Future Integrative Medicine **2024** vol. 3(4) | 239–250
DOI: 10.14218/FIM.2024.00031

Original Article

Radioprotective Effects of Licochalcone B: DNA Protection, Cytokine Inhibition, and Antioxidant Boost

Boyuan Ren[1#], Jiayan Jin[2#], Yichen Wang[3], Xiao Xu[4], Yun Liu[5], Hongfan Ding[4], Qiang Li[1*] and Ji-de Jin[1*]

[1]Beijing Institute of Radiation Medicine, Beijing, China; [2]Beijing Institute of Basic Medical Sciences, Beijing, China; [3]Chinese PLA General Hospital, Beijing, China; [4]Senior Department of Burns and Plastic Surgery, the Fourth Medical Center of PLA General Hospital, Beijing, China; [5]Beijing Technology of University, Beijing, China

Received: June 20, 2024 | **Revised:** July 23, 2024 | **Accepted:** August 06, 2024 | **Published online:** August 30, 2024

Abstract

Background and objectives: Radiation injury poses a serious threat to human health, causing complex and multifaceted damage to cells and tissues. Such injury can be caused by various factors, including nuclear accidents, medical radiation therapy, and space travel. Currently, finding effective treatment methods and drugs to mitigate the harmful effects of radiation injury on the human body is a crucial research direction. This study aimed to explore the protective effects and mechanisms of Licochalcone B (Lico B) on radiation-induced cell damage and radiation-induced mortality in mice.

Methods: HaCaT cells, THP-1 cells, and HAEC cells were irradiated with a 10 Gray (Gy) dose of X-rays, while RAW 264.7 cells were irradiated with a 10 Gy dose of γ-rays. The cells were pre-treated with Lico B for 2 h before irradiation, and samples were collected 2 h after irradiation. Cell proliferation viability, oxidative stress levels, DNA damage, expression levels of inflammatory factors, matrix metalloproteinases, guanylate cyclase, and iron death-related factors were measured. C57BL/6 mice were exposed to total-body irradiation with a dose of 8 Gy or a combined dose of 6 Gy + 8 Gy of γ-rays to induce radiation injury. Lico B was injected intraperitoneally one day before irradiation and then administered for two consecutive days, with continuous observation for 20 days.

Results: Mechanistically, Lico B significantly improved antioxidant levels, reduced DNA damage, and lowered the expression of inflammatory factors in HaCaT, THP-1, HAEC, and RAW 264.7 cells. Therapeutically, Lico B increased cell proliferation capacity and significantly extended the survival time of irradiated mice, demonstrating a strong radioprotective effect.

Conclusions: Lico B exhibits significant radioprotective effects and may serve as a potential radioprotective agent.

Introduction

Radiation injury is a common health threat, caused by factors such as radiation therapy, nuclear accidents, and radiological terrorist attacks. Radiation injury refers to a series of pathological changes and physiological dysfunctions that occur in humans or other organisms after exposure to or contamination by radioactive substances (such as X-rays, gamma rays, and radioactive isotopes). This issue endangers human health by causing complex and multifaceted damage to cells and tissues.[1] The primary mechanisms of radiation injury include the ionization or excitation of electrons within atoms or molecules by radioactive substances, triggering a series of biochemical reactions that lead to DNA, protein, and lipid damage. This damage results in gene mutations, inflammatory responses,[2] and immune function impairment. These changes can subsequently cause various diseases, including leukemia, tumors, immune system damage,[3] and reproductive system injuries.[4] Currently, finding effective treatments and drugs to mitigate the harm of radiation injury is an important research topic in radiomedicine.

In recent years, the research field of natural products as anti-radiation injury drugs has garnered significant attention. *licorice* (*Glycyrrhiza uralensis* Fisch.) is a common herb widely used in traditional Chinese medicine and as a food additive.[5–7] Licochalcone B (Lico B), primarily found in the roots of *licorice*,[8] is one

Keywords: Licochalcone B; Radiation injury; Antioxidant; DNA damage; Inflammatory response; Radioprotective agents.

***Correspondence to:** Qiang Li and Ji-de Jin, Beijing Institute of Radiation Medicine, Beijing 100850, China. ORCID: https://orcid.org/0000-0003-3610-6934 (QL); https://orcid.org/0000-0003-1816-2879 (JJ). Tel: +86-10-66931425; Fax: +86-10-68158311, E-mails: StylizationTCM-Gu302@outlook.com (QL); jinjide505@163.com (JJ)
[#]Contributed equally to this work.

How to cite this article: Ren B, Jin J, Wang Y, Xu X, Liu Y, Ding H, *et al*. Radioprotective Effects of Licochalcone B: DNA Protection, Cytokine Inhibition, and Antioxidant Boost. *Future Integr Med* 2024;3(4):239–250. doi: 10.14218/FIM.2024.00031.

of the main active components of *licorice*, exhibiting various pharmacological activities and biological effects such as antioxidant, anti-inflammatory, antiviral, antitumor, and immunomodulatory properties.[9] Studies have shown that Lico B can reduce oxidative stress-induced cellular damage by inhibiting the production and scavenging of reactive oxygen species.[10] Lico B can also inhibit inflammatory responses by reducing the release of inflammatory mediators and the infiltration of inflammatory cells, thereby alleviating symptoms of inflammation-related diseases.[11–13] Additionally, Lico B possesses antiviral activity, capable of inhibiting viral replication and spread, showing potential for treating viral infections.[14–16] Moreover, Lico B has demonstrated antitumor activity, inhibiting the proliferation and invasion of tumor cells and promoting tumor cell apoptosis.[17] Recent research has also found that Lico B can modulate immune system functions, enhancing the body's immunity.[18] Due to its multiple biological activities and pharmacological effects, Lico B holds broad potential applications in the medical field.[19]

Radiation injury often leads to DNA damage, the production of excessive reactive oxygen species, and excessive inflammatory responses in the body. Whether Lico B can provide protection against radiation injury by reducing DNA damage, eliminating reactive oxygen species, and inhibiting inflammatory responses has not been reported so far. In this study, the protective effects of Lico B against radiation injury were investigated using cell and mouse models to provide a theoretical basis for its application in the treatment of radiation injury.

Materials and methods

Reagents and Instruments

Lico B (HY-N0373) was purchased from MedChemExpress. Dulbecco's Modified Eagle Medium (DMEM; Gibco, 04242), DMEM/F12 Medium (Gibco, 01503), RPMI Medium 1640 (Gibco, 01351), and fetal bovine serum (FBS; Gibco, FND500) were obtained from Gibco (CA, USA). Penicillin-streptomycin was sourced from M&C GENE TECHNOLOGY (BEIJING) LTD (Beijing, China). Glutathione (GSH) Assay Kit (Beyotime, S0053), DNA Damage Detection Kit (γ-H2AX Immunofluorescence Method; Beyotime, C2035S), Cell Counting Kit-8 (CCK-8) Assay Kit (Bimake, C6005M), RNA Rapid Extraction Kit (Shanghai Yishan Biotechnology Co., Ltd., RN001), Fast Reverse Transcription Kit (Shanghai Yishan Biotechnology Co., Ltd., RT001), Hieff UNICON® Universal Blue quantitative polymerase chain reaction (qPCR) SYBR Green Master Mix (Shanghai Yeasen Biotechnology Co., Ltd., 11201ES08), and qPCR Primers (synthesized by Beijing Tianyi Huiyuan Biotechnology Co., Ltd.) were used. Cell culture consumables were provided by Corning. The Varioskan Flash Microplate Reader was purchased from Thermo (MA, USA), the 7500 Fast Real-time PCR System from ABI (CA, USA), the Power Pac164-5050 Electrophoresis Apparatus from Bio-Rad (CA, USA), the Tanon5200 Automatic Chemiluminescence Imaging System from Shanghai Tianneng (Shanghai, China), and the R2000 X-ray Irradiator from Rad Source (GE, USA).

Animals

Sixty C57BL/eight male mice, Specific pathogen free grade, aged six to eight weeks and weighing 18–22 g, were purchased from SPF Biotechnology Co., Ltd. This study was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Experimental Animal Management and Use Committee of the Academy of Military Medical Sciences (Animal Ethics Approval Number: AMMSLAC-S-FT054-V3.0-R01). All surgical procedures were carried out under sodium pentobarbital anesthesia, and every effort was made to minimize suffering.

