MATERIAL AND METHOD

**Animals.** Specific pathogen-free female Wistar rats (180±20 g; n=50) were purchased from the Research Laboratory Animal Center of Gansu University of Chinese Medicine (SYXK (Gan) 2015-0005; Lanzhou, China). They were kept in standard conditions. All animal experiment procedures were approved by the Animal Ethics Committee of Gansu University of Chinese Medicine (approval no. 2017-010).

**Preparation of HQD.** HQD was prepared using *Astragali Radix* (30 g), *Rehmanniae Radix* (15 g), *Ophiopogonis Radix*

(15 g), *Ginseng Radix et Rhizoma* (15 g), *Fructus Lycii* (15 g) and *Fructus Schisandrae Chinensis* (10 g). The herbs were decocted two times, with 1 h of each time. The decoction was collected and made into crude drug with concentration of 0.198g crude drug/ml. According to the ratio of human to rat body surface area, the high dose, medium dose and low dose of HQD was determined at 18 g crude drug/kg, 9 g crude drug/kg and 4.5 g crude drug/kg, respectively.

**Animal grouping and treatment.** Fifty rats were randomly divided into the following five groups: the normal control (NC), radiation-alone model (RAM), and high-dose HQD (HQDH, 18 g/kg), medium-dose HQD (HQDM, 9 g/kg) and low-dose HQD (HQDL, 4.5 g/kg) groups, with 10 rats in each group. HQD was administered by continuous gavage for 1 week, with the NC and RAM rats given equal volumes of double distilled water for gavage. With the exception of the NC group, the other groups received single-dose irradiation of 4 Gy  $^{12}\text{C}^{6+}$  ion beams in the whole brain according to previous description, which was conducted at the Institute of Modern Physics, Chinese Academy of Sciences (Lanzhou, China). On day 7 after radiation, blood was collected from the femoral artery and the serum was separated by centrifuging at 1500 rpm for 15 min at 4°C. Then, the rats were sacrificed by 3 % pentobarbital (30 mg/kg), and the brain and kidney tissues were collected.

**Haematoxylin and eosin staining.** The brain and kidney tissues were fixed in 4 % paraformaldehyde, embedded in paraffin and sectioned using a microtome (CM1520, Leica Biosystems, Wetzlar, Germany). According to routine procedure, the sections were stained with haematoxylin and eosin (H&E) to evaluate the pathological changes in brain and kidney. Photomicrographs of the H&E-stained sections were taken at 200× magnification under microscope (BX53, Olympus, Tokyo, Japan).

**Measurement of peripheral blood cells.** Changes in the peripheral blood were measured using an automatic animal blood cell analyser (BC2800Vet, Shenzhen Mindray Biomedical Electronics Co. Ltd., Shenzhen, China). The levels of white blood cell (WBC), neutrophil, lymphocyte, and monocyte were measured.

**ELISA analysis of cytokines and hormones.** Serum levels of interleukin (IL)-1 $\beta$ , IL-2, IL-6, corticosterone (CORT), corticotropin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) were measured using corresponding kits (FEIYA BIOTECHNOLOGY, Jiangsu, China). The absorbance at 490 nm was detected on the Multiskan Sky Microplate Spectrometer (Thermo Fisher Scientific Technology Co. Ltd., Massachusetts, USA).

**Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining.** TUNEL staining was performed on brain tissues using TUNEL Apoptosis Detection Kit (FITC) (T3901120, Yeasen Biotech, Shanghai, China). Briefly, brain tissue sections were permeabilized by proteinase K solution (20 mg/ml) at 37 °C for 30 min. After washing with PBS three times for 10 min each time, the terminal deoxynucleotidyl transferase and fluorescein were added and incubated at 37°C for 1 h. Finally, the brain sections were sealed with anti-fluorescence quenching agent. Apoptosis of neuronal cells was then detected at 100× magnification under an inverted fluorescence microscope (IX73-A12FL/PH, Olympus, Japan). Image-Pro Plus software 6.0 was used to determine the numbers of apoptotic neurons, and proportions were calculated with the following equation: percentage of apoptotic neurons (%) = number of apoptotic cells/total number of cells × 100 %.

**Real-time PCR analysis of PCNA expression.** Total RNA from the brain and kidney tissues was isolated using TRIzol (T9926840, Yeasen Biotech, Shanghai, China), and the RNA concentration was determined using an ultra-micro ultraviolet-visible spectrophotometer (Q5000, Quawell Technology Inc., San Jose, CA, USA). The cDNA was synthesized using the Reverse Transcription Kit (H9001100, Yeasen Biotech Co. Ltd., Shanghai, China). Real-time PCR was performed on ABI 7500 (Applied Biosystems, Foster City, CA, USA). Primers for  $\beta$ -actin and PCNA were synthesized by Takara (Tokyo, Japan). The primer sequences were as follows:  $\beta$ -actin upstream primer: 5'-TGTGAcgTTGACATCC GTAAAG-3', downstream primer 5'-GGCAGtaatCTCCTTCTGCATC-3'; and, PCNA upstream primer 5'-ACTCCACTGTCTCCTACAGTAA-3', downstream primer 5'-TCGATCTTGGGAGCCAAATAAT-3'. The reaction conditions were as follows: pre-denaturation at 95 °C for 2 min, denaturation at 95 °C for 40 s, annealing at 58 °C for 50 s, and extension at 60 °C for 2 min for a total of 42 cycles. The  $\beta$ -actin gene served as the internal reference gene, and the  $2^{-\Delta\Delta Ct}$  method was used to determine the relative expression level of each gene.

**Western blot analysis of PCNA expression.** Total protein from the brain and kidney tissues was isolated after lysis with RIPA buffer (20140801, Solarbio, Beijing, China). Protein concentration was determined using an ultra-micro ultraviolet-visible spectrophotometer (Q5000, Quawell Technology Inc. San Jose, CA, USA). Equal amounts of protein (40  $\mu$ g) were resolved in 10 % acrylamide gels, and then electro-transferred to polyvinylidene difluoride membranes. The membranes were blocked in 5 % bovine serum albumin and incubated overnight at 4 °C with the following antibodies: rabbit polyclonal anti-PCNA (42046, GeneTex) and rabbit polyclonal anti-GAPDH (42977,

GeneTex). After incubating the membranes with horseradish peroxidase-conjugated secondary antibody (B0201, ImmunoWay Biotechnology Co., Plano, TX, USA), the proteins were detected with chemiluminescence, and densitometry was measured using the ChemiDoc XRS<sup>+</sup> gel imaging system (1708265, Bio-Rad, Hercules, CA, USA).

