Acupuncture and Herbal Medicine

Isofraxidin alleviated radiation-induced testicular damage *via* the Nrf2/HO-1-NLRP3/ASC axis

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Abstract

Objective: Radiotherapy is used to treat patients with tumors; however, radiation (IR)-induced testicular injury, which has no effective treatment approved in clinical practice, significantly influences their prognosis and quality of life. The protective effects and underlying mechanisms of action of isofraxidin (IF) against IR-induced testicular injury were investigated.

Methods: A mouse testis injury model was established using 5 Gy irradiation. Hematoxylin and eosin (H&E) staining, immunofluorescence staining, and enzyme-linked immunosorbent assay were used to measure DNA damage, apoptosis, inflammatory reactions, and oxidative stress in the testes of mice after irradiation. The effectiveness of IF irradiation on testicular injury was evaluated, and the mechanisms of the related oxidative stress and inflammatory response pathways were discussed.

Results: IF can improve IR-induced testicular injury by inhibiting the increased levels of DNA damage, apoptosis rate, oxidative stress, and inflammatory factors. The radioprotective effects of IF on testicular injury are mediated by the stimulation of nuclear factor E2-related factor 2 (Nrf2)/heme oxidase-1 (HO-1) or suppression of NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome signaling pathways. In addition, crosstalk between the Nrf2/HO-1 and NLRP3 inflammasome signaling pathways was elucidated, in which the inhibition of the NLRP3 inflammasome was mediated by the activation of Nrf2 signaling with IF upon IR exposure.

Conclusion: IF can be a potent radioprotective agent to mitigate testicular damage, and may provide a new therapeutic option to alleviate the side effects of radiotherapy in male patients with tumors.

Keywords: Isofraxidin, Radiation, Testicular

Graphical abstract: http://links.lww.com/AHM/A142.

Introduction

Radiotherapy is widely used for the treatment of male tumor patients, including those with testicular cancer, prostate cancer, and Hodgkin lymphoma[1,2]. Although radiotherapy can improve the therapeutic effect on tumors, it can also increase the incidence of male infertility and sexual dysfunction. The testis is a radiosensitive tissue that can cause transient azoospermia at radiation doses less than 0.35 Gy[3,4]. Such effects must be recognized when deciding on the treatment plan. Irradiation with a dose of more than 2 Gy can cause temporary infertility for 10 to 24 months, and the recovery time is positively correlated with the irradiation dose. Lifelong sterilization can result from irradiation with >6 Gy[5]. Radioactive testicular injury (RITI) is characterized by atrophy of the spermatogonial tubules and a reduction in sperm quality. RITI can cause either temporary or permanent sterility. Radioprotective agents mitigate radiation damage prior to exposure[6]. Food and Drug Administration-approved radioprotective agents such as amifostine and fexofenadine have been used clinically to mitigate organ toxicity induced by radiotherapy[7–9]. However, no radioprotective drugs can effectively target radiation-induced testicular injury. Radiotherapy-induced reproductive injury can significantly affect the quality of life of patients[10]. New radioprotective agents are needed that can benefit normal tissues and cells and improve the outcomes of cancer treatment in clinical practice.

Isofraxidin (IF), also known as 7-hydroxy-6,8-dimethoxychromen-2-one, is a hydroxycoumarin-like monomer that is commonly found in the roots, stems, leaves, and fruits of Acanthopanax spicata. It has pharmacological activities such as anti-inflammatory, antioxidant, and antitumor properties. Research indicates that

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IF attenuates the nuclear factor-κ-gene binding (NF-κB) and NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammatory vesicle-mediated inflammatory response, and has strong antioxidant activity[11–16]. IF has positive effects on several types of cancers, including such as lung, breast, colon, leukemia, and hepatocellular carcinoma, and may inhibit apoptosis, metastasis, and invasive signaling pathways[17–19]. Reactive oxygen species (ROS) and free radicals produced through water electrolysis are the primary mediators of radiation-induced testicular damage[20]. These agents induce cell damage, apoptosis, necrosis, and inflammatory responses by damaging DNA bases, oxidizing proteins, and causing the peroxidation of cell membrane lipids. This disrupts the metabolism, proliferation, and differentiation of germ cells, ultimately resulting in oligospermia and azoospermia. Possibly, IF can mitigate radiation-induced testicular damage owing to its anti-inflammatory and antioxidant properties.

This study investigated the protective effects of IF against radiation-induced testicular injury and its mechanism of action in relation to inflammation and oxidative stress. This involves IF attenuation of radiation-induced testicular injury through the nuclear factor E2-related factor 2 (NRF2)/heme oxidase-1 (HO-1)-NLRP3/ASC signaling axis. This may provide a new intervention for preventing radiation-induced testicular injury in male patients undergoing RT.

Materials and methods

Chemicals and reagents

IF (HY-N0774), nigericin (HY-100381), and Nrf2-IN-1 (HY-101025) were purchased from MedChemExpress (NJ, USA), dissolved in saline, and intraperitoneally injected into the mice (10, 20, 40 mg/kg)[11–13].

Mice and irradiation

Eight-week-old male BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal experiments were approved by the Animal Care and Use Committee of the Animal Center in Academy (IACUC-DWZX-2021-557). The mice were divided into groups randomly (n = 6 per group) and maintained in a 12-hour light/dark cycle at 22°C to 25°C with free access to autoclaved food and water. They were dosed before and after irradiation according to a predetermined schedule, and subjected to radiation with a cobalt radioactive source (5 Gy) at the Beijing Institute of Radiation Medicine.

Enzyme-linked immunosorbent assay

The concentrations of testosterone, 8-hydroxy-2'-deoxyguanosine (8-OHDG), malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), ROS, NOD-like receptor family pyrin domain-containing 3 (NLRP3), and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) in serum were evaluated. The presence of ASC, interleukin-1 beta (IL-1β), interleukin-18 (IL-18), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in the serum was determined using the enzyme-linked immunosorbent assay (ELISA) technique. Following irradiation, the mice were humanely killed with carbon dioxide within 1 or 2 days, and their serum was extracted by centrifugal force. Subsequently, the serum extracts were subjected to a serial dilution process prior to being placed in plates for incubation at 37°C for 1 h, following the introduction of horseradish peroxidase (HRP)-conjugated antibodies. Following washing, color development, and reaction cessation, the samples' optical density was recorded.