Cell preparation and irradiation

Human Epidermal Cell Lines HaCaT cells were generously provided by Professor Wenxia Zhou's laboratory at the Academy of Military Medical Sciences. Human monocyte-macrophage cell lines THP-1 and RAW 264.7 cells were obtained from the frozen cell stocks of the Experimental Hematology and Biochemistry Laboratory, Institute of Radiation Medicine, Academy of Military Medical Sciences. The study complied with the ethical requirements of the Academy of Military Medical Sciences. THP-1 cells were cultured in RPMI 1640 medium (containing 10% FBS and 1% penicillin-streptomycin) and induced to differentiate into macrophages using 100 nmol/L phorbol-12-myristate-13-acetate (PMA).[20] HaCaT cells were cultured in DMEM/F12 complete medium containing 10% FBS and 1% penicillin-streptomycin, with passaging every two to three days. RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained in a cell culture incubator at 37°C with 5% CO_2, with media changes every two to three days. Experimental groups included: control group, irradiation group, irradiation + Lico B 20 μmol/L group, and irradiation + Lico B 40 μmol/L group. HaCaT cells were seeded at 5×10^4 cells/mL, RAW 264.7 cells at 5×10^5 cells/mL, and THP-1 cells at 1.2×10^6 cells/mL in culture plates and incubated overnight. Cells were treated with Lico B for 2 h and then exposed to X-ray/γ-ray irradiation. The irradiation dose was 10 Gray (Gy), with an X-ray dose rate of 1.175 Gy/m and a γ-ray dose rate of 68.47 R/m. Culture dishes were returned to the incubator, and cells were cultured for an additional 2 h at 37°C with 5% CO_2 after irradiation. Cells were then collected for analysis of DNA damage, intracellular GSH, and RNA extraction for gene expression analysis. Irradiation experiments were conducted in compliance with radiation safety regulations and under the guidance of professionals. The experimental protocol was conducted according to guidelines approved by the Academy of Military Medical Sciences.

Cell proliferation assay

The CCK-8 assay kit was used to measure the proliferation of HaCaT cells under different doses of X-ray irradiation (10 Gy, 20 Gy) at various time points (0 h, 24 h, 48 h, 72 h, 96 h).[21] The culture medium was removed from the treated cells in a 96-well plate, and 100 μL of CCK-8 reaction solution (CCK-8 reagent: DMEM/F12 = 1:9) was added to each well, following the kit instructions. The cells were then incubated in a cell culture incubator for 2 h, and absorbance at 450 nm was measured. Cell proliferation activity was calculated using the formula: A_{450nm} value of the experimental group - A_{450nm} value of the blank control group.[22]

DNA damage detection

The DNA damage detection kit (phosphorylated histone H2AX (γ-H2AX) immunofluorescence method) was used to assess DNA double-strand breaks in cells. According to the kit instructions, cells were fixed with a fixative, permeabilized with a wash buffer, and incubated with a specific anti-γ-H2AX antibody. After washing with wash buffer to remove unbound antibodies, the cells were incubated with a fluorescently labeled anti-rabbit 488 secondary antibody. The cells were washed again with wash buffer to remove unbound secondary antibodies. The labeled cells were then ob-

served under a fluorescence microscope for imaging and fluorescence quantitative analysis.

GSH detection

GSH and oxidized glutathione (GSSG) are crucial redox pairs within cells. Following the assay kit instructions, GSH and GSSG were detected. Cells were washed with PBS buffer, digested with the digestive solution for 2 m, and centrifuged to collect the cells, discarding the supernatant. The cell pellet was resuspended in three times the volume of the protein removal reagent and thoroughly vortexed. The samples underwent two rapid freeze-thaw cycles using liquid nitrogen and a 37°C water bath, followed by incubation on ice or at 4°C for 5 m. The samples were then centrifuged at 10,000 g for 10 m at 4°C, and the supernatant was used for total glutathione measurement. For the prepared samples, a dilution of GSH removal auxiliary solution was added at a ratio of 20 μL per 100 μL of sample and vortexed immediately. Next, GSH removal reagent working solution was added at a ratio of 4 μL per 100 μL of sample and vortexed immediately, followed by a 60-m reaction at 25°C. This reaction can remove up to 50 μmol/L of GSH; if the GSH content in the sample is too high, the sample needs to be appropriately diluted before the GSH removal operation. The treated samples were used for subsequent measurements. Absorbance was measured once after a 25-m reaction, using a single-point measurement method. A standard curve was plotted based on the absorbance of different concentration standards. The total glutathione or oxidized glutathione content was calculated by comparing the sample absorbance to the standard curve (GSSG concentration obtained from the standard curve multiplied by 2). GSH = Total Glutathione - GSSG×2.

qPCR detection of gene expression

To detect mRNA of inflammatory cytokines using qPCR, the following steps were performed: Cell collection: Cells from treated six-well plates were collected, and the supernatant was aspirated. The cells were washed twice with PBS. RNA extraction: Total RNA was purified using an RNA extraction kit, following the kit instructions. Reverse transcription: The extracted total RNA was reverse transcribed into cDNA using reverse transcriptase and primers, as per the kit instructions. qRT-PCR: qRT-PCR was performed to detect the mRNA expression of inflammation-related factors *interleukin* (*IL*)*-1β*, *IL-1α*, *Caspase-1*, *tumor necrosis factor* (*TNF*) *-α*, *IL-18*, *IL-6*; *matrix metalloproteinases* (*MMP*) *1 and 9*; *guanosine triphosphate cyclohydrolase 1* (*GCH1*); and *amino acid transporter - solute carrier family 7 member 11* (*SLC7A11*). Primer sequences are listed in Table 1. PCR reaction setup: A 20 μL reaction system was prepared in a PCR reaction tube, including 0.2 μL of cDNA template, 0.8 μL of forward and reverse primers each, 10 μL of Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix, and an appropriate amount of RNase-free water. PCR reaction program: The PCR reaction program was set on an ABI Prism 7500 Fast instrument, with a pre-denaturation step at 95°C for 30 s, denaturation at 95°C for 5 s, and annealing and extension at 60°C for 30 s, for a total of 40 cycles. The melting curve program was set according to the instrument's instructions. Housekeeping gene and data analysis: The human *β-Actin* gene was used as the housekeeping gene. Target gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Protection of Lico B on lethal irradiation in mice

Experiment 1: Eight-week-old male C57BL/six mice were randomly divided into three groups, with 10 mice in each: normal group, model group, and experimental group. The mice in the experimental group were given intraperitoneal injections of Lico B (40 mg/kg), while the mice in the normal and model groups received injections of an equal volume of solvent (5% dimethyl sulfoxide, 5% Tween 80, and saline). Animals were administered injections once daily before γ-ray irradiation. The mice in the model and experimental groups were then exposed to total-body γ-ray irradiation at a dose of 8 Gy. After irradiation, the mice continued to receive drugs or solvent for an additional two days. The number of deaths and the survival status of the mice were monitored for 20 consecutive days, and the survival curve was plotted using the Kaplan-Meier method.[23]

Experiment 2: All procedures were the same as in experiment 1, except that the mice in the experimental group were exposed to a combined dose of 6 Gy + 8 Gy γ-rays.[24–26]

Table 1. Primer sequences of qPCR

Gene name	Primer sequences (5′-3′)
β-Actin	F: TCT CCC AAG TCC ACA CAG G R: GGC ACG AAG GCT CAT CA
Caspase-1	F: TTTCCGCAAGGTTCGATTTTCA R: GGCATCTGCGCTCTACCATC
IL-1β	F: ATGATGGCTTATTACAGTGGCAA R: GTCGGAGATTCGTAGCTGGA
IL-1α	F: TGGTAGTAGCAACCAACGGGA R: ACTTTGATTGAGGGCGTCATTC
TNF-α	F: CCTCTCTCTAATCAGCCCTCTG R: GAGGACCTGGGAGTAGATGAG
IL-18	F: TCTTCATTGACCAAGGAAATCGG R: TCCGGGGTGCATTATCTCTAC
IL-6	F: ACTCACCTCTTCAGAACGAATTG R: CCATCTTTGGAAGGTTCAGGTTG
MMP-1	F: AAAATTACACGCCAGATTTGCC R: GGTGTGACATTACTCCAGAGTTG
MMP-9	F: TGTACCGCTATGGTTACACTCG R: GGCAGGGACAGTTGCTTCT
GCH1	F: ACGAGCTGAACCTCCCTAAC R: GAACCAAGTGATGCTCACACA
SLC7A11	F: TCTCCAAAGGAGGTTACCTGC R: AGACTCCCCTCAGTAAAGTGAC

Caspase-1, Cysteine-aspartic acid protease 1; *GCH1*, guanosine triphosphate cyclohydrolase 1; *IL*, interleukin; *MMP*, matrix metalloproteinases; *qPCR*, quantitative polymerase chain reaction; *SLC7A11*, solute carrier family 7 member 11; *TNF*, tumor necrosis factor.