**Statistical analysis.** All data are expressed as the mean  $\pm$  standard deviation. Statistical and graphical analyses were performed using SPSS version 22.0 software. Statistical significance was determined using one-way analysis of variance followed by LSD method (for variance with homogeneity) or Dunnett-t method (for variance without homogeneity).  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

**HQD can significantly improve the pathological damage of brain and kidney in rats with  $^{12}C^{6+}$  irradiation.** HE staining was used to evaluate pathological changes in brain and kidney tissues. As shown in Fig. 1A, the neurons in the brains of the NC rats exhibited an orderly arrangement with normal morphology and structure. The RAM group had loose brain tissue, decreased number of neurons, condensed and hyperchromatic neuronal nuclei, and widened perivascular and perineuronal spaces. The HQDL and HQDM groups had loose brain tissue, decreased number of neuronal cells, and a widened perivascular space. The pathological changes such as loose brain tissue and decreased number of neurons in the HQDH group were obviously improved compared with the RAM group. However, the morphology and structures of brain neurons in the HQDH group did not completely return to a normal state as those in NC group. As shown in Fig. 1B, the glomeruli and tubules were normal in the NC group. Proliferation of glomerular mesangial cells, dilation and congestion of renal tubular interstitial vessels, and a narrow and irregular renal tubular lumen were observed in the RAM group. The HQDL and HQDM groups had similar characteristics, except that the renal tubular arrangement was disordered. Glomerular mesangial cell proliferation was obviously improved and renal tubules were clearly defined in the HQDH group. These results indicate that HQD can significantly reduce the brain and kidney injury of  $^{12}C^{6+}$  irradiation model rats.

**HQD alleviates the damage of hematopoietic system in rats with  $^{12}C^{6+}$  irradiation.** To determine the damage of hematopoietic system in rats with  $^{12}C^{6+}$  radiation, the numbers of WBC, neutrophil, lymphocyte, and monocyte were checked. Compared to NC group, the numbers of WBC

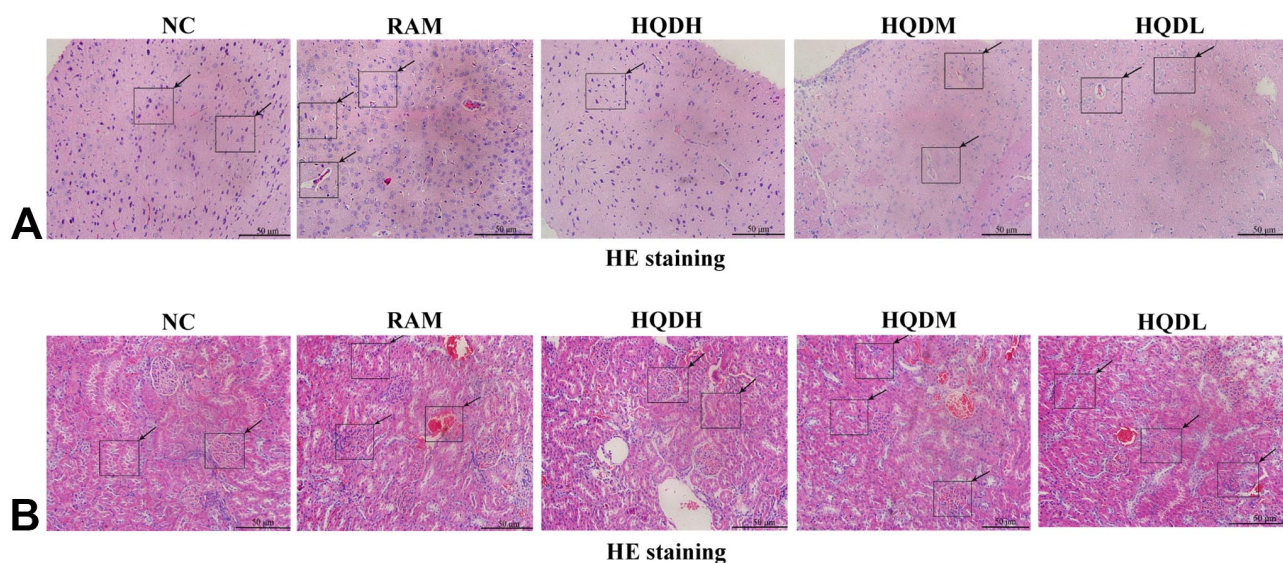


Fig. 1. Pathological changes in the brain and kidney in  $^{12}\text{C}^{6+}$ -irradiated rats. (A) H&E staining showing the pathological changes of brain tissues in each group. (B) H&E staining showing the pathological changes of kidney tissues in each group. Scale bars, 50  $\mu\text{m}$ .

and monocyte in the peripheral blood were decreased in the RAM group ( $P < 0.01$ ), but no significant changes were observed for neutrophils and lymphocytes ( $P > 0.05$ ) (Fig. 2A-2D). Compared with the RAM group, the numbers of monocyte and lymphocyte increased in the HQDH group,

the number of WBC increased in the HQDM and HQDH groups, and the number of neutrophils was higher in the HQD groups ( $P < 0.01$ , respectively; Fig. 2A-2D). These results indicate that HQD may alleviate the damage of hematopoietic system in  $^{12}\text{C}^{6+}$  irradiation model rats.