Histopathology and immunofluorescence

After euthanizing the mice, the spleen specimens were excised and preserved in a 4% paraformaldehyde solution for 24 h. Subsequently, the specimens were subjected to a dehydration process involving successive immersions in ethanol of varying concentrations. Afterward, they were embedded within a paraffin matrix and subsequently sectioned into 3-μm slices. A routine histopathological assessment was conducted utilizing hematoxylin and eosin (H&E) staining. Furthermore, the apoptotic index within the spleen tissue was quantified through the application of the Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) technique, by the protocol provided with the TUNEL kit (product number 12156792910) by Roche (Basel, Switzerland). In the context of the immunofluorescence experiment, the tissue sections were initially treated with normal goat serum to block non-specific binding, followed by incubation with antibodies targeting Nrf2 (ab62352), γ-H2AX (ab81299), HO-1. The primary antibodies used were anti-NLRP3 (ab270449), anti-ASC (ab283684), and anti-caspase-1 (ab189491), all diluted 1:100, obtained from Signalway Antibody LLC. The proteins were visualized through incubation with an appropriate secondary antibody (Proteintech, diluted 1:1,000).

Statistical analysis

The quantification of immunofluorescence imagery was achieved through Image J. Graph Pad Prism 5 (provided by GraphPad Software Inc.) was employed for statistical evaluation, necessitating the repetition of the experiment on three independent experiments. To ascertain significant disparities among the distinct groups, a t test was conducted, with a P value <0.05 deemed statistically noteworthy.

Results

Ixofraxidin (IF) reduces radiation-induced testicular damage. To determine the radioprotective effect of IF on the testes, we administered 10/20/40 mg/kg/mouse *via* intraperitoneal injection 12 h before, 2 h after, and 12 h after 5 Gy irradiation. Testes were then removed 24 h after irradiation and subjected to H&E staining. Immunofluorescence staining for γ-H2AX and TUNENL was performed. The results indicated that in the control and IF groups, the seminiferous tubules in the testicular tissue exhibited regular shapes. Spermatogenic cells at all levels (spermatogonia, primary spermatocytes, secondary spermatocytes, and round spermatocytes) were arranged in a hierarchically ordered manner, spermatogenesis was abundant in the lumen, and the rate of apoptosis and DNA

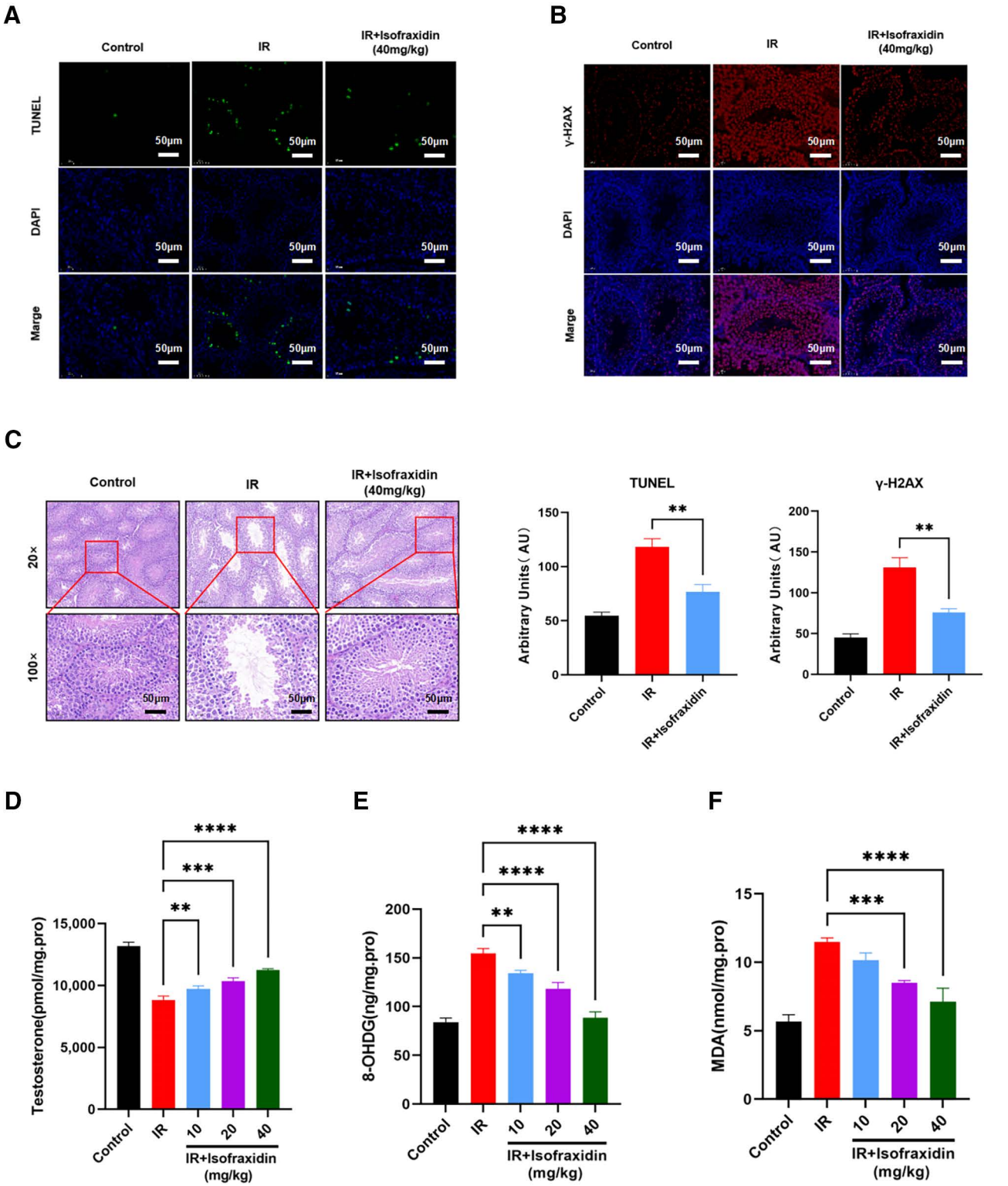

Figure 1. Isofraxidin attenuates radiation-induced testicular damage by immunofluorescence staining to monitor DNA damage. (A) and the extent of apoptosis in the testes (B) H&E staining to observe the testicular structure of the groups (C), and enzyme immunoassay to determine testosterone (D), 8-OHDG (E), and MDA (F) levels in the testes (F). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ represent significance as compared to the exposed group. 8-OHDG: 8-Hydroxy-2'-deoxyguanosine; DAPI: 4',6-Diamidino-2-phenylindole; H&E: Hematoxylin and eosin; IR: Radiation; MDA: Malondialdehyde; TUNEL: Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling.

damage was significantly reduced compared to that in the irradiated group (Figure 1A–C). Testosterone, 8-OHDG, and MDA levels were measured using enzyme immunoassays. The results showed that, in comparison to the IR group, the IF group demonstrated a notable elevation in testosterone content within the testis, which exhibited a dose-dependent response. Additionally, the IF group exhibited a substantial reduction in 8-OHDG and MDA content (Figure 1D–F), indicating that IF exerts a significant protective effect against radiation-induced testicular damage. This indicated that IF has a significant protective effect against radiation-induced testicular damage.