Statistical analysis

Data were represented as mean plus or minus the standard error of the mean (mean ± SEM). For comparisons among multiple groups, one-way analysis of variance with Dunnett's post-hoc test was used (GraphPad Prism, USA). The log-rank test was used for survival analysis. $P < 0.05$ was considered statistically significant.[23]

Results

Lico B promotes cell proliferation

The results of cell proliferation indicated that the growth of HaCaT cells was significantly inhibited after X-ray irradiation. However,

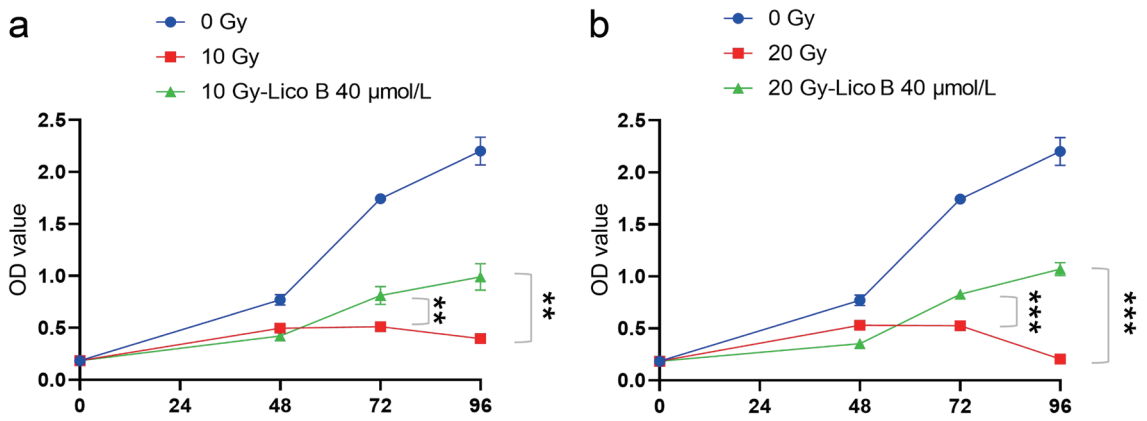

Fig. 1. Licochalcone B promoted cell proliferation. Radiation injury of HaCaT cells was induced with X-ray irradiation at doses of 10 Gy (a) and 20 Gy (b). Cell proliferation was detected using the CCK-8. Data are presented as mean ± SEM of biological replicates (n = 3). $^{**}P < 0.01$ and $^{***}P < 0.001$ (One-way analysis of variance with Dunnett's post hoc test). CCK-8, Cell Counting Kit-8; Gy, Gray; Lico B, licochalcone B; OD, optical density; SEM, standard error of the mean.

compared to the irradiation group, the inhibitory effect on cell proliferation was markedly reduced in the Lico B group (Fig. 1a, b). This suggests that Lico B can mitigate the damaging effects of radiation on HaCaT cell proliferative capacity, thereby playing a protective role against radiation.

Lico B reduces DNA damage

γH2AX is a marker of DNA damage and repair, with increased levels reflecting the extent of DNA damage.[27] After irradiation, γH2AX levels were elevated in both HaCaT cells and THP-1 cells, with a more pronounced increase observed in THP-1 cells. However, in the Lico B treatment group, γH2AX levels were significantly reduced in both HaCaT cells (Fig. 2a, b) and THP-1 cells (Fig. 2c, d), showing a dose-dependent effect. These results indicate that Lico B treatment effectively reduces radiation-induced DNA double-strand breaks.

Lico B enhances cellular antioxidant capacity

GSH is an important intracellular antioxidant that plays a crucial role in protecting cells from oxidative stress-induced damage.[28] When cells are exposed to oxidative stress, GSH captures and neutralizes free radicals, protecting DNA, proteins, and lipids from oxidative damage. Under normal conditions, the ratio of GSH to its oxidized form GSSG is high, maintaining a reductive environment within the cell. However, under oxidative stress conditions, GSH is oxidized to GSSG, leading to a decrease in the GSH/GSSG ratio. In this irradiation experiment, both the GSH content and the GSH/GSSG ratio in RAW 264.7 cells significantly decreased after irradiation. In contrast, after Lico B treatment, the GSH content and the GSH/GSSG ratio in cells were significantly increased compared to the irradiation group, showing dose dependency (Fig. 3a, b).

Lico B reduces the expression of cellular inflammatory factors

Caspase-1 is a key molecule in inflammasome activation. Its upregulation participates in inflammasome activation and the production of pro-inflammatory cytokines such as IL-1β and IL-18. Apoptosis-associated speck-like protein containing a CARD (ASC) acts as an adaptor protein, linking the nucleotide oligomerization domain-like receptor family, pyrin domain-containing protein 3, and Caspase-1, thus being involved in inflammasome assembly. Cytokines such as IL-1α, IL-1β, IL-18, TNF-α, and IL-6 play significant roles in immune regulation and inflammatory responses. In this irradiation experiment, these inflammatory factors in THP-1 cells significantly increased after X-ray irradiation. However, after Lico B treatment, the levels of *IL-1β*, *IL-18*, *Caspase-1*, and *TNF-α* were significantly reduced (Fig. 4a–d). Similarly, after X-ray irradiation, these inflammatory factors in HaCaT cells significantly increased, but Lico B treatment significantly reduced the levels of *IL-1β*, *IL-18*, *Caspase-1*, *TNF-α*, and *IL-6* (Fig. 5a–e). Additionally, after γ-ray irradiation, HaCaT cells also showed an increase in the expression of inflammatory factors, while Lico B treatment significantly reduced the levels of *IL-1α*, *IL-1β*, and *IL-6* (Fig. 5f–h). These results suggest that Lico B alleviated radiation-induced inflammatory responses.

Lico B reduces the expression of matrix metalloproteinases

MMP-1 and MMP-9 belong to the matrix metalloproteinase family, which is involved in the degradation of the extracellular matrix.[29] They play crucial roles in DNA damage repair and inflammatory responses. Radiation-induced DNA damage and inflammatory responses led to increased gene expression and activity of *MMP-1* and *MMP-9*. However, after administration of Lico B, the expression levels of *MMP-1* and *MMP-9* were significantly reduced (Fig. 6a, b), which could be attributed to the anti-inflammatory and antioxidant properties of Lico B.

Lico B promotes the expression of GCH1

GCH1 is a key enzyme in the synthesis of tetrahydrobiopterin (BH4), which is involved in biopterin synthesis in organisms. A decrease in GCH1 activity reduces BH4 production, causing the uncoupling of nitric oxide synthase and leading to increased levels of Reactive Oxygen Species. Studies have found that radiation therapy can reduce *GCH1* expression, possibly due to cell and DNA damage caused by radiation, which in turn affects the transcription and expression of the *GCH1* gene.[30,31] In this study, radiation led to reduced *GCH1* expression, but after administration of Lico B, *GCH1* expression significantly increased (Fig. 6c).

Lico B promotes the expression of amino acid transporters

SLC7A11 is an amino acid transporter involved in intracellular glutathione metabolism.[32] This study found that the expression of *SLC7A11* was reduced in HaCaT cells after irradiation, while administration of Lico B significantly promoted the expression of