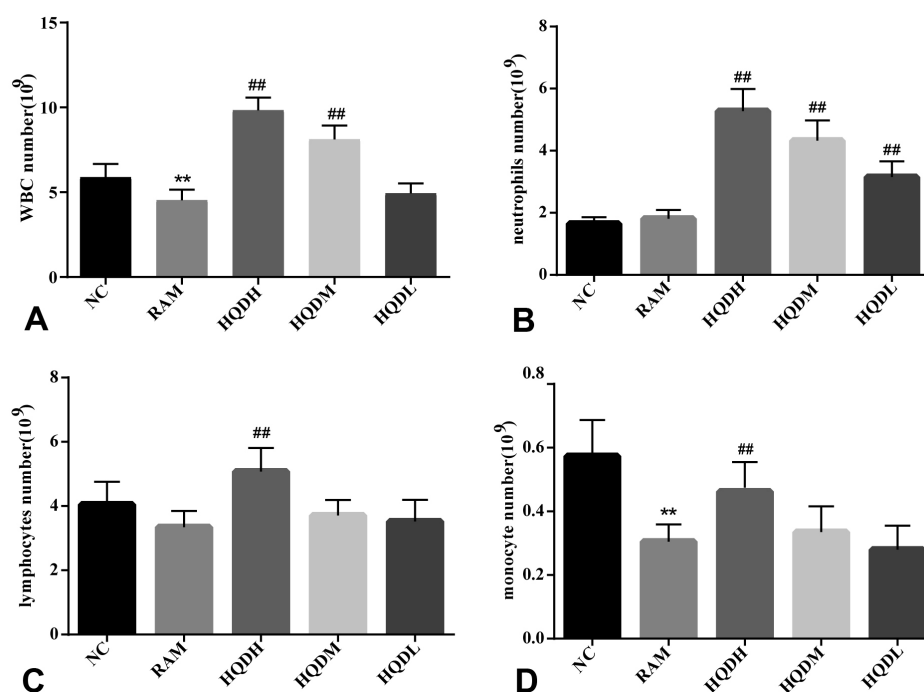


Fig. 2. Effects of HQD on blood cells in  $^{12}\text{C}^{6+}$ -irradiated rats. Automatic hemocyte analyzer was conducted to assess the count of blood cells. (A) Effect of HQD on the number of WBC. (B) Effect of HQD on the number of neutrophils. (C) Effect of HQD on the number of lymphocytes. (D) Effect of HQD on the number of monocyte. Data are expressed as the mean  $\pm$  standard deviation (SD). \*\* $P < 0.01$  vs. NC group; ## $P < 0.01$  vs. RAM group.

**HQD can significantly inhibit the inflammatory reaction of  $^{12}\text{C}^{6+}$  irradiation model rats.** The inflammatory factors were analyzed with ELISA. The results showed that, compared with NC, the levels of IL-1 $\beta$  and IL-6 were higher in the RAM group, but that of IL-2 was lower in the RAM group ( $P < 0.01$ ; Fig. 3A-3C). The levels of IL-1 $\beta$  and IL-6 in the HQD groups were lower than those in the RAM group, and the levels of IL-2 were higher than that in the RAM group ( $P < 0.05$  or  $P < 0.01$ , respectively; Fig. 3A-3C). These results indicate that HQD may inhibit the inflammatory reaction in  $^{12}\text{C}^{6+}$  irradiation model rats by reducing the secretion of inflammatory factors.

**HQD may indirectly affect the endocrine function of rats with  $^{12}\text{C}^{6+}$  irradiation.** Levels of endocrine hormones detected with ELISA. The serum levels of CORT and ACTH were higher in the RAM group than those in the NC group, whereas the CRH content was lower ( $P < 0.01$ ; Fig. 4A-4C). The levels of CORT and ACTH were lower in the HQDH group than those in the RAM group, and the level of CRH was higher in the HQDH and HQDM groups than that in the RAM group ( $P < 0.05$  or  $P < 0.01$ , respectively; Fig. 4A-4C). These results indicate that HQD may indirectly affect the endocrine function of rats with  $^{12}\text{C}^{6+}$  irradiation.

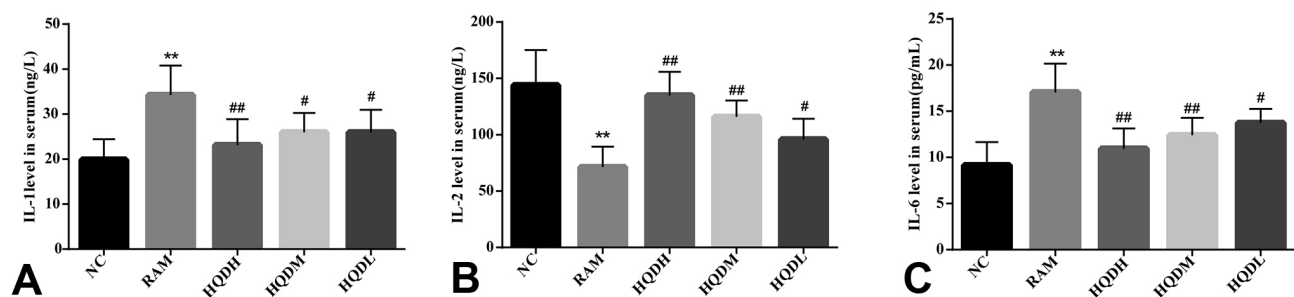


Fig. 3. Effects of HQD on serum cytokine levels in  $^{12}\text{C}^{6+}$ -irradiated rats. ELISA was conducted to assess cytokine level. (A) Effect of HQD on the levels of IL-1. (B) Effect of HQD on the levels of IL-2. (C) Effect of HQD on the levels of IL-6. Data are expressed as the mean  $\pm$  standard deviation (SD). \*\* $P < 0.01$  vs. NC group; # $P < 0.01$ , ## $P < 0.01$  vs. RAM group.

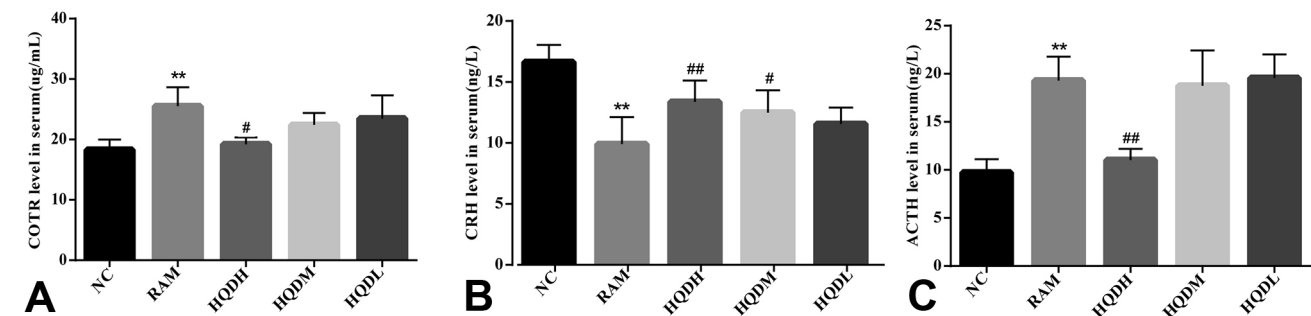


Fig. 4. Effects of HQD on serum hormones in  $^{12}\text{C}^{6+}$ -irradiated rats. ELISA was conducted to assess serum hormones. (A) Effect of HQD on the levels of CORT. (B) Effect of HQD on the levels of CRH. (C) Effect of HQD on the levels of ACTH. Data are expressed as the mean  $\pm$  standard deviation (SD). \*\* $P < 0.01$  vs. NC group; # $P < 0.01$ , ## $P < 0.01$  vs. RAM group.