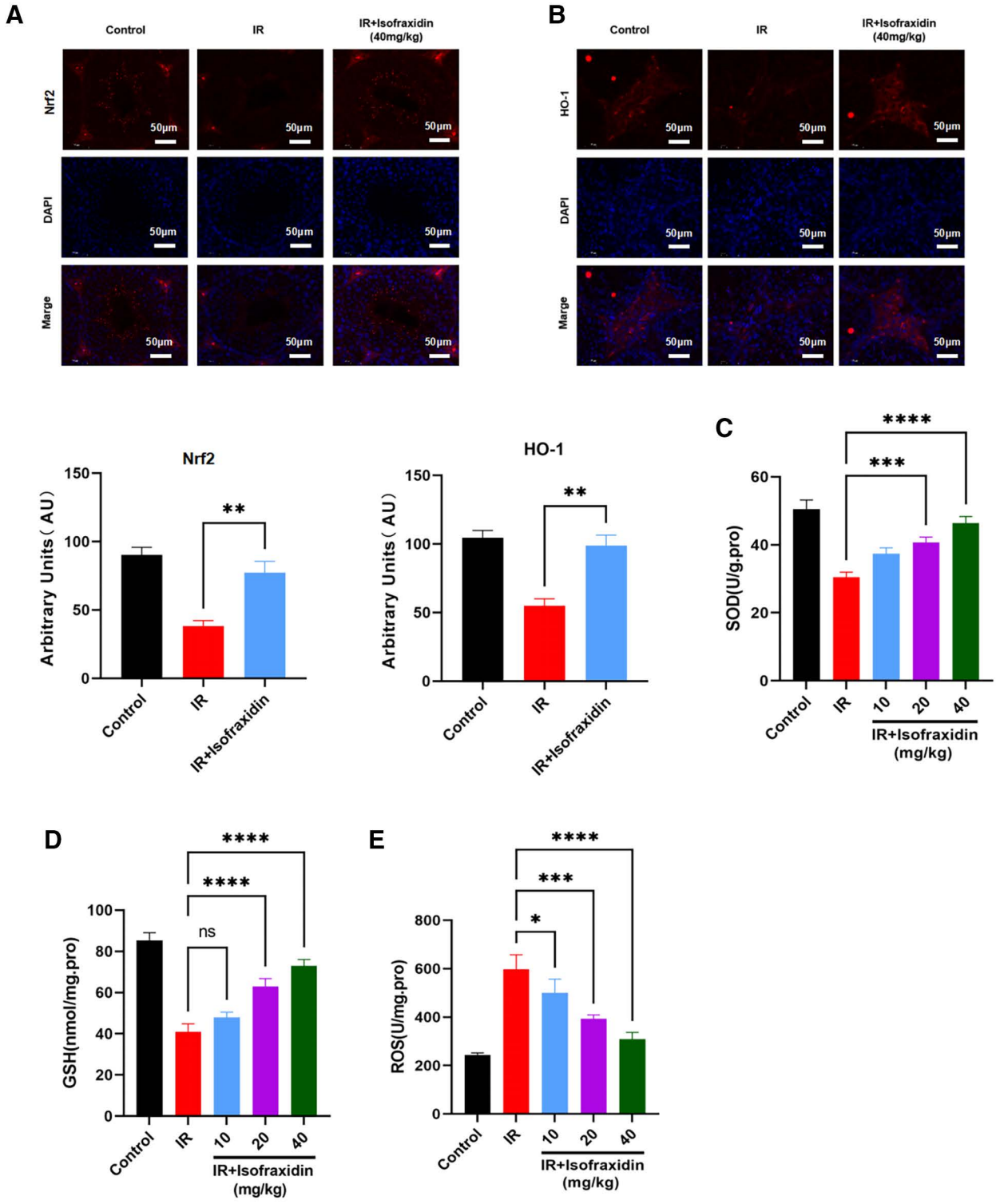

Figure 2. Isofraxidin attenuates radiation-induced oxidative stress damage in the testis, with immunofluorescence staining for Nrf2 (A) and HO-1 expression (B), and enzyme-linked immunoassay for SOD (C), GSH (D), and ROS levels (E) in the testis.*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 represent significance as compared to the exposed group. DAPI: 4',6-Diamidino-2-phenylindole; GSH: Reduced glutathione; HO-1: Heme oxidase-1; IR: Radiation; Nrf2: Nuclear factor E2-related factor 2; ROS: Reactive oxygen species; SOD: Superoxide dismutase.

IF attenuates oxidative stress damage in the testis induced by radiation

Oxidative stress is an essential regulatory mechanism of radiation injury. We evaluated the protective effect of IF on radiation-induced oxidative stress injury in the testes by enzyme immunoassay to determine the content of SOD, GSH, and ROS in the testes. We then explored the specific mechanism mediating the protective effect of IF against radiation-induced oxidative stress by immunofluorescence staining and found that IF attenuated radiation-induced oxidative stress injury in the testis through the Nrf2/HO-1 signaling pathway (Figure 2A, B). The results showed that, in comparison to the IR group, the IF group exhibited the capacity to elevate SOD levels in a dose-dependent manner, while concurrently reducing GSH and ROS levels. This resulted in the modulation of

radiation-induced oxidative stress disorders in the testicular tissue (Figure 2C–E).

The protective effect of IF against radiation damage is mediated by the Nrf2/HO-1 signaling pathway

To confirm that Nrf2/HO-1 is the primary signaling pathway responsible for the protective effects of IF against radiation damage, we administered Nrf2-IN-1, a specific inhibitor of Nrf2, to IF. The results showed that IF significantly reduced radiation-induced intratesticular structural damage, DNA damage, and apoptosis in the testes, maintained testosterone levels, and decreased MDA and 8-OHDG levels. However, these protective effects were inhibited when IF was treated with Nrf2-IN-1 (Figure 3A–F). This suggests that the Nrf2/HO-1 pathway is a critical signaling pathway that mediates the protective effects of IF against radiation.

Inhibition of radiation-induced activation of the NLRP3 inflammasome in the testis by IF

IF inhibits the activation of NLRP3 inflammatory vesicles, thereby reducing the inflammatory response induced by myocardial infarction. We investigated the mechanism underlying the anti-inflammatory action of IF using immunofluorescence staining and enzyme immunoassays. The results showed that IF significantly ameliorated the disturbance of inflammatory factors in the testes after irradiation in a dose-dependent manner (Figure 4F–H). In addition, it significantly reduced the expression of NLRP3, ASC, and IL-1β (Figure 4A–E). After treatment with Nigericin, an NLRP3-specific agonist, and IF, the regulation of inflammatory factor disorders was weakened (Figure 5A–D). The protective effect of IF against radiation-induced testicular injury was weakened (Figure 6A–F). These results suggest that IF inhibits the activation of inflammatory responses in the testes after irradiation *via* the NLRP3 inflammatory pathway and has a protective role against irradiation.