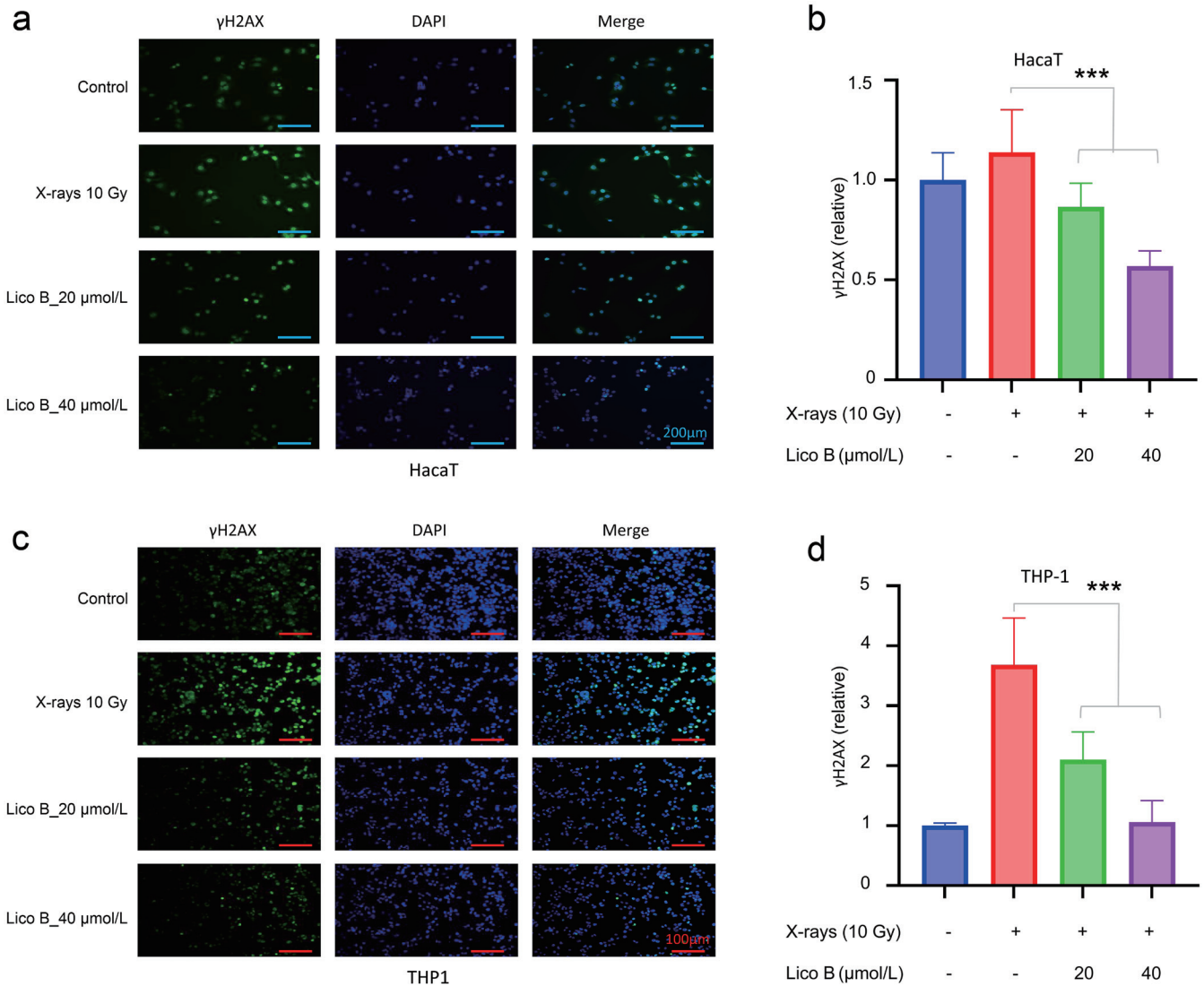

Fig. 2. Licochalcone B reduced DNA damage. Lico B reversed DNA damage in HaCaT (a–b) and THP-1 (c–d) cells induced by X-ray irradiation. DNA damage was detected using the γ-H2AX immunofluorescence method. Data are presented as mean ± SEM of biological replicates (n = 3). ***P < 0.001 (One-way analysis of variance with Dunnett's post hoc test). Gy, Gray; Lico B, licochalcone B; SEM, standard error of the mean; γ-H2AX, gamma-H2A histone family member X.

SLC7A11 (Fig. 6d). A consistent phenomenon was also observed in HAEC cells (Fig. 6e). These findings indicate the important role of Lico B in regulating the intracellular and extracellular GSH balance to counter oxidative stress by regulating the expression of *SLC7A11*. Additionally, the cytotoxicity of Lico B was evaluated, and the results showed that it did not exhibit any cytotoxicity at concentrations up to 80 μmol/L (Fig. 6f).

Lico B increases the survival rate of mice induced by radiation

The study results showed that Lico B treatment significantly increased the survival time of mice treated with γ-ray radiation. Statistical analysis revealed that, compared to the model group, the experimental group of mice exhibited longer survival times whether they received a solo 8 Gy radiation (Fig. 7a) or combined 6 Gy + 8 Gy irradiation (Fig. 7b). In the model group subjected to 8 Gy irradiation, all mice died within 10 days after irradiation. However, in the model group that received combined 6 Gy + 8 Gy irradiation, no mouse deaths were observed by day 10 after treatment with 6 Gy of irradiation, and after an additional 8 Gy of irradiation on day 11, all mice died within six days. These results indicate that Lico B had a protective effect against γ-ray irradiation-induced lethality in mice.

Discussion

Radiation-induced damage poses a serious health threat, particularly in the context of nuclear accidents, radiotherapy, or radiological terrorism. Radiation can cause cellular DNA damage, tissue inflammation, and apoptosis, ultimately leading to organ dysfunction and life-threatening consequences.[33,34] The current scarcity of radioprotective drugs presents challenges for clinical treatment. Common methods for managing radiation damage include remov-

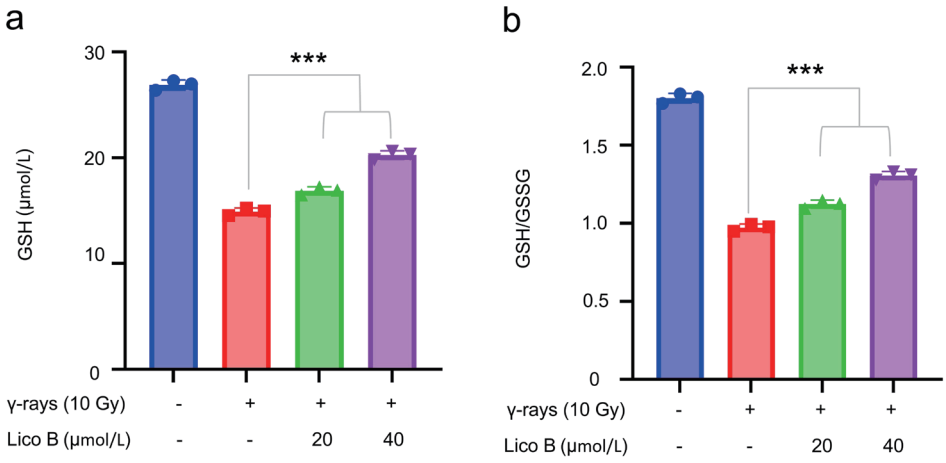

Fig. 3. Licochalcone B counteracted the inhibitory effect of γ-ray irradiation on GSH content in RAW 264.7 cells. The content of GSH (a) and the ratio of GSH/GSSG (b) were measured using a chemical colorimetric method. Data are presented as mean ± SEM of biological replicates (n = 3). ***P < 0.001 (One-way analysis of variance with Dunnett's post hoc test). Lico B, licochalcone B; GSH, glutathione; GSSG, glutathione disulfide; SEM, standard error of the mean.

ing radioactive substances, symptomatic treatment, bone marrow transplantation, growth factor therapy, and radiation therapy.[35] However, these treatments may have side effects, such as immunosuppression, increased risk of infection, and organ damage.[36,37] Consequently, there is an urgent need to explore new treatment methods and drugs. Current research directions include developing

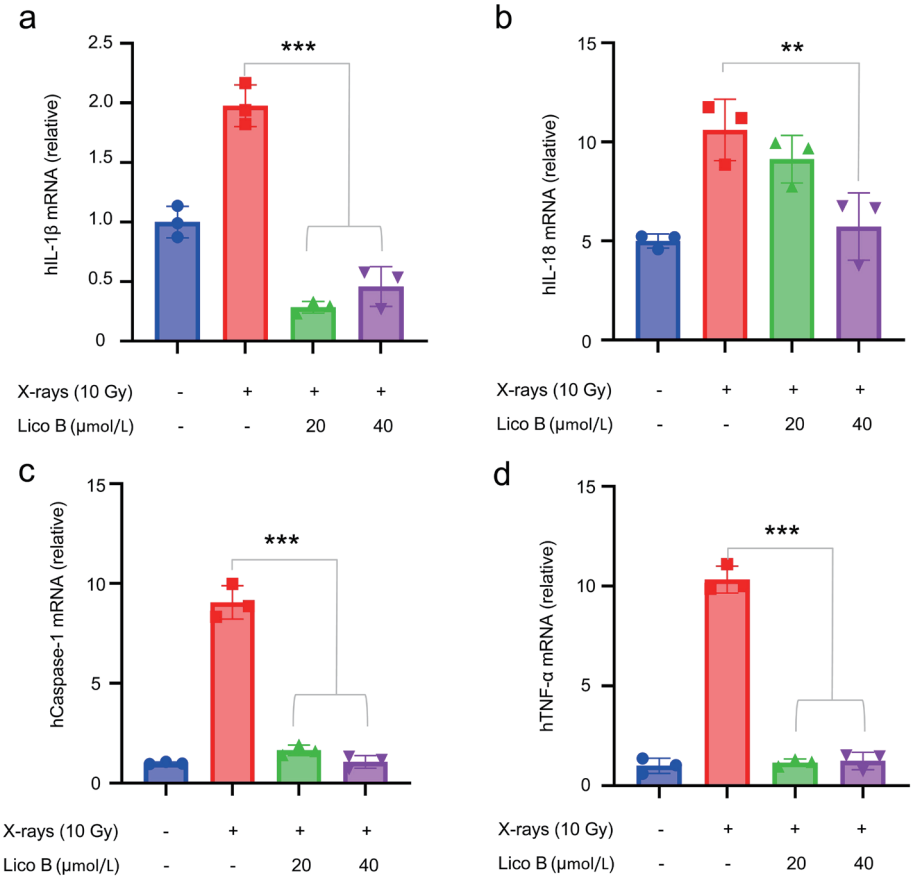

Fig. 4. Licochalcone B inhibited the release of inflammatory factors induced by X-ray in THP-1 cells. The expression levels of *IL-1β* (a), *IL-18* (b), *Caspase-1* (c), and *TNF-α* (d) in THP-1 cells were detected using qPCR. Data are presented as mean ± SEM. P < 0.05 indicates significant differences. **P < 0.01, ***P < 0.001 (One-way analysis of variance with Dunnett's post hoc test). *Caspase-1*, Cysteine-aspartic acid protease 1; *IL*, interleukin; Lico B, licochalcone B; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; *TNF-α*, tumor necrosis factor alpha.