**HQD may reduce the apoptosis of nerve cells in the brain tissue of  $^{12}\text{C}^{6+}$  irradiation model rats.** TUNEL staining showed obvious apoptosis of neurons in the RAM and HQDL groups, whereas apoptosis was reduced in the HQDM and HQDH groups (Fig. 5A). Statistically, the apoptosis rate of nerve cells in brain tissue of RAM group was significantly higher than that in NC group ( $P < 0.01$ ; Fig. 5B). However, the apoptosis rate in HQDM group and HQDH group was significantly decreased than that in RAM

group ( $P < 0.01$ ; Fig. 5B). These results indicate that HQD may play a preventive and therapeutic role by reducing the apoptosis of nerve cells in the brain tissue of  $^{12}\text{C}^{6+}$  radiation model rats.

**HQD may inhibit the proliferation of nerve cells by regulating the mRNA expression of PCNA in the brain tissue of  $^{12}\text{C}^{6+}$  irradiation model rat.** Real-time PCR was performed to PCNA mRNA expression in the brain and

kidney tissues of rats. As shown in Figure 6A-6B, PCNA mRNA expression was in the brain tissue and kidney tissue significantly lower in the RAM group than those in the NC group ( $P < 0.01$ ). However, PCNA mRNA expression in the brain tissue and kidney tissue was significantly higher in the HQDM and HQDH groups than those in the RAM group ( $P < 0.01$ ; Fig. 6A-6B).

### HQD may inhibit the proliferation of nerve cells by regulating the protein expression of PCNA in the brain

**tissue of  $^{12}\text{C}^{6+}$  irradiation model rat.** Western blot analysis demonstrated that PCNA protein expression in the brain tissue and kidney tissue was significantly lower in the RAM group than those in the NC group ( $P < 0.01$ ; Fig. 6C-6F). PCNA protein expression in the brain tissue was significantly higher in the HQDL, HQDM and HQDH groups than in the RAM group ( $P < 0.01$ ; Fig. 6C and 6E). PCNA expression levels in the kidney tissue were significantly higher in the HQDM and HQDH groups than that in the RAM group ( $P < 0.01$ ; Fig. 6D and 6F).