IF protects against radiation-induced testicular injury through the Nrf2/HO-1-NLRP3/ASC signaling pathway

Finally, we investigated the crosstalk between the Nrf2/HO-1 and NLRP3 inflammatory signaling pathways in mediating the radioprotective effects of IF. Our results showed that the inhibitory effect of IF on the activation of the NLRP3 inflammatory vesicle signaling pathway was attenuated by Nrf2-IN-1 treatment (Figures 7 and 8), suggesting that in the mechanism mediating the radioprotective effects of IF, the Nrf2/HO-1 pathway is upstream of the NLRP3 inflammatory vesicle signaling pathway, suggesting that IF ameliorates radiation-induced testicular damage *via* the Nrf2/HO-1-NLRP3/ASC signaling axis pathway.

Discussion

Radiotherapy is commonly used to treat cancer, particularly testicular cancer[21]. One positive effect is the inhibition of tumor growth through local radiation effects. However, in most cancer treatments, radiotherapy can negatively affect the tumor area and other healthy organs and tissues. Exposure of the testis to radiation can result in damage due to a lack of spermatogonia, decreased testosterone levels, inflammation, and oxidative stress. These factors can ultimately lead to oligozoospermia or infertility, reducing the quality of life of patients[22]. This study aimed to examine the protective effects of IF against radiation-induced testicular injury and to propose new preventative measures for clinical radiotherapy.

Radiation-induced DNA double-strand breaks in testis spermatogonia has been found to activate DNA repair mechanisms or trigger cell death by recognizing exposed DNA-damaged fragments through cell cycle arrest[23,24]. The loss of germ cells induced by ionizing radiation occurs primarily through apoptosis, with proliferating spermatogonia being the most vulnerable[25]. This study showed that IF effectively reduced radiation-induced oxidative DNA damage and apoptosis in the testis and decreased spermatogenic cell loss (Figure 1A, B). Testosterone is crucial for regulating the structural and functional integrity of male reproductive organs, particularly during spermatogenesis. Impaired testosterone synthesis can lead to a decrease in testicular weight and spermatogenesis disorders, leading to infertility[26,27]. IF significantly mitigated the radiation-induced reduction in testosterone levels in a dose-dependent manner and had a significant protective effect against radiation-induced testicular damage (Figure 1).

Although the mechanism of action of ionizing radiation on tissues is not yet fully understood, several studies have investigated its effects on oxidative stress[28,29]. An increase in ROS levels induced by radiation can lead to testicular tissue damage. Oxidative stress induces lipid peroxidation[30,31]. SOD and MDA are essential markers for evaluating lipid peroxidation and radiation damage[32–35]. GSH is an important marker of oxidative stress. The reduced content of this substance may be linked to reactive oxygen radicals produced by the intracellular electrolysis of water following radiation[36,37]. In addition, 8-OHDG is commonly used as a biomarker for oxidative DNA damage[38]. Our study demonstrated that IF administration reduced the levels of MDA, 8-OHDG (Figure 1E), GSH, and ROS (Figure 2D, E) in a dose-dependent manner, elevated the levels of SOD (Figure 2C), scavenged oxygen-free radicals in post-irradiation testes, and significantly ameliorated oxidative stress damage in post-irradiation testes.

The regulation of radiation-induced oxidative stress damage and cellular dysfunction is carried out by Nrf2[39]. The Nrf2/HO-1 pathway is considered a primary defense mechanism against oxidative stress. It regulates the expression of a variety of antioxidant genes such as HO-1, quinone oxidoreductase 1 (NQO1), CAT, and SOD[40,41]. These antioxidant enzymes protect the cells from radiation-induced oxidative stress. Under normal physiological conditions, Bach1 occupies the regulatory site of HO-1 initiation and inhibits HO-1 expression. However, Bach1 dissociates from the regulatory site of HO-1 initiation under oxidative stress. Nrf2 is then transferred from the cytoplasm to the nucleus where it binds to the antioxidant response element (ARE) region, initiating HO-1 expression. This process promotes the