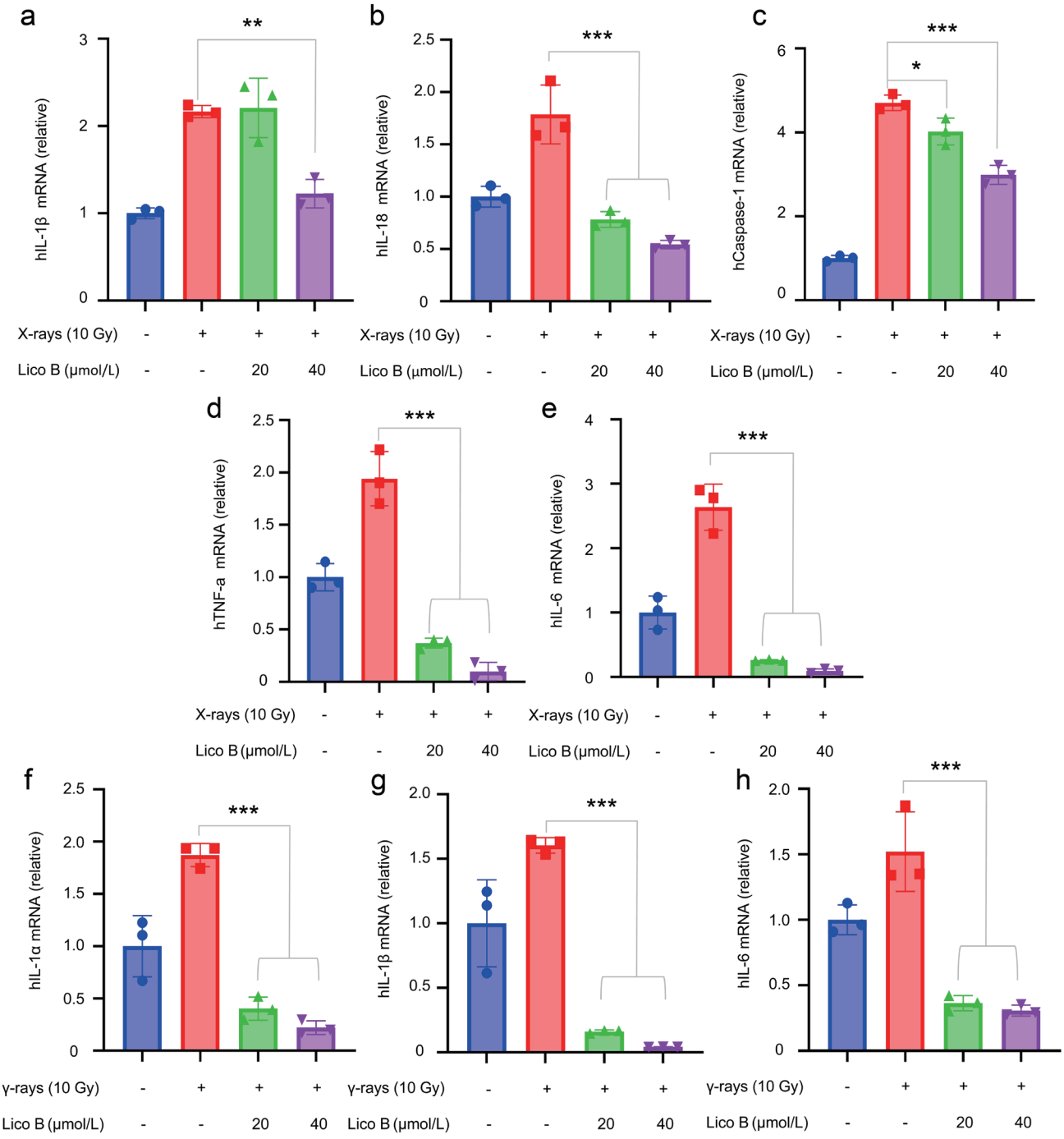

Fig. 5. Licochalcone B inhibited the release of inflammatory factors induced by X-ray and γ-ray in Hacat cells. Cell radiation injury was induced with 10 Gy of X-ray or γ-ray, and the expression of cytokines was detected using qPCR. The levels of *IL-1β* (a), *IL-18* (b), *Caspase-1* (c), *TNF-α* (d), and *IL-6* (e) in HaCaT cells treated with X-ray. The expression levels of *IL-1α* (f), *IL-1β* (g), and *IL-6* (h) in HaCaT cells treated with γ-ray. Data are presented as mean ± SEM. $P < 0.05$ indicates significant differences. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (One-way analysis of variance with Dunnett's post hoc test). *Caspase-1*, Cysteine-aspartic acid protease 1; *IL*, interleukin; Lico B, licochalcone B; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; *TNF-α*, tumor necrosis factor alpha.

small molecule drugs from traditional Chinese medicine, stem cell therapy, gene editing technology to repair damaged cells, and using biomaterials to promote tissue regeneration. Advances in these areas are expected to lead to breakthroughs in treating radiation damage, providing safer and more effective options for patients.

In this study, we investigated the protective effects of Lico B against radiation using cell and mouse models. We found that Lico B significantly alleviated radiation-induced cellular damage and