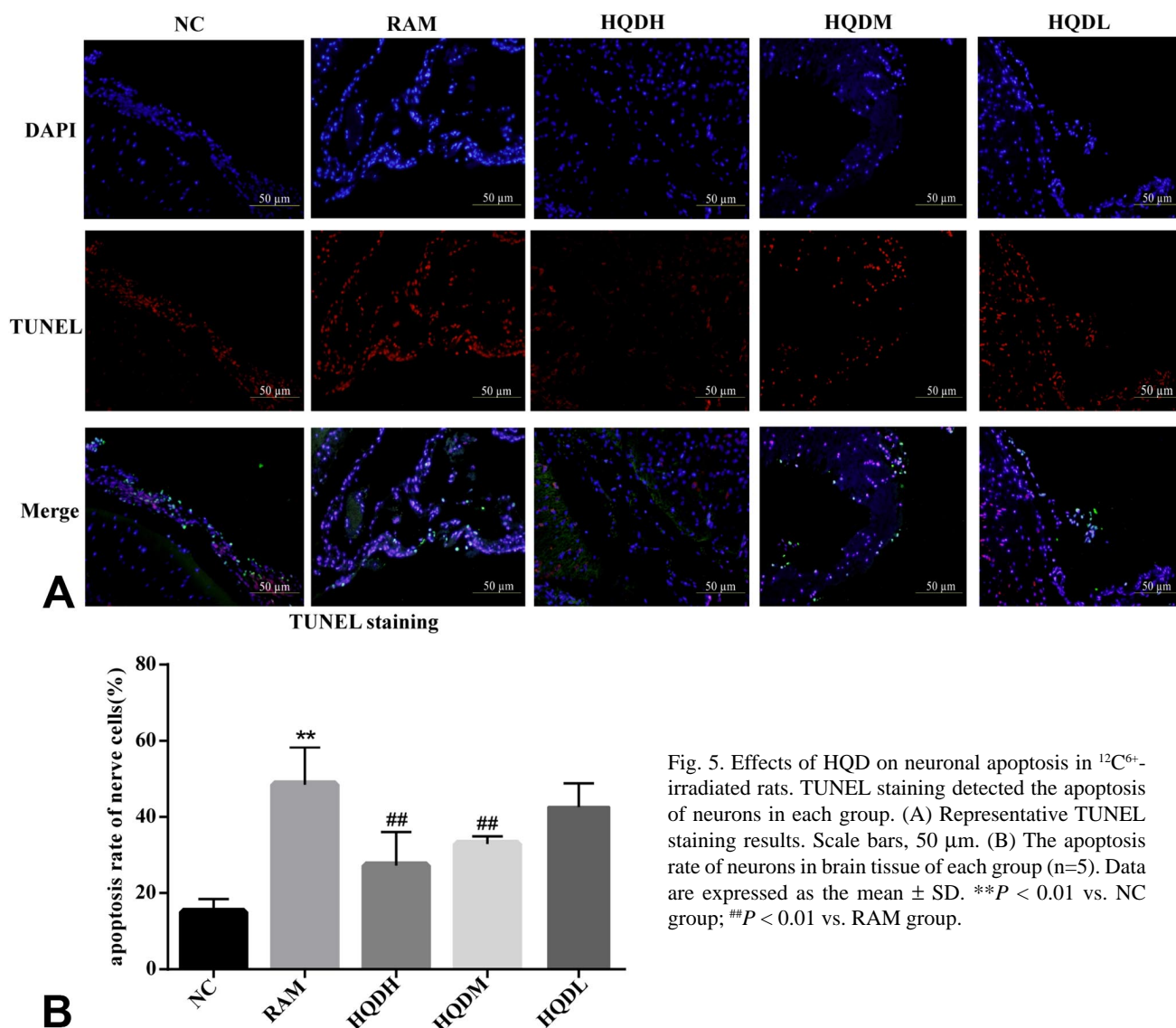


Fig. 5. Effects of HQD on neuronal apoptosis in  $^{12}\text{C}^{6+}$ -irradiated rats. TUNEL staining detected the apoptosis of neurons in each group. (A) Representative TUNEL staining results. Scale bars, 50  $\mu\text{m}$ . (B) The apoptosis rate of neurons in brain tissue of each group (n=5). Data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$  vs. NC group; ## $P < 0.01$  vs. RAM group.

## DISCUSSION

Heavy ions are widely applied for tumour radiotherapy as they have good tumour-killing effects, but the associated bystander effects remain an issue (Liang *et*

*al.*, 2019). Radiation-induced in vivo bystander effects can occur in adjacent tissues and distant organs of irradiated tissues, and mainly include DNA damage, apoptosis and



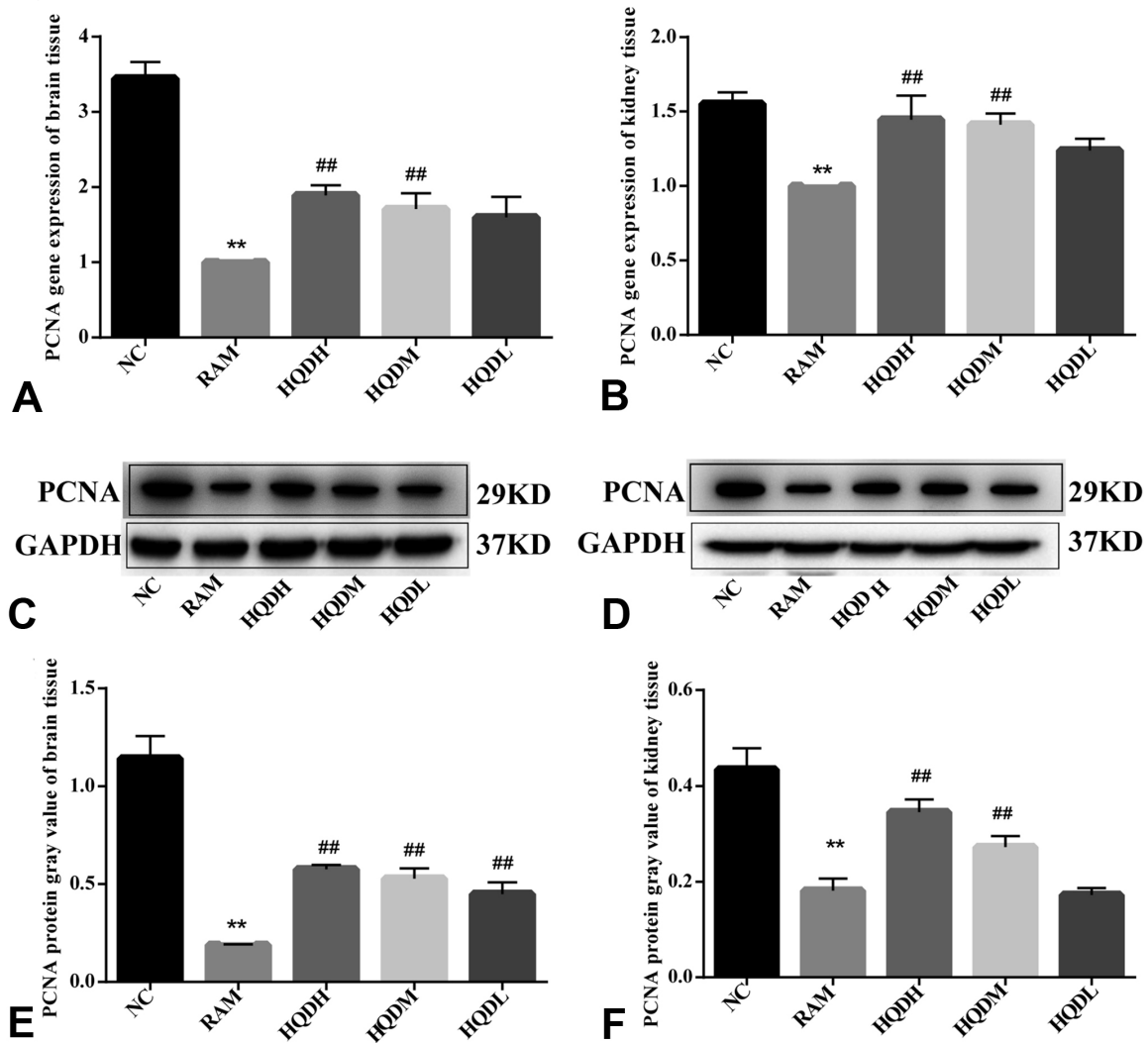


Fig. 6. PCNA expression in brain and kidney tissues in  $^{12}\text{C}^{6+}$ -irradiated rats. (A) Effect of HQD on PCNA mRNA expression in brain tissues as detected by real-time PCR. (B) Effect of HQD on PCNA mRNA expression in kidney tissues as detected by real-time PCR. (C) Effects of HQD on PCNA protein expression in brain tissues were analyzed by Western blot. (D) Effects of HQD on PCNA protein expression in kidney tissues were analyzed by Western blot. (E) Relative grey value of PCNA protein in brain tissues in each group. (F) Relative grey value of PCNA protein in kidney tissues in each group. Data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$  vs. NC group; ## $P < 0.01$  vs. RAM group.

epigenetic changes (Koturbash *et al.*, 2006, 2007; Mancuso *et al.*, 2008). The para-radiation effect is a ‘non-target’ effect in which unirradiated cells produce reactions similar to those of irradiated cells (Feiok *et al.*, 2016; Siva *et al.*, 2016). Our previous research found that HQD exhibited protective effects against  $^{12}\text{C}^{6+}$  irradiation-induced side effects, but the underlying molecular mechanism is not clear.