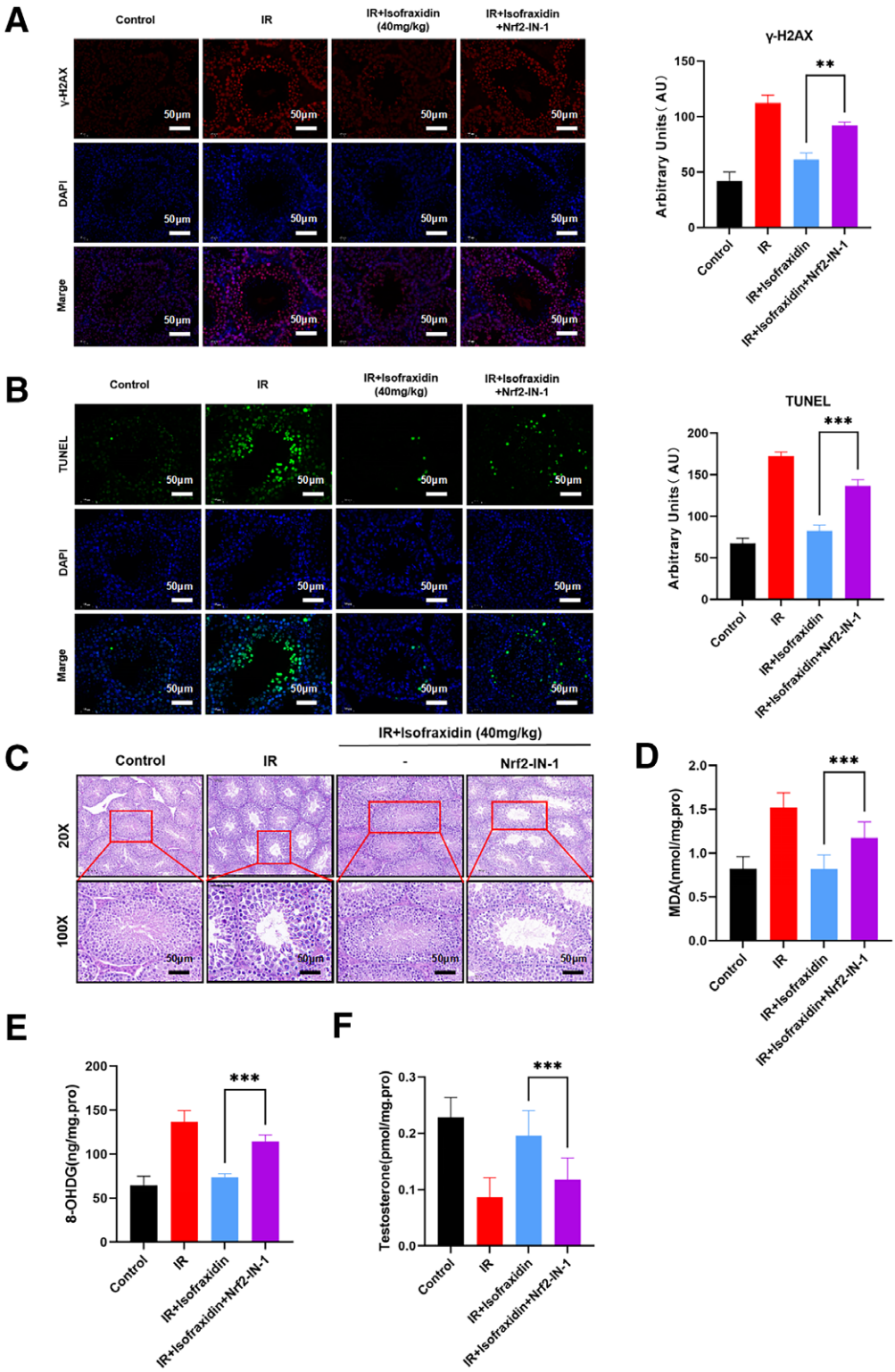

Figure 3. Isofraxidin alleviates oxidative stress damage in testis caused by radiation through Nrf2/HO-1 signaling pathway, DNA damage (A) and apoptosis degree (B) in testis are detected by immunofluorescence staining, testis structure is observed by H&E staining (C), and MDA (D), 8-OHDG (E), and testosterone (F) content in testis are determined by enzyme-linked immunosorbent assay. $^{**}P < 0.01$ and $^{***}P < 0.001$ represent significance as compared to the exposed group. DAPI: 4',6-Diamidino-2-phenylindole; H&E: Hematoxylin and eosin; HO-1: Heme oxidase-1; IR: Radiation; MDA: Malondialdehyde; Nrf2: Nuclear factor E2-related factor 2.

scavenging of ROS, maintains redox balance, inhibits inflammation, and repairs damaged DNA[42]. We investigated whether this pathway is involved in the protective effect of IF against radiation-induced testicular injury. Treatment with IF significantly increased NRF2 and HO-1 expression. Our findings suggest that IF protects against radiation-induced testicular injury by upregulating NRF2 and HO-1 expression (Figure 2). Furthermore, we utilized Nrf2-IN-1, a targeted inhibitor of NRF2, to investigate whether the activation of the NRF2/HO-1 pathway is necessary for mediating the radioprotective effect of IF. The results showed that inhibition of Nrf2

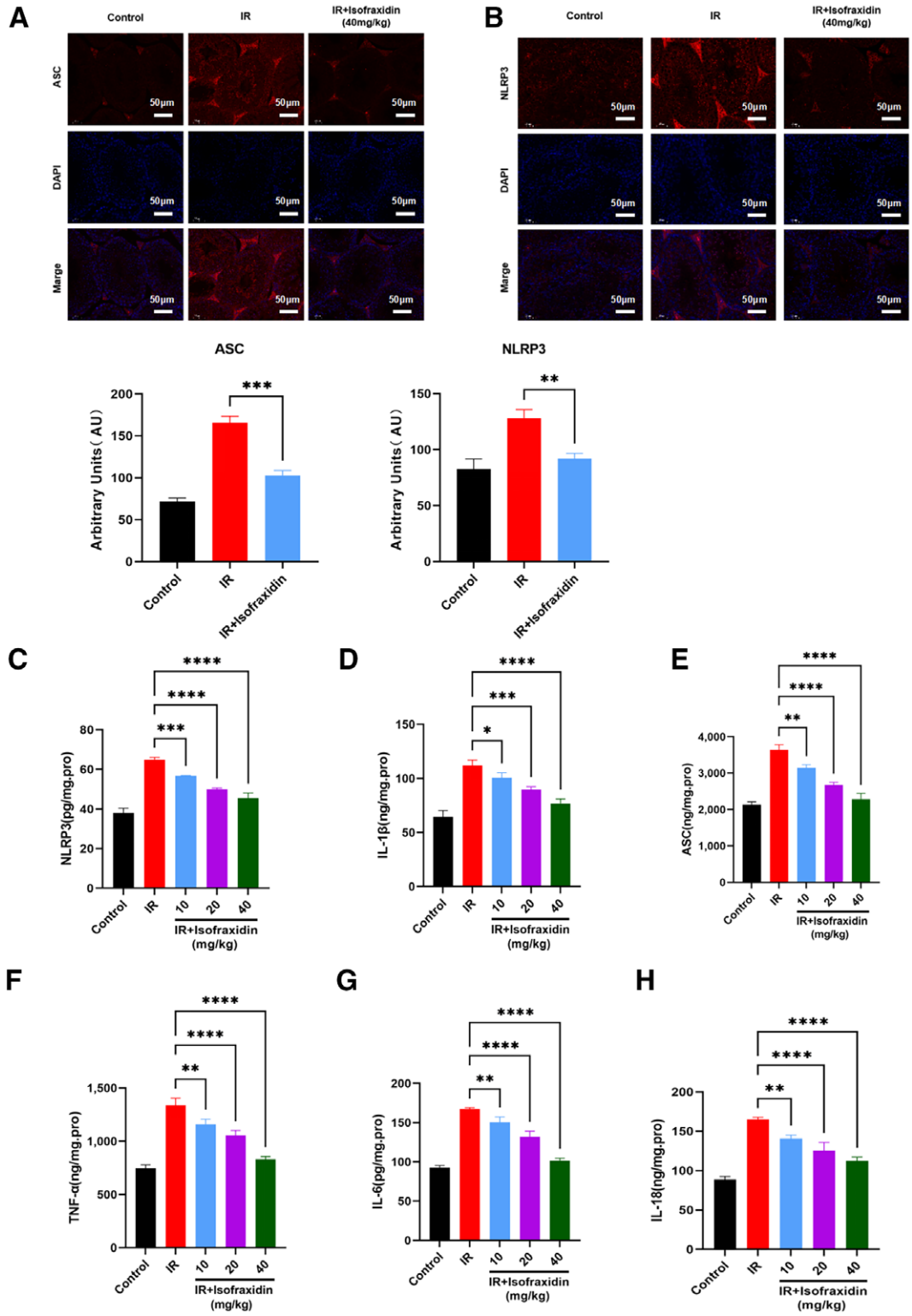

Figure 4. Isofraxidin exerts a radiolucent anti-inflammatory effect by reducing NLRP3 inflammatory vesicle activation. The expression of ASC (A) and NLRP3 (B) was determined by immunofluorescence staining, and the levels of NLRP3 (C), IL-1β (D), ASC (E), TNF-α (F), IL-6 (G), and IL-18 (H) were measured by enzyme-linked immunosorbent assay in the testis. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 represent significance as compared to the exposed group. ASC: Apoptosis-associated speck-like protein containing a caspase recruitment domain; DAPI: 4',6-Diamidino-2-phenylindole; IL-1β: Interleukin-1 beta; IR: Radiation; TNF-α: Tumor necrosis factor-alpha; NLRP3: NOD-like receptor family pyrin domain-containing 3.

with Nrf2-IN-1 significantly decreased the protective effects of IF against radiation-induced testicular injury, oxidative stress, apoptosis, and DNA damage (Figure 3). This suggests that activation of the NRF2-mediated HO-1 pathway is crucial for the radioprotective effects of IF.

