Hesperidin relieves irradiation-induced cognitive dysfunction via regulation of HMGB1-mediated neuroinflammation

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Research Article

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Abstract

**Background:** High mobility group box1 (HMGB1) is a chromatin-binding protein that especially regulates inflammatory signaling cascades. Several reports have demonstrated the anti-inflammatory effect of hesperidin. Whether hesperidin attenuates radiation-induced brain injury via inhibiting HMGB1-mediated neuroinflammation remains unclear.

**Methods:** Morris water maze test and the step-down passive avoidance test were applied to evaluate whether hesperidin could relieve the irradiation-induced cognitive dysfunction. Nissl staining, western blotting and immunofluorescence were performed to uncover the mechanisms.

**Results:** In this study, we found that radiation reduced the neuronal number and increased the content of the proinflammatory cytokines in the hippocampus, and hesperidin significantly reversed these changes. More importantly, hesperidin significantly improved the learning and memory abilities of X-ray-stimulated mice. We also found that radiation markedly increased Iba-1 expression in the hippocampus and resulted in substantial translocation of HMGB1 from the nucleus to the cytoplasm in the hippocampus and BV-2 cell, and hesperidin reversed the radiation-induced upregulation of Iba-1 and the cytoplasmic translocation of HMGB1. Moreover, hesperidin rescued the radiation-induced the upregulations in the phosphorylation levels of ERK, p38 and p65 in the hippocampus.

**Conclusions:** This study demonstrated that hesperidin alleviated the radiation-induced cognitive dysfunction via inhibiting HMGB-mediated neuroinflammation, and indicated that hesperidin could be a promising candidate for treatment of radiation-induced brain injury.

Background

With the development and application of nuclear energy and radiation technology in various fields, a growing number of people are being in danger of radiation following exposure to radioactive leaks from medical, industrial and nuclear facilities, and radiotherapy[1]. As a treatment for various primary and metastatic brain tumors, whole brain radiotherapy is helpful for extending survival for the majority of patients. However, both low and high doses of ionizing radiation (IR) cause detrimental effects in the central nervous system (CNS) because of the inevitable exposure of surrounding healthy tissue[2]. IR could cause direct or indirect DNA damage by generating excessive free radicals, and the general mechanism of radiation-induced cell death is related to double-stranded DNA breaks leading to mitotic catastrophe. IR is considered to lead to breakdown of the blood brain barrier (BBB) through the effect on brain microvascular endothelial cells and as a potential risk factor for cognitive dysfunction. The most severe form of injury produced from radiation-induced stress is radionecrosis, producing a brisk neuroinflammatory reaction[3]. Emerging evidence suggests neuroinflammation is linked with the development of many CNS diseases, such as stroke, Parkinson disease, Alzheimer disease as well as radiation-induced brain injury (RBI)[4].
High-mobility group box 1 (HMGB1) serves as a damage-related signal molecule which mediates cross-talk in damaged cells and induces inflammation [5]. HMGB1 is expressed in various eukaryotic cells and characterized as a nonhistone nuclear protein in general circumstances. Once the cells are stimulated by physical or biochemical stress, HMGB1 could translocate from nucleus to cytosol, and be released into the extracellular environment to further activate inflammatory signaling pathway[6]. The excess release of HMGB1 has been proved to lead to tissue damage and organ dysfunction[7]. In response to cell stress, damage, and death, HMGB1, as a crucial endogenous danger signal and an important proinflammatory mediator, will bind toll-like receptor 4 (TLR4) and triggers TLR4 downstream inflammatory signaling pathway[8]. TLR4, a pattern recognition receptor in the innate immune system, may induce microglial activation and sickness behavior after being activated by its preferential ligand HMGB1[9].