**Brain is most vulnerable to radiation.** After high dose or long-term radiation, synaptic structure damage, decreased neuron density, cognitive impairment and memory decline may occur (Kim *et al.*, 2008; Qiao *et al.*, 2014). In severe

cases, behavior changes, nervous system diseases or brain tumors may even occur (Zhi *et al.*, 2017). The abnormal structure and function of neurons caused by radiation will inevitably damage the function and structural plasticity of synapses, and then affect the high-level brain function. The results of our study found that the RAM group had loose brain tissue, decreased number of neurons, condensed and hyperchromatic neuronal nuclei, and widened perivascular and perineuronal spaces, indicating the pathological damages caused by radiation. After HQD treatment, the pathological changes were alleviated. This shows that HQD may have protective effect on radiation-induced brain injury.

**Kidney is one of the most serious radiation-sensitive organs in abdomen.** Compared with the epithelial cells of other tissues, renal tubular epithelial cells are more sensitive to radiation, which can lead to a variety of pathological changes of kidney and result in the decline of renal function. Therefore, it is of great significance to explore the mechanism of radiation-induced renal injury and to develop effective prevention and treatment measures. In this study, proliferation of glomerular mesangial cells, dilation and congestion of renal tubular interstitial vessels, and a narrow and irregular renal tubular lumen were observed in the RAM group. These changes were attenuated by HQD treatment, suggesting the protective effect of HQD on radiation-induced kidney injury.

**Radiation can also lead to haematopoietic, immune and genitourinary injuries** (Li *et al.*, 2016). In the haematopoietic system, radiation can inhibit the proliferation of bone marrow haematopoietic cells by interfering with cell apoptosis, ultimately leading to a reduction in peripheral blood cells (Hu *et al.*, 2021). Therefore, changes in the number of peripheral blood cells can be an indirect indicator of the degree of radiation damage to some extent. Wang *et al.* (2020) have confirmed that the number of WBC, red blood cells, platelets, neutrophils and other blood cells in the peripheral blood of X-ray model mice and bone marrow mononuclear cells and number of colony forming cells in bone marrow decreased significantly after radiation, thus causing various infections, anaemia and even death. Consistently, this study showed that the number of WBC and monocytes in the peripheral blood of rats in the RAM group decreased significantly. The numbers of WBC, neutrophils, lymphocyte and monocyte in the peripheral blood of rats increased significantly after intervention with HQD, possibly because the production of peripheral blood cells and proliferation of bone marrow cells were promoted.

**Radiation may cause a dynamic imbalance in proinflammatory factors and anti-inflammatory factors.** IL-1 $\beta$ , IL-6 and tumor necrosis factor alpha are proinflammatory cytokines secreted by neutrophils, eosinophils, mononuclear phagocytes and macrophages, which are mainly involved in various physiological and pathological inflammatory processes. As an important proinflammatory factor, IL-1 $\beta$  is upstream of the inflammatory response and can promote the secretion of other proinflammatory factors and the production of effector molecules such as IL-6 (Yu *et al.*, 2017). IL-6 is an endogenous inflammatory factor that can promote a systemic inflammatory response, catalyse and amplify the inflammatory response of the body, and regulate the acute phase inflammatory response by facilitating the secretion of a variety of acute phase proteins (Riehl *et al.*, 2012).

Ionizing radiation can cause IL-1 $\beta$  to increase and cause an oxidative stress reaction (Gao *et al.*, 2019). IL-1 $\beta$  is an important inflammatory mediator of radiation-induced brain injury (Tong *et al.*, 2016). Radiation injury can increase the expression of proinflammatory factors such as IL-6, IL-17 and transforming growth factor 1 beta (Li *et al.*, 2015). The content of IL-2 in the spleen is reduced after radiation injury. Our results showed that the contents of IL-1 $\beta$  and IL-6 in the serum of RAM rats increased significantly, whereas that of IL-2 decreased significantly. After treatment with HQD, the serum levels of IL-1 $\beta$  and IL-6 in the intervention group decreased significantly, whereas that of IL-2 increased significantly. Thus, HQD can inhibit the expression of inflammatory factors that is part of the radiation-induced bystander effect of heavy ion radiation.

**Radiotherapy can also prolong the cell cycle, and induce apoptosis in radiation-exposed cells.** PCNA, as a cell cycle related protein, is almost not expressed in the G0 phase of cell cycle, but it increases at G1 stage, reaches peak in S phase and decreases in G2-M phase. Meanwhile, PCNA participates in many important cellular events such as chromatin remodeling, DNA damage repair, apoptosis, sister chromatid aggregation, etc. (Kannouche *et al.*, 2004; de Medina-Redondo & Meraldi, 2011). Therefore, PCNA is a key indicator of cell proliferation status. The results of this study showed that the rate of apoptosis of neurons in brain tissue of RAM group was significantly increased, whereas the gene and protein expression of PCNA in the brain tissue and kidney tissue was significantly decreased in the RAM group. These data indicate that heavy ion radiation may inhibit proliferation by inhibiting the expression of PCNA. However, after treatment with HQD, the rates of apoptosis of neurons in the intervention groups decreased significantly, and the expression of PCNA in the brain tissue and kidney tissue increased significantly. Another anti-radiation mechanism of HQD may be the inhibition of cell apoptosis and the promotion of cell proliferation in organs and tissues.