NLRP3 inflammation has recently received significant attention because of its crucial role in regulating radiation-induced injury[43–46]. With external stimulation, NLRP3 oligomerizes through interactions between its nucleotide-binding and oligomerization (NACHT)

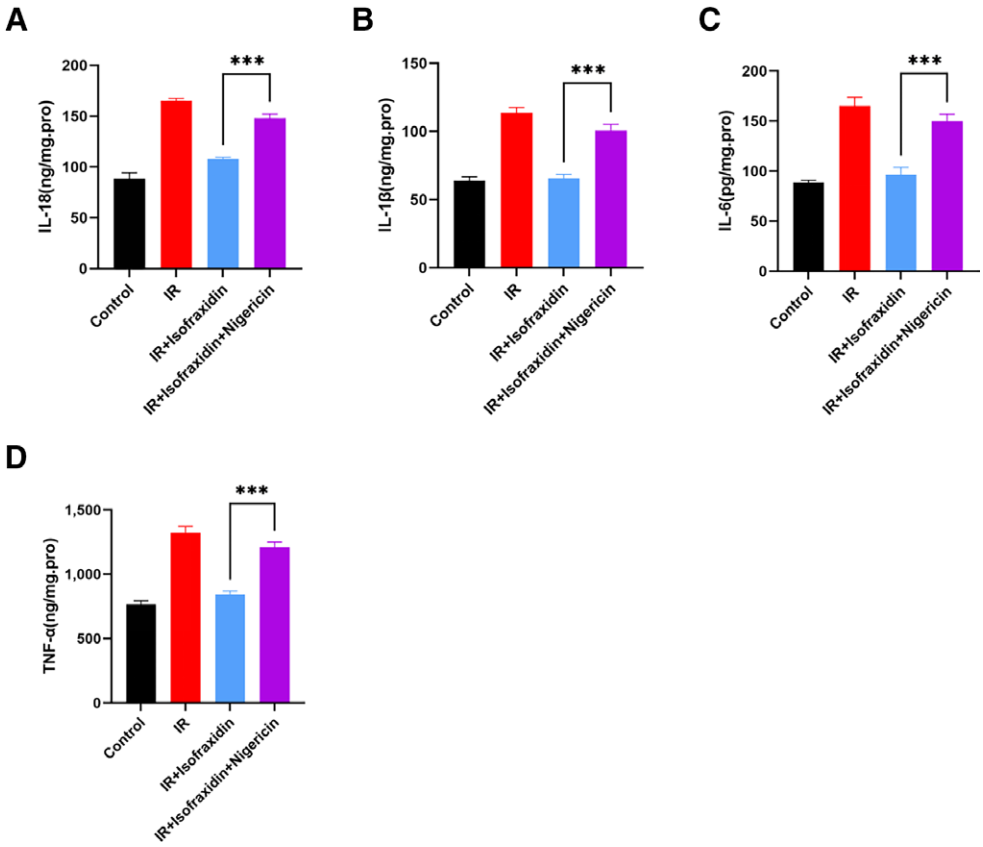

Figure 5. The role of NLRP3 inflammasome signaling in mediating the radioprotective effects of isofraxidin is significant, enzyme immunoassay to determine the magnitude of IL-18 (A), IL-1β (B), IL-6 (C), and TNF-α (D) levels in the testis. ***$P < 0.001$ represents significance as compared to the isofraxidin group. IL-1β: Interleukin-1 beta; IR: Radiation; NLRP3: NOD-like receptor family pyrin domain-containing 3; TNF-α: Tumor necrosis factor-alpha.

structural domains. This leads to homotypic interactions between the N-terminal pyridine structural domain (PYD)-PYD and the formation of nucleated helical ASC filaments that recruit ASC articulators[47]. Subsequently, cystatinase-1 is recruited to the assembled ASC *via* cystatinase activation and recruitment structural domain (CARD)–CARD interactions. This leads to neighborhood-induced self-cleavage and activation[48]. Activated cystatins will cleave the cytokines pro-IL-1β and pro-IL-18, producing the potent pro-inflammatory mediators IL-1β and IL-18. IF can reduce inflammatory injury caused by myocardial infarction through the NLRP3 inflammasome[49]. However, the mechanisms underlying the anti-inflammatory effects of IF radiation are unclear. Therefore, we hypothesized that the NLRP3 inflammasome signaling pathway is involved in the radioprotective effects of IF. The results indicated that the activation of the NLRP3 inflammasome was significantly inhibited by IF treatment (Figure 4), and the regulation of testicular injury was significantly reduced by treatment with the NLRP3-specific agonist nigericin, which aligns with the expected outcomes (Figure 5). Treatment with the NLRP3-specific agonist nigericin significantly reduced testicular injury, apoptosis, DNA damage, and oxidative stress (Figure 6). This suggests that the inhibition of the NLRP3 inflammasome pathway is a crucial mechanism that mediates the anti-inflammatory effects of IF radiation damage.

Nrf2 is a classical transcription factor that activates the expression of antioxidant proteins. It also possesses antioxidant and anti-inflammatory protective properties[50]. Inflammation and oxidative stress are closely associated with the pathophysiology of radiation-induced injuries. Nrf2-mediated inhibition of NLRP3 occurs mainly through a reduction in ROS-induced activation of NLRP3. Nrf2 reduces the expression of NLRP3 by cleaving caspase-1, thereby reducing IL-1β levels. In addition, Nrf2 induces the expression of NQO1, which inhibits NLRP3 inflammatory vesicles. NQO1 is a flavoprotein that mediates the reduction of quinones to hydrogenated anthraquinones, thereby protecting the plasma membrane from lipid peroxidation and free radical attacks. It works with HO-1 to form the NOQ1/HO-1 pathway, reducing cellular ROS levels in an Nrf2/ARE-dependent manner[51–52]. Chen et al found that in carbon tetrachloride-induced acute liver injury, antioxidants reduced ROS levels and inhibited the activation of NLRP3 inflammatory vesicles. ROS levels were closely related to the regulation of the Nrf2/HO-1 pathway, and the up-regulation of HO-1 exerted a protective effect on the elevated ROS levels[53–55]. Based on the above studies, we investigated whether Nrf2/HO-1-regulated oxidative stress and the NLRP3 inflammatory vesicle pathway interact with radiation injury. The results showed that treatment with Nrf2-IN-1 attenuated the inhibitory effect of IF on the NLRP3 inflammatory vesicle pathway (Figures 7 and 8). This suggests that IF ameliorates radiation-induced testicular injury *via* the Nrf2/HO-1-NLRP3/ASC signaling axis.