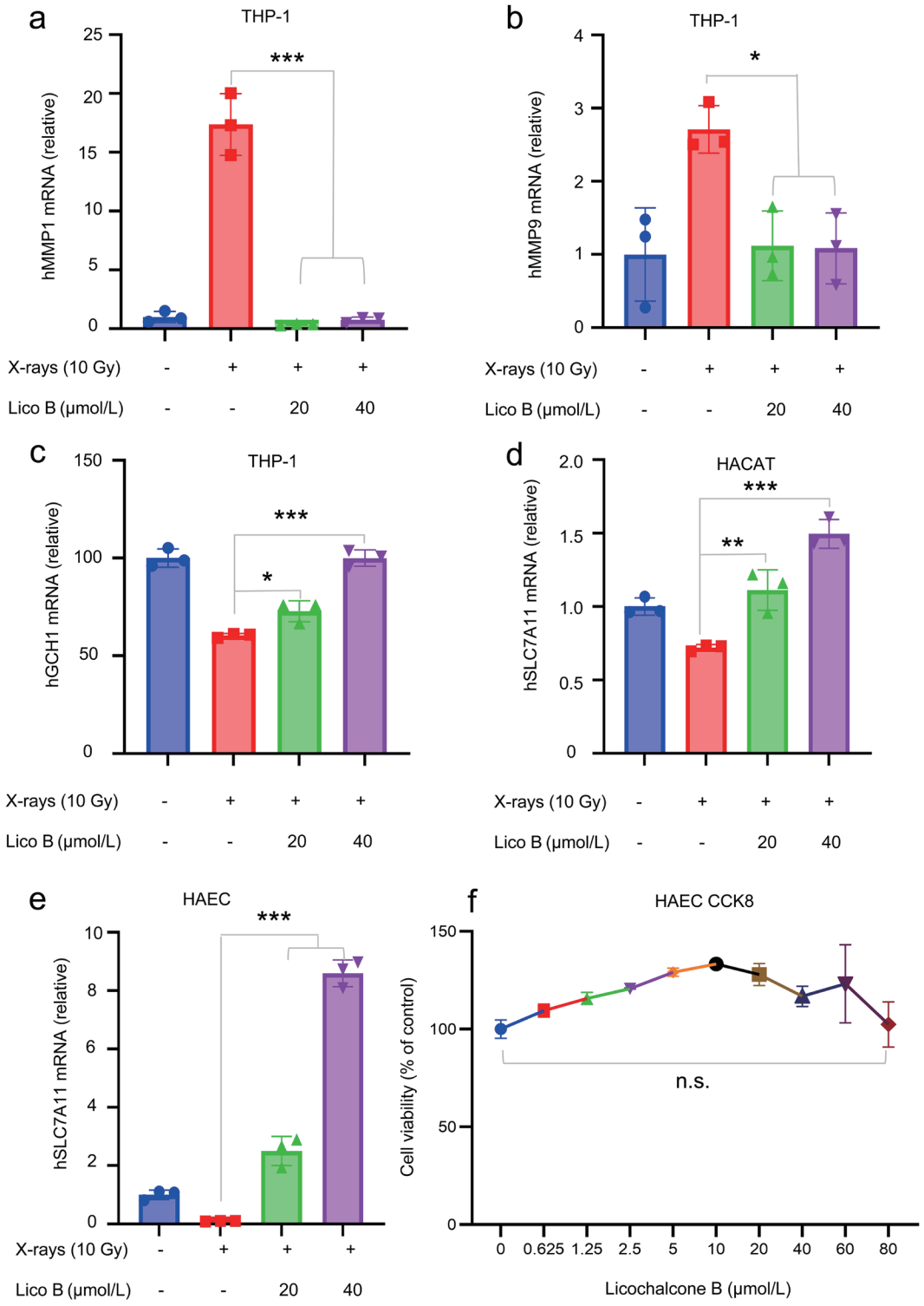

Fig. 6. Licochalcone B inhibited the expression of matrix metalloproteinases and promoted the expression of *GCH1* and *SLC7A11* in cells treated with X-ray irradiation. After cells were treated with 10 Gy of X-ray irradiation, gene expression was detected using qPCR. The expression levels of *MMP-1* (a), *MMP-9* (b), and *GCH1* (c) in X-ray-induced THP-1 cells were measured. The expression of *SLC7A11* (d) in X-ray-induced HaCaT and HAEC cells was also detected using qPCR. The cytotoxicity of Lico B treatment at different concentrations for 24 h in HAEC cells was measured using the CCK-8 assay. Data are presented as mean ± SEM. $P < 0.05$ indicates significant differences. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and n.s.: no significant difference (One-way analysis of variance with Dunnett's post hoc test). CCK-8, Cell Counting Kit-8; *GCH1*, GTP Cyclohydrolase 1; Lico B, licochalcone B; *MMP*, matrix metallopeptidase; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; *SLC7A11*, solute carrier family 7 member 11.

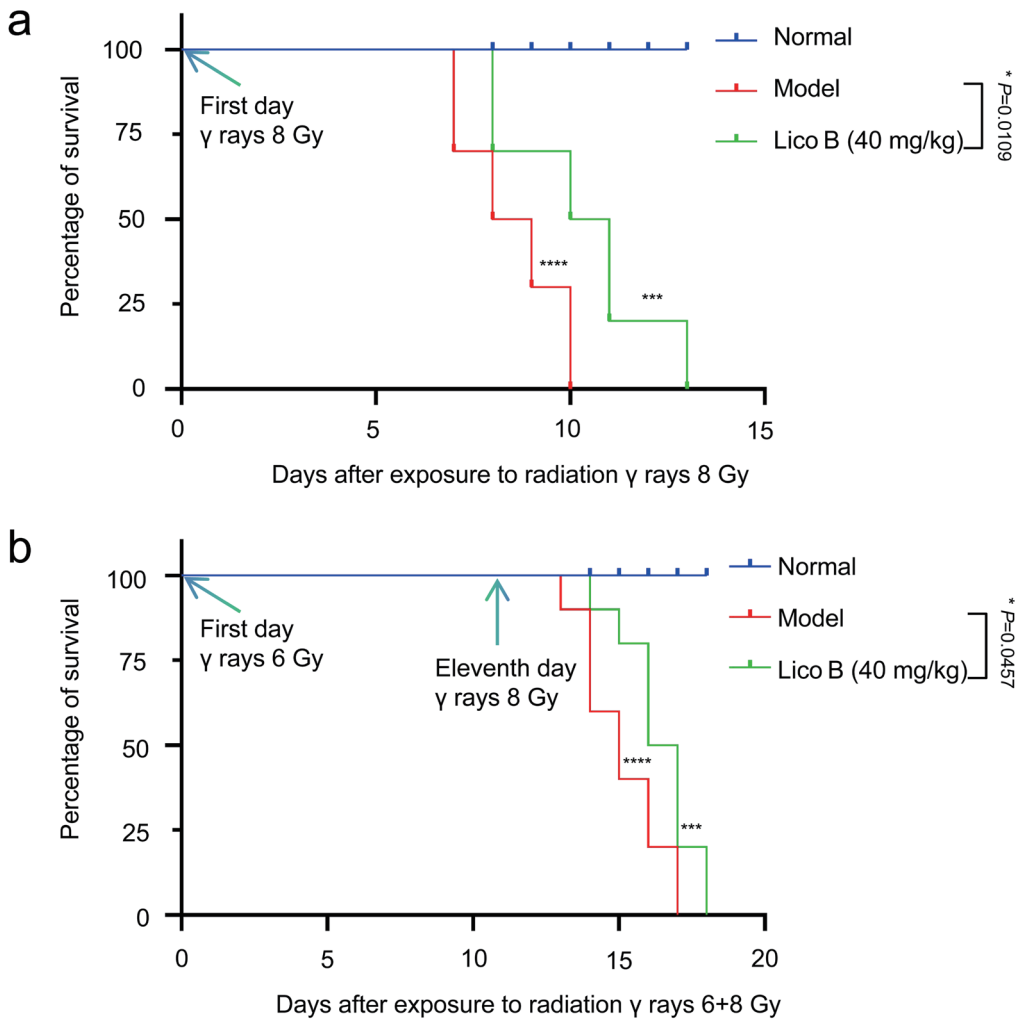

Fig. 7. Lico B significantly prolonged the survival time of mice exposed to γ-ray radiation (n = 10). (a) Survival curves of mice after treatment with 8 Gy γ-ray irradiation. (b) Survival curves after treatment with combined 6 Gy + 8 Gy γ-ray irradiation. The significance of survival analysis differences was determined using a log-rank test. $*P < 0.05$, $***P < 0.001$. Lico B, licochalcone B; Gy, Gray.

inflammatory responses, reduced DNA damage, restored immune function, and improved radiation damage *in vivo*, extending the survival time of irradiated mice. These findings highlight the potential application of Lico B in radiation protection and treatment.

Radiation is a common carcinogen and teratogen that causes both direct and indirect damage to DNA.[38] DNA damage can lead to halted cell division and growth, as well as difficulties in repairing damaged DNA.[39] Failure to repair DNA damage can result in cell death, mutations, and radiation-induced tumor formation. Therefore, reducing DNA damage is crucial for protecting the body from radiation damage.[40] Lico B promoted cell proliferation following radiation exposure, which aids in cell repair and proliferation.

Further mechanistic studies revealed that Lico B reduced DNA damage by inhibiting the expression of γH2AX, thereby offering additional protection against radiation damage. DNA damage can lead to mutations in cellular genetic material, affecting normal cell function and proliferation. Our study found that Lico B alleviated radiation-induced DNA damage through several pathways. First, Lico B exhibited antioxidant properties by promoting the synthesis of intracellular GSH, enhancing the synthesis of *SLC7A11* to balance intracellular and extracellular GSH levels, and scavenging free radicals. Reducing oxidative stress after Lico B treatment was crucial for minimizing radiation-induced oxidative stress and cellular damage. Second, Lico B inhibited the inflammatory response following DNA damage, thereby reducing inflammation-induced cellular damage. Additionally, Lico B promoted DNA repair by regulating the expression of DNA repair-related genes, thereby decreasing the accumulation of damage. Consequently, Lico B maintained DNA stability and integrity by regulating GSH levels, enhancing GSH synthesis, inhibiting inflammation following DNA damage, and promoting the DNA repair process.

Oxidative stress is a significant mechanism of radiation damage. Lico B alleviated radiation-induced oxidative stress and cellular damage through multiple pathways. Lico B promoted the synthesis of intracellular GSH, enhanced the synthesis of *GCH1* and BH4, balanced intracellular and extracellular GSH levels, and scavenged free radicals. GSH is a major intracellular antioxidant that neutralizes oxygen free radicals and reduces oxidative stress damage. Studies showed that during ferroptosis, intracellular iron

overload increased oxidative stress, resulting in cellular damage and death.[41] The expression level of *SLC7A11* is closely related to intracellular GSH levels; low expression of *SLC7A11* can decrease intracellular GSH levels, increasing cellular sensitivity to oxidative stress. Our study found that Lico B increased GSH synthesis by enhancing the expression of *SLC7A11*, thereby reducing radiation-induced oxidative stress. This mechanism may be a crucial pathway through which Lico B protects cells from radiation damage.

Radiation-induced inflammatory responses are another important mechanism of radiation damage.[42,43] Radiation can trigger cellular inflammatory responses by activating the production of cytokines and chemokines, leading to cellular and tissue damage. Our study demonstrated that Lico B significantly reduced radiation-induced inflammatory responses by inhibiting the expression of inflammation-related genes, such as *Caspase-1*, *IL-1β*, *IL-1α*, *TNF-α*, *IL-18*, and *IL-6*. Additionally, Lico B inhibited the expression of *matrix metalloproteinases* (*MMP-1/MMP-9*), proteases associated with tissue damage and inflammation. This further supports the protective role of Lico B against radiation-induced tissue injury. By alleviating radiation-induced inflammatory responses, Lico B further reduced cellular and tissue damage, which could be a key mechanism of its radioprotective effect.