**The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in maintaining and regulating stress responses in animals.** ACTH secreted by the HPA axis promotes the growth and development of the adrenal cortex and regulates CORT secretion (Holubová *et al.*, 2016). CRH, which is secreted by hypothalamic neurons, is mainly responsible for stimulating the release of ACTH. Under the intervention of stress factors such as radiation, and cold and heat, the level of CORT in blood decreases, possibly because these stress factors increase ACTH and CORT contents due to the enhanced activity of the pituitary-adrenal axis (Spencer & Deak, 2017). Similarly, when the body encounters stresses such as radiation, a pituitary-adrenal cortex system reaction can occur via the regulation of CRH secretion. The results



of this study revealed that the secretion of serum CORT and ACTH increased and the secretion of CRH decreased in the RAM group, which might be due to the fact that heavy ion radiation can improve the excitability of the HPA axis and further promotes the release of CRH and  $\beta$  endorphin ( $\beta$ -EP) by the pituitary to increase the secretion of ACTH (Holubová *et al.*, 2016). However, the increased secretion of ACTH and the damage to pituitary cells caused by radiation weakens the synthesis ability of the pituitary itself, and thus the expression of CRH in neuronal cells is reduced, the contents of CRH and  $\beta$ -EP in the pituitary are decreased, and the over-synthesis of CRH and  $\beta$ -EP is blocked (Warille *et al.*, 2017). After treatment with HQD, the secretion of serum CORT and ACTH was significantly reduced, and the secretion of CRH was significantly increased in the intervention groups. These results suggest that the anti-radiation mechanism of HQD may be achieved by regulating endocrine hormones in the HPA axis.

## CONCLUSION

In conclusion, the mechanisms underlying the protective effects of HQD against heavy ion radiation-induced damage may be as follows. 1) HQD may alleviate damage to the haematopoietic system caused by radiation by increasing the number of WBC, neutrophils, lymphocyte and monocyte. 2) HQD may inhibit radiation-induced oxidative stress injury by inhibiting the secretion of inflammatory factors. 3) HQD may inhibit apoptosis and increase PCNA expression, thereby reducing radiation-induced pathological damage to brain tissue and kidney tissue. 4) HQD may attenuate radiation-induced damage to the endocrine system by regulating the secretion of the endocrine hormones ACTH, CORT and CRH in the HPA axis. Our results may provide a basis for HQD as a radiation-protection agent.

## Ethics Approval and Consent to Participate

All animal experiment procedures were approved by the Animal Ethics Committee of Gansu University of Traditional Chinese Medicine (approval no. 2017-010).

AN, F.; YAN, C.; LI, J.; LV, X.; WANG, C.; DENG, J.; SHI, B.; ZHANG, J.; CHE, W. & ZHENG, W. Efectos protectores y mecanismos de la decocción de Huangqi contra los efectos secundarios inducidos por la radiación. *Int. J. Morphol.*, 41(5):1527-1536, 2023.

**RESUMEN:** La irradiación con haz de iones pesados  $^{12}\text{C}^{6+}$  puede provocar efectos secundarios. Las citoquinas inflamatorias, las hormonas endocrinas y las proteínas apoptóticas pueden estar

involucradas en los efectos secundarios inducidos por la irradiación  $^{12}\text{C}^{6+}$ . Este estudio caracterizó los efectos y mecanismos protectores de la decocción de Huangqi (HQD) contra los efectos externos inducidos por la radiación  $^{12}\text{C}^{6+}$ . Las ratas Wistar se dividieron aleatoriamente en grupos control, modelo de irradiación de iones pesados  $^{12}\text{C}^{6+}$  y grupos de dosis alta/media/baja de HQD. La tinción con HE evaluó los cambios patológicos del cerebro y el riñón. Se detectaron indicadores químicos de sangre periférica, así como factores inflamatorios y hormonas endocrinas. La apoptosis se midió con TUNEL. La expresión del antígeno nuclear de células en proliferación (PCNA) se determinó mediante PCR en tiempo real y transferencia Western blot. La irradiación indujo daños patológicos en los tejidos cerebrales y renales. Después de la irradiación, disminuyó el número de glóbulos blancos (WBC) y monocitos, y la expresión de interleucina (IL)-2, hormona liberadora de corticotropina (CRH) y PCNA. El daño estuvo acompañado por una mayor expresión de IL-1 $\beta$ , IL-6, corticosterona (CORT) y hormona adrenocorticotrópica (ACTH), así como un aumento de la apoptosis neuronal. Estas alteraciones fueron indicativas de efectos inducidos por la radiación. La administración de HQD atenuó el daño patológico a los tejidos cerebrales y renales, y aumentó el número de leucocitos y monocitos, así como la expresión de IL-2, CRH y PCNA. También disminuyó la expresión de IL-1 $\beta$ , IL-6, CORT y ACTH, así como la apoptosis neuronal. HQD exhibe mecanismos protectores contra los efectos externos inducidos por la radiación  $^{12}\text{C}^{6+}$ . El mecanismo subyacente puede implicar la promoción de la producción de células sanguíneas periféricas, la inhibición de factores inflamatorios y la apoptosis y la regulación de hormonas endocrinas.

**PALABRAS CLAVE:** Decocción de Huangqi; Irradiación  $^{12}\text{C}^{6+}$ ; Factores inflamatorios; Hormona endocrina; Apoptosis.

## REFERENCES

- Blakely, E. A. & Kronenberg, A. Heavy-ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness. *Radiat. Res.*, 150(5 Suppl.):S126-45, 1998.
- Burdak-Rothkamm, S.; Rothkamm, K.; McClelland, K.; Al Rashid, S. T. & Prise, K. M. BRCA1, FANCD2 and Chk1 are potential molecular targets for the modulation of a radiation-induced DNA damage response in bystander cells. *Cancer Lett.*, 356(2 Pt. B):454-61, 2015.
- de Medina-Redondo, M. & Meraldi, P. The spindle assembly checkpoint: clock or domino? *Results Probl. Cell Differ.*, 53:75-91, 2011.
- Feiock, C.; Yagi, M.; Maidman, A.; Rendahl, A.; Hui, S. & Seelig, D. Central nervous system injury - A newly observed bystander effect of radiation. *PLoS One*, 11(9):e0163233, 2016.
- Fernandez-Palomo, C.; Schultke, E.; Smith, R.; Brauer-Krisch, E.; Laissue, J.; Schroll, C.; Fazzari, J.; Seymour, C. & Mothersill, C. Bystander effects in tumor-free and tumor-bearing rat brains following irradiation by synchrotron X-rays. *Int. J. Radiat. Biol.*, 89(6):445-53, 2013.
- Gao, X. L.; Zhang, Q. & Huo, H. X. Anti-inflammatory effect of Chinese agarwood essential oil via inhibiting p-STAT3 and IL-1 $\beta$ /IL-6. *Chin. Pharm. J.*, 54(23):1951-7, 2019.
- Holubová, A.; Stofková, A.; Jurcovicová, J. & Slamberová, R. The effect of neonatal maternal stress on plasma levels of adrenocorticotrophic hormone, corticosterone, leptin, and ghrelin in adult male rats exposed to acute heterotypic stressor. *Physiol. Res.*, 65(Suppl. 5):S557-66, 2016.