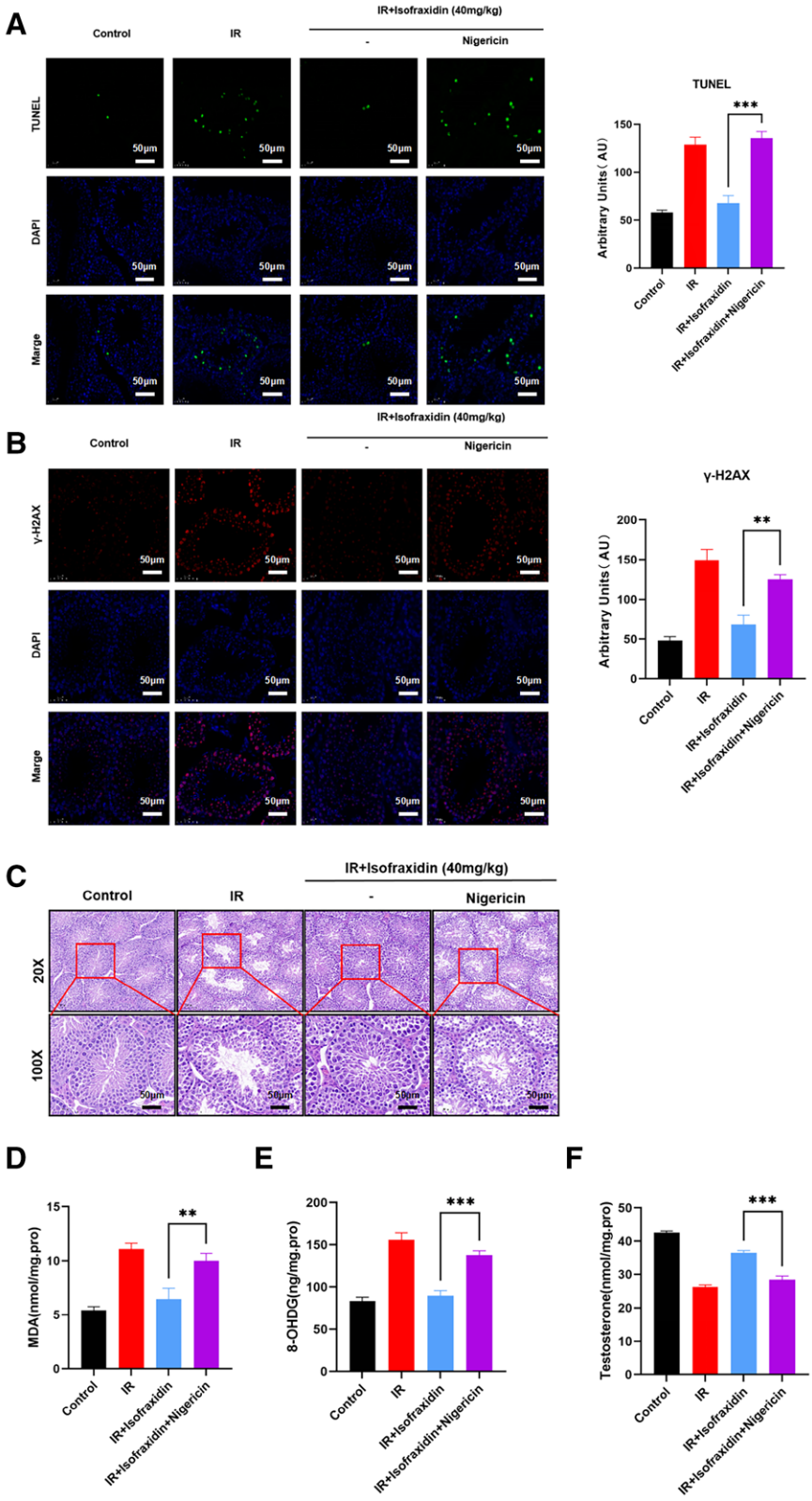

Figure 6. The role of NLRP3 inflammasome signaling in mediating the radioprotective effects of isofraxidin is significant. DNA damage (A) and apoptosis degree (B) in testis are detected by immunofluorescence staining, testis structure is observed by H&E staining (C), MDA (D), 8-OHDG (E), and testosterone (F) content in testis are determined by enzyme-linked immunosorbent assay $^{**}P < 0.01$, $^{***}P < 0.001$ represent significance as compared to the isofraxidin group. 8-OHDG: 8-Hydroxy-2'-deoxyguanosine; DAPI: 4',6-Diamidino-2-phenylindole; H&E: Hematoxylin and eosin; IR: Radiation; MDA: Malondialdehyde; NLRP3: NOD-like receptor family pyrin domain-containing 3; TUNEL: Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling.

Conclusion

IF can regulate DNA damage, apoptosis, oxidative stress, and inflammatory factor disorders *via* the Nrf2/HO-1-NLRP3/ASC signaling axis. This can mitigate radiation-induced testicular injury and possibly protect against radiation-induced testicular injury. Research is