TLR4 is associated with learning and memory processes and is also a key regulator in the pathogenesis of neurodegenerative diseases, which relates to the production of inflammatory cytokines[10]. It has been shown that TLR4 inhibitor could effectively in improving neurological function, reduced the expression of iNOS and TNF-α in APP/PS1 transgenic Alzheimer's Disease (AD) mice[11]. Therefore, we hypothesized that inhibiting HMGB1 translocation from the nucleus to cytosol, decreasing the release of HMGB1, and further regulating TLR4-mediated inflammatory signaling pathways play an important role in the pathophysiology of brain damage.

Hesperidin, a dietary bioavonoid possesses many pharmacological functions such as neuroprotective, anti-inflammation, antioxidant, and anti-hypertensive. Besides, hesperidin could cross the BBB and exert neuroprotective function under different neurodegenerative diseases[12]. Previous study has proved that hesperidin reduced oxidative stress and enhanced hippocampal neurogenesis in methotrexate-treated rats[13]. Hesperidin could improve neurobehavioral impairment and restoration in brain biochemical changes in fluoride-stimulated rats [14]. Moreover, hesperidin attenuated LPS-induced neuroinflammation, apoptosis and memory impairments through regulating TLR4 expression in vivo and in vitro [15]. Whether the neuroprotective effect of hesperidin against brain damage after ionizing radiation is related to inhibition of HMGB1 translocation from the nucleus to the cytosol is still unclear. Therefore, the purpose of this study was to explore the role of hesperidin in radiation brain injury and its potential mechanism of action that involves HMGB1.

**Methods**

**Chemicals**

Hesperidin was obtained from Sigma-Aldrich (St. Louis, MO, USA).

**Animals**

The male C57BL/6 mice (4–6 weeks, 22–25 g) were provided by the Qinglong Mountain Animal Center. Animals have free access to food and water. All experiments utilizing animals were reviewed and approved by the Ethical Committee and Animal Welfare Committee of Drum Tower Hospital, the Affiliated
Hospital of Nanjing University Medical School. Animals were then randomly divided into three groups: Control group, Radiation, and Radiation + hesperidin group.

**Morris water maze test**

The spatial cognitive performance of mice was evaluated by Morris water maze. A circular swimming pool (138 cm in diameter and 45 cm in height) was filled with opaque water made by white nontoxic paint to a depth of 33 cm at 24 ± 2°C. Four starting points around the edge of the pool were designated as N, E, S, and W, dividing the pool into four quadrants. A platform (6 cm in diameter) was located in a constant position in the middle of one quadrant. To render it invisible to the mice, platform was submerged 1.2 cm below the surface of the water. The task for the mice was to escape from the water by locating the hidden platform. At Day 1, mice were trained to escape from the water by locating the platform which was above the water with a red flag on it. Then, one block of four trails was given for 5 consecutive days. For each trial, the mouse was placed in the water facing the wall of the pool at one of four starting points and allowed to swim for a maximum of 60 s. If the mice found the platform, they were allowed to remain on it for 10 s; the mice not finding the platform were guided to it and allowed to remain there for 10 s. At day 7, mice were given 60 s retention probe test in which the platform was removed from the pool. During retention, the number of crossings of the platform location and the time spent in the target quadrant were measured.

**Step-down passive avoidance test**

The step-down passive avoidance test was applied to evaluate learning and memory function, composed of a 5 min training session, followed 24 h later by a 5 min test session. The plastic box (30×30×40 cm) was used. The floor consisted of an electrified grid of parallel 0.1-cm copper bars spaced 0.5 cm apart and an elevated wooden platform (4×4×4 cm) in center of the plastic box. During the training session, mice were placed onto the platform, and once stepping down with their four paws on the electrified grid, the mice received an immediate electric shock (36V, AC). Instinctively, they showed a tendency to jump up the platform to avoid the shock. In the test session, the same procedures were conducted. The time it took the mouse to step down from the platform onto the grid (step-down latency) and the number of times stepped down during the training period (error counts) were recorded.

**Cell culture**

The murine BV-2 microglial cells were maintained in DME/F12 medium, containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. The BV-2 cells were incubated at 37°C in 5% CO₂. The cells were incubated with hesperidin (50