The type and dose of radiation significantly impact radiation damage. X-rays and γ-rays are common types of radiation that can cause damage. Our study utilized these two radiation sources to evaluate the protective effect of Lico B. The results showed that Lico B exhibited significant protective effects in models of both X-ray and γ-ray radiation-induced damage, indicating its broad-spectrum radioprotective effect.[44,45]

In animal models, we further confirmed the radioprotective effect of Lico B *in vivo*. First, Lico B-treated mice showed a significantly improved survival rate after receiving a lethal dose of radiation. Additionally, a higher survival rate was observed in mice irradiated with the combined dose of 6 Gy + 8 Gy compared with the model group. The doses of 8 Gy and the combined dose of 6 Gy + 8 Gy had different effects on mouse survival. A single dose of 8 Gy is lethal, with all mice dying within 10 days of exposure. In contrast, the combined dose involving an initial non-lethal 6 Gy dose followed by a lethal 8 Gy dose resulted in all mice dying within six days after the second irradiation. This indicates the cumulative and additive effects of radiation on the body, meaning that the initial 6 Gy dose followed by the 8 Gy dose increased the mice's sensitivity to radiation, leading to quicker mortality. Lico B treatment provided significant protection against both the single 8 Gy dose and the combined dose, though the protective effect was less pronounced with the latter due to the cumulative effects presenting greater challenges. The combined dose offered a more complex model that mimics real-world scenarios of repeated radiation exposure, highlighting the cumulative effects and the need for more robust protective strategies. In conclusion, the combined dose of 6 Gy + 8 Gy increased the sensitivity of mice to subsequent exposures and provided a more challenging model for testing protective agents, which was valuable for understanding radiation sensitivity mechanisms and developing treatments for severe radiation sickness.

Our study showed that Lico B exhibited significant protective effects against radiation-induced damage in cell and animal models through multiple pathways, including reducing DNA damage, exhibiting antioxidant effects, inhibiting inflammation, and regulating ferroptosis. This provides valuable information for further research and development of radioprotective strategies and offers new ideas and methods for protecting humans from radiation damage.

However, despite the positive results of our study, there are still some limitations. First, most of our research was conducted *in vitro* and in mouse models; further clinical studies are needed to validate these findings. Second, the safety and efficacy of Lico B need further evaluation to ensure its feasibility for clinical application.

Conclusions

This study demonstrated that Lico B has the potential to reduce DNA damage and improve radiation damage in organisms. This lays the foundation for further research into the application of Lico B in radiation protection and treatment and offers insights for the development of other drugs with similar effects.

Acknowledgments

We would like to express our gratitude to the research team of the article "10.15252/embr.202153499" for their inspiration and foundational work.

Funding

This work was supported by Military Logistics Research Projects (JKAWS22J1005 and AWS21J003).

Conflict of interest

All authors declare that there are no conflicts of interest related to the research project or the writing of this article.

Author contributions

Conducting main experiments and data analysis, writing the manuscript (BYR, QL, JYJ, YCW), participating in the experiments (JYJ, YCW, YL, HFD), literature review, supervision, validation of the study (QL, BYR, XX), designing and supervising the progress of experiments, and revising the manuscript (QL, JDJ). All authors have read and approved the final manuscript.

Ethical statement

This study was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Experimental Animal Management and Use Committee of the Academy of Military Medical Sciences (Animal Ethics Approval Number: AMMSLAC-S-FT054-V3.0-R01). All surgical procedures were carried out under sodium pentobarbital anesthesia, and every effort was made to minimize suffering.

Data sharing statements

The data used to support the findings of this study are available from the corresponding author upon request.

References

[1] Zhang D, He J, Cui J, Wang R, Tang Z, Yu H, *et al*. Oral Microalgae-Nano Integrated System against Radiation-Induced Injury. ACS Nano 2023;