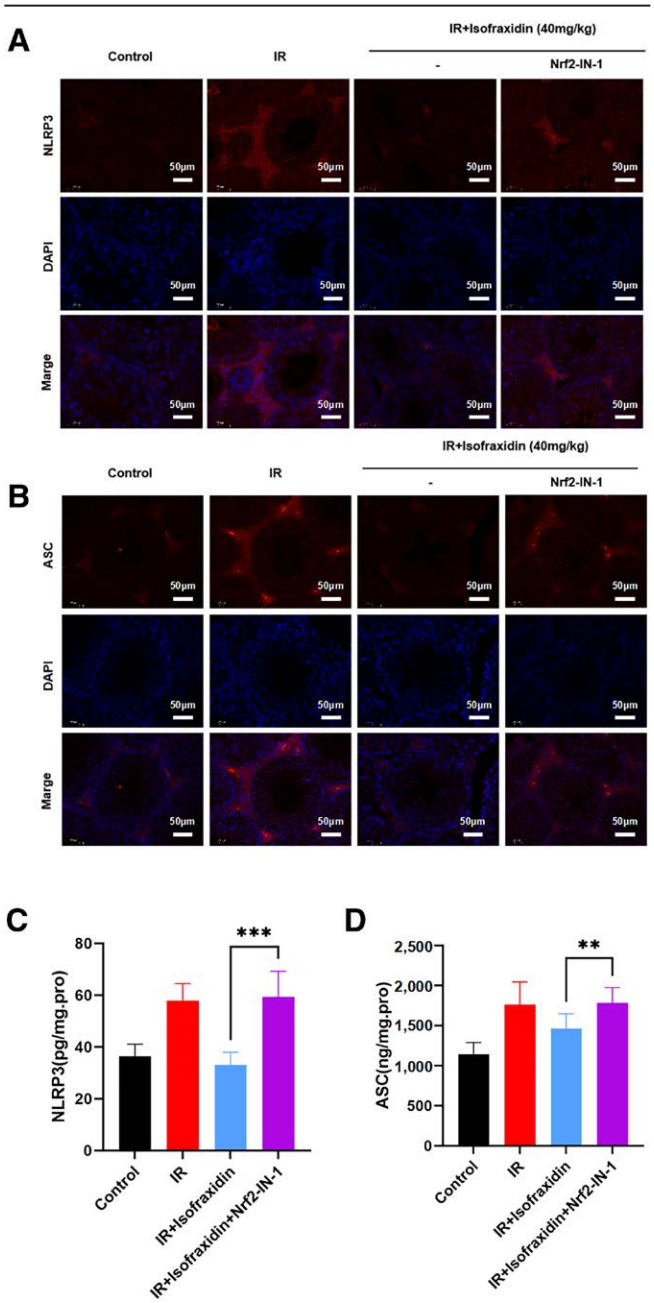

Figure 7. Isofraxidin protects against radiation-induced testicular injury through the Nrf2/HO-1-NLRP3/ASC signaling pathway. Immunofluorescence to determine the expression level of NLRP3 (A) and ASC (B) in testis, enzyme immunoassay to determine the size of NLRP3 (C) and ASC (D) content in testis. $^{**}P < 0.01$ and $^{***}P < 0.001$ represent significance as compared to the isofraxidin group. ASC: Apoptosis-associated speck-like protein containing a caspase recruitment domain; DAPI: 4',6-Diamidino-2-phenylindole; HO-1: Heme oxidase-1; IR: Radiation; NLRP3: NOD-like receptor family pyrin domain-containing 3; Nrf2: Nuclear factor E2-related factor 2.

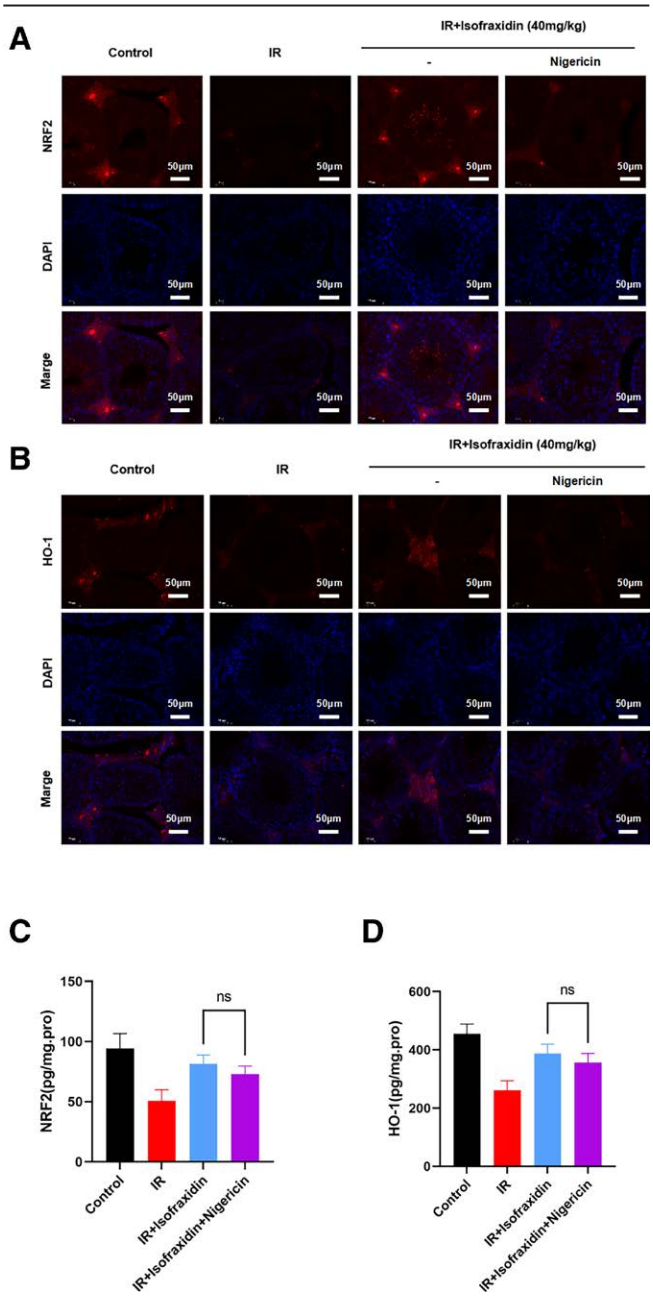

Figure 8. Isofraxidin protects against radiation-induced testicular injury through the Nrf2/HO-1-NLRP3/ASC signaling pathway. Immunofluorescence to determine the expression level of Nrf2 (A) and HO-1 (B) in testis, enzyme immunoassay to determine the size of Nrf2 (C) and HO-1 (D) content in testis. ns represent no significance as compared to the isofraxidin group. ASC: Apoptosis-associated speck-like protein containing a caspase recruitment domain; DAPI: 4',6-Diamidino-2-phenylindole; HO-1: Heme oxidase-1; IR: Radiation; NLRP3: NOD-like receptor family pyrin domain-containing 3; Nrf2: Nuclear factor E2-related factor 2.

required for more understanding of these mechanisms. The findings suggest a novel therapeutic possibility to reduce the reproductive risk for male patients undergoing radiotherapy. Nevertheless, the research methods employed in this study to elucidate the protective mechanism of IF against radiation-induced damage to the reproductive system are relatively straightforward. However, further investigation is required to identify the specific mechanisms and vital molecular events involved.

Conflict of interest statement

Yue Gao is the editorial board member of this journal and other authors declare no conflicts of interest.

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Author contributions

Zengchun Ma, Zebin Liao, and Yue Gao contributed to the study concept and design, manuscript review, experiments, and funding. Changkun Hu performed the experiments and data analyses, prepared the manuscript, and prepared the figures. Liangliang Zhang and Zekun Wu performed the experiments and data analysis.

Ethical approval of studies and informed consent

All animal experiments were approved by the Animal Care and Use Committee of the Animal Center in Academy (IACUC-DWZX-2021-557).

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None.

Data availability

All data generated or analyzed during this study are included in this published article.

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