- Hu, M.; Xu, H. & Chang, Z. Protective effect of black garlic polysaccharide on mice damaged by x-ray radiation. *J. Beihua University (Nat. Sci.)*, 22(2):202-6, 2021.
- Kannouche, P. L.; Wing, J. & Lehmann, A. R. Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. *Mol. Cell*, 14(4):491-500, 2004.
- Kim, J. S.; Lee, H. J.; Kim, J. C.; Kang, S. S.; Bae, C. S.; Shin, T.; Jin, J. K.; Kim, S. H.; Wang, H. & Moon, C. Transient impairment of hippocampus-dependent learning and memory in relatively low-dose of acute radiation syndrome is associated with inhibition of hippocampal neurogenesis. *J. Radiat. Res.*, 49(5):517-26, 2008.
- Koturbash, I.; Boyko, A.; Rodriguez-Juarez, R.; McDonald, R. J.; Tryndyak, V. P.; Kovalchuk, I.; Pogribny, I. P. & Kovalchuk, O. Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. *Carcinogenesis*, 28(8):1831-8, 2007.
- Koturbash, I.; Rugo, R. E.; Hendricks, C. A.; Loree, J.; Thibault, B.; Kutanzi, K.; Pogribny, I.; Yanch, J. C.; Engelward, B. P. & Kovalchuk, O. Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo. *Oncogene*, 25(31):4267-75, 2006.
- Li, Q.; Xu, X.; Zhong, W.; Du, Q.; Yu, B. & Xiong, H. IL-17 induces radiation resistance of B lymphoma cells by suppressing p53 expression and thereby inhibiting irradiation-triggered apoptosis. *Cell. Mol. Immunol.*, 12(3):366-72, 2015.
- Li, W.; Pang, H.; Zhou, J.; Wu, H. & Jiang, R. Effects of lyceum barbarum polysaccharide (LBP) on peripheral blood and bone marrow mononuclear cells of X ray irradiated mice. *Radiat. Prot.*, 36(4):218-23, 2016.
- Liang, J.; Li, J.; Li, J.; Zeng, Q.; Du, X.; Hu, R.; Liu, F.; An, Y.; Wang, J.; Sun, X.; *et al.* Effects of lung and kidney bystander effect of Guiqi Yiyuan Ointment on rat with radiation damage by 12C6+ beam based on transcriptome. *China J. Tradit. Chin. Med. Pharm.*, 34(5):2130-4, 2019.
- Mancuso, M.; Pasquali, E.; Leonardi, S.; Tanori, M.; Rebessi, S.; Di Majo, V.; Pazzaglia, S.; Toni, M. P.; Pimpinella, M.; Covelli, V.; *et al.* Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum. *Proc. Natl. Acad. Sci. U. S. A.*, 105(34):12445-50, 2008.
- Qiao, S.; Peng, R.; Yan, H.; Gao, Y.; Wang, C.; Wang, S.; Zou, Y.; Xu, X.; Zhao, L.; Dong, J.; *et al.* Reduction of phosphorylated synapsin I (ser-553) leads to spatial memory impairment by attenuating GABA release after microwave exposure in Wistar rats. *PLoS One*, 9(4):e95503, 2014.
- Riehl, T. E.; Foster, L. & Stenson, W. F. Hyaluronic acid is radioprotective in the intestine through a TLR4 and COX-2-mediated mechanism. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 302(3):G309-16, 2012.
- Siva, S.; Lobachevsky, P.; MacManus, M. P.; Kron, T.; Möller, A.; Lobb, R. J.; Ventura, J.; Best, N.; Smith, J.; Ball, D.; *et al.* Radiotherapy for non-small cell lung cancer induces DNA damage response in both irradiated and out-of-field normal Tissues. *Clin. Cancer Res.*, 22(19):4817-26, 2016.
- Smith, R. W.; Wang, J.; Schultke, E.; Seymour, C. B.; Brauer-Krisch, E.; Laissue, J. A.; Blattmann, H. & Mothersill, C. E. Proteomic changes in the rat brain induced by homogenous irradiation and by the bystander effect resulting from high energy synchrotron X-ray microbeams. *Int. J. Radiat. Biol.*, 89(2):118-27, 2013.
- Spencer, R. L. & Deak, T. A users guide to HPA axis research. *Physiol. Behav.*, 178:43-65, 2017.
- Tong, F.; Zhang, J.; Liu, L.; Gao, X.; Cai, Q.; Wei, C.; Dong, J.; Hu, Y.; Wu, G. & Dong, X. Corilagin attenuates radiation-induced brain injury in mice. *Mol. Neurobiol.*, 53(10):6982-96, 2016.
- Wang, J. F.; Gao, Y.; Wang, F.; Wang, G. Y.; Zhao, Q. L. & Yang, C. Y. Protective effect of tectorigenin on hematopoietic system injury induced by radiation in mice. *Chin. J. Lab. Diagn.*, 24(12):2024-8, 2020.
- Warille, A. A.; Altun, G.; Elamin, A. A.; Kaplan, A. A.; Mohamed, H.; Yurt, K. K. & El Elhaj, A. Skeptical approaches concerning the effect of exposure to electromagnetic fields on brain hormones and enzyme activities. *J. Microsc. Ultrastruct.*, 5(4):177-84, 2017.
- Yu, J.; Zhang, Y. & Diao, B. Effects of JAK-STAT signaling pathway, IL-1b and IL-6 on injury of PC12 cells with X-ray irradiation. *Chin. J. Pathophysiol.*, 33(1):174-8, 2017.
- Zhao, L. Z.; Yang, B.; Sun, X. M.; Zhang, T. L.; Yu, D. H.; Li, W. L. & Yan, X. J. Chemical constituents of Huangqi decoction and their mechanisms in the treatment of non-alcoholic fatty liver disease. *Acta Pharm. Sin.*, 56(11):3082-90, 2021.
- Zhi, W. J.; Wang, L. F. & Hu, X. J. Recent advances in the effects of microwave radiation on brains. *Mil. Med. Res.*, 4:29, 2017.

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