17(11):10560–10576. doi:10.1021/acsnano.3c01502, PMID:37253200.
[2] Sun B, Kitchen S, Tang N, Garza A, Jacob S, Pulliam L. Engineered induced-pluripotent stem cell derived monocyte extracellular vesicles alter inflammation in HIV humanized mice. Extracell Vesicles Circ Nucl Acids 2022;3(2):118–132. doi:10.20517/evcna.2022.11, PMID:37067894.
[3] Kannan M, Singh S, Chemparathy DT, Oladapo AA, Gawande DY, Dravid SM, *et al*. HIV-1 Tat induced microglial EVs leads to neuronal synaptodendritic injury: microglia-neuron cross-talk in NeuroHIV. Extracell Vesicles Circ Nucl Acids 2022;3(2):133–149. doi:10.20517/evcna.2022.14, PMID:36812097.
[4] Ameixa J, Bald I. Unraveling the Complexity of DNA Radiation Damage Using DNA Nanotechnology. Acc Chem Res 2024;57(11):1608–1619. doi:10.1021/acs.accounts.4c00121, PMID:38780304.
[5] Kan W, Li Q, Li P, Ren L, Mu W, Lin L, *et al*. Glycyrrhiza uralensis polysaccharides ameliorate acute lung injury by inhibiting the activation of multiple inflammasomes. J Funct Foods 2023;100:105386. doi:10.1016/j.jff.2022.105386.
[6] Wen J, Qin S, Li Y, Zhang P, Zhan X, Fang M, *et al*. Flavonoids derived from licorice suppress LPS-induced acute lung injury in mice by inhibiting the cGAS-STING signaling pathway. Food Chem Toxicol 2023;175:113732. doi:10.1016/j.fct.2023.113732, PMID:36958387.
[7] Wen J, Mu W, Li H, Yan Y, Zhan X, Luo W, *et al*. Glabridin improves autoimmune disease in Trex1-deficient mice by reducing type I interferon production. Mol Med 2023;29(1):167. doi:10.1186/s10020-023-00754-y, PMID:38066431.
[8] Lin Y, Kuang Y, Li K, Wang S, Song W, Qiao X, *et al*. Screening for bioactive natural products from a 67-compound library of Glycyrrhiza inflata. Bioorg Med Chem 2017;25(14):3706–3713. doi:10.1016/j.bmc.2017.05.009, PMID:28522265.
[9] Cao Y, Xu W, Huang Y, Zeng X. Licochalcone B, a chalcone derivative from Glycyrrhiza inflata, as a multifunctional agent for the treatment of Alzheimer's disease. Nat Prod Res 2020;34(5):736–739. doi:10.1080/14786419.2018.1496429, PMID:30345819.
[10] Zhou B, Wang H, Zhang B, Zhang L. Licochalcone B attenuates neuronal injury through anti-oxidant effect and enhancement of Nrf2 pathway in MCAO rat model of stroke. Int Immunopharmacol 2021;100:108073. doi:10.1016/j.intimp.2021.108073, PMID:34454290.
[11] Li Q, Feng H, Wang H, Wang Y, Mou W, Xu G, *et al*. Licochalcone B specifically inhibits the NLRP3 inflammasome by disrupting NEK7-NLRP3 interaction. EMBO Rep 2022;23(2):e53499. doi:10.15252/embr.202153499, PMID:34882936.
[12] Zhan X, Li Q, Xu G, Xiao X, Bai Z. The mechanism of NLRP3 inflammasome activation and its pharmacological inhibitors. Front Immunol 2022;13:1109938. doi:10.3389/fimmu.2022.1109938, PMID:36741414.
[13] Luo W, Song Z, Xu G, Wang H, Mu W, Wen J, *et al*. LicochalconeB inhibits cGAS-STING signaling pathway and prevents autoimmunity diseases. Int Immunopharmacol 2024;128:111550. doi:10.1016/j.intimp.2024.111550, PMID:38232536.
[14] Xu H, Li S, Liu J, Cheng J, Kang L, Li W, *et al*. Bioactive compounds from Huashi Baidu decoction possess both antiviral and anti-inflammatory effects against COVID-19. Proc Natl Acad Sci U S A 2023;120(18):e2301775120. doi:10.1073/pnas.2301775120, PMID:37094153.
[15] Sruthi D, Jishna JP, Dhanalakshmi M, Deepanraj SP, Jayabaskaran C. Medicinal Plant Extracts and Herbal Formulations: Plant Solutions for the Prevention and Treatment of COVID-19 Infection. Future Integr Med 2023;2(4):216–226. doi:10.14218/FIM.2023.00079.
[16] Chai R, Fan Y, Li Q, Cui H, Liu H, Wang Y, *et al*. Traditional Chinese medicine: an important broad-spectrum anti-coronavirus treatment strategy on COVID-19 background? Tradit Med Res 2022;7(3):19. doi:10.53388/TMR20220330274.
[17] Huang Z, Jin G. Licochalcone B Induced Apoptosis and Autophagy in Osteosarcoma Tumor Cells via the Inactivation of PI3K/AKT/mTOR Pathway. Biol Pharm Bull 2022;45(6):730–737. doi:10.1248/bpb.b21-00991, PMID:35431285.
[18] Wang M, Yu B, Wang J, Wang Y, Liang L. Exploring the role of Xingren on COVID-19 based on network pharmacology and molecular docking. J Food Biochem 2022;46(10):e14363. doi:10.1111/jfbc.14363.
[19] Dong S, Liu Z, Chen H, Ma S, Wang F, Shen H, *et al*. A synergistic mechanism of Liquiritin and Licochalcone B from Glycyrrhiza uralensis against COPD. Phytomedicine 2024;132:155664. doi:10.1016/j.phymed.2024.155664, PMID:38870751.
[20] Ren L, Li Q, Li H, Zhan X, Yang R, Li Z, *et al*. Polysaccharide extract from Isatidis Radix inhibits multiple inflammasomes activation and alleviate gouty arthritis. Phytother Res 2022;36(8):3295–3312. doi:10.1002/ptr.7514, PMID:35666808.
[21] Ai Y, Shi W, Zuo X, Sun X, Chen Y, Wang Z, *et al*. The Combination of Schisandrol B and Wedelolactone Synergistically Reverses Hepatic Fibrosis Via Modulating Multiple Signaling Pathways in Mice. Front Pharmacol 2021;12:655531. doi:10.3389/fphar.2021.655531, PMID:34149411.
[22] Lin L, Chen Y, Li Q, Xu G, Ding K, Ren L, *et al*. Isoxanthohumol, a component of Sophora flavescens, promotes the activation of the NLRP3 inflammasome and induces idiosyncratic hepatotoxicity. J Ethnopharmacol 2022;285:114796. doi:10.1016/j.jep.2021.114796, PMID:34740771.
[23] Fang ZE, Wang Y, Bian S, Qin S, Zhao H, Wen J, *et al*. Helenine blocks NLRP3 activation by disrupting the NEK7-NLRP3 interaction and ameliorates inflammatory diseases. Phytomedicine 2024;122:155159. doi:10.1016/j.phymed.2023.155159, PMID:37931457.
[24] Down JD, Boudewijn A, van Os R, Thames HD, Ploemacher RE. Variations in radiation sensitivity and repair among different hematopoietic stem cell subsets following fractionated irradiation. Blood 1995;86(1):122–127. PMID:7795217.
[25] Wu S, Tang F, Chen Q, Xia X, Zhang X, Cao J. [Study on difference of rhG-CSF rescue for 8Gy, 10Gy and 15Gy radiated mice]. Suzhou University Journal of Medical Science 2012;32(4):489–493.
[26] Demyashkin G, Karakaeva E, Saakian S, Tarusova N, Guseinova A, Vays A, *et al*. Comparative Characterisation of Proliferation and Apoptosis of Colonic Epithelium after Electron Irradiation with 2 GY and 25 GY. Int J Mol Sci 2024;25(2):1196. doi:10.3390/ijms25021196, PMID:38256269.
[27] Li C, Liu W, Wang F, Hayashi T, Mizuno K, Hattori S, *et al*. DNA damage-triggered activation of cGAS-STING pathway induces apoptosis in human keratinocyte HaCaT cells. Mol Immunol 2021;131:180–190. doi:10.1016/j.molimm.2020.12.037, PMID:33423764.
[28] Farina M, Aschner M. Glutathione antioxidant system and methylmercury-induced neurotoxicity: An intriguing interplay. Biochim Biophys Acta Gen Subj 2019;1863(12):129285. doi:10.1016/j.bbagen.2019.01.007, PMID:30659883.
[29] Cui B, Wang Y, Jin J, Yang Z, Guo R, Li X, *et al*. Resveratrol Treats UVB-Induced Photoaging by Anti-MMP Expression, through Anti-Inflammatory, Antioxidant, and Antiapoptotic Properties, and Treats Photoaging by Upregulating VEGF-B Expression. Oxid Med Cell Longev 2022;2022:6037303. doi:10.1155/2022/6037303, PMID:35028009.
[30] Xue J, Yu C, Sheng W, Zhu W, Luo J, Zhang Q, *et al*. The Nrf2/GCH1/BH4 Axis Ameliorates Radiation-Induced Skin Injury by Modulating the ROS Cascade. J Invest Dermatol 2017;137(10):2059–2068. doi:10.1016/j.jid.2017.05.019, PMID:28596000.
[31] Feng Y, Feng Y, Gu L, Mo W, Wang X, Song B, *et al*. Tetrahydrobiopterin metabolism attenuates ROS generation and radiosensitivity through LDHA S-nitrosylation: novel insight into radiogenic lung injury. Exp Mol Med 2024;56(5):1107–1122. doi:10.1038/s12276-024-01208-z, PMID:38689083.
[32] Ye Y, Chen A, Li L, Liang Q, Wang S, Dong Q, *et al*. Repression of the antiporter SLC7A11/glutathione/glutathione peroxidase 4 axis drives ferroptosis of vascular smooth muscle cells to facilitate vascular calcification. Kidney Int 2022;102(6):1259–1275. doi:10.1016/j.kint.2022.07.034, PMID:36063875.
[33] McBride WH, Schaue D. Radiation-induced tissue damage and response. J Pathol 2020;250(5):647–655. doi:10.1002/path.5389, PMID:31990369.
[34] Jackson IL, Doyle-Eisele M. Animal Model Considerations for Medical Countermeasure Development for Radiation and Sulfur Mustard Exposures: Animal models for radiation and HD exposures. Disaster Med Public Health Prep 2023;18:e81. doi:10.1017/dmp.2023.180, PMID:37937347.
[35] Ladbury C, Eustace N, Amini A, Dandapani S, Williams T. Biology-Guided Radiation Therapy: An Evolving Treatment Paradigm. Surg Oncol Clin N

Am 2023;32(3):553–568. doi:10.1016/j.soc.2023.02.006, PMID:37182992.
[36] Luo Y, Zeng Z, Liu Y, Liu A. Reflecting on the cardiac toxicity in non-small cell lung cancer in the era of immune checkpoint inhibitors therapy combined with thoracic radiotherapy. Biochim Biophys Acta Rev Cancer 2023;1878(6):189008. doi:10.1016/j.bbcan.2023.189008, PMID:37913939.
[37] Oh JH, Craft JM, Townsend R, Deasy JO, Bradley JD, El Naqa I. A bioinformatics approach for biomarker identification in radiation-induced lung inflammation from limited proteomics data. J Proteome Res 2011;10(3):1406–1415. doi:10.1021/pr101226q, PMID:21226504.
[38] Jang WH, Shim S, Wang T, Yoon Y, Jang WS, Myung JK, *et al*. In vivo characterization of early-stage radiation skin injury in a mouse model by two-photon microscopy. Sci Rep 2016;6:19216. doi:10.1038/srep19216, PMID:26755422.
[39] Wang C, Yao S, Zhang T, Sun X, Bai C, Zhou P. RNA N6-Methyladenosine Modification in DNA Damage Response and Cancer Radiotherapy. Int J Mol Sci 2024;25(5):2597. doi:10.3390/ijms25052597, PMID:38473842.
[40] Akamatsu K, Shikazono N, Saito T. Fluorescence anisotropy study of radiation-induced DNA damage clustering based on FRET. Anal Bioanal Chem 2021;413(4):1185–1192. doi:10.1007/s00216-020-03082-w, PMID:33245399.
[41] Guo K, Cao Y, Qian L, Daniels M, Tian Y, Li Y, *et al*. Dihydroartemisinin induces ferroptosis in pancreatic cancer cells by the regulation of survival prediction-related genes. Tradit Med Res 2023;8(12):67. doi:10.53388/TMR20230618001.
[42] Arroyo-Hernández M, Maldonado F, Lozano-Ruiz F, Muñoz-Montaño W, Nuñez-Baez M, Arrieta O. Radiation-induced lung injury: current evidence. BMC Pulm Med 2021;21(1):9. doi:10.1186/s12890-020-01376-4, PMID:33407290.
[43] Balkrishna A, Sharma N, Srivastava D, Kukreti A, Srivastava S, Arya V. Exploring the Safety, Efficacy, and Bioactivity of Herbal Medicines: Bridging Traditional Wisdom and Modern Science in Healthcare. Future Integr Med 2024;3(1):35–49. doi:10.14218/FIM.2023.00086.
[44] Fornalski KW, Adamowski Ł, Dobrzyński L, Jarmakiewicz R, Powojska A, Reszczyńska J. The radiation adaptive response and priming dose influence: the quantification of the Raper-Yonezawa effect and its three-parameter model for postradiation DNA lesions and mutations. Radiat Environ Biophys 2022;61(2):221–239. doi:10.1007/s00411-022-00963-9, PMID:35150289.
[45] Maalouf M, Granzotto A, Devic C, Bodgi L, Ferlazzo M, Peaucelle C, *et al*. Influence of Linear Energy Transfer on the Nucleo-shuttling of the ATM Protein: A Novel Biological Interpretation Relevant for Particles and Radiation. Int J Radiat Oncol Biol Phys 2019;103(3):709–718. doi:10.1016/j.ijrobp.2018.10.011, PMID:30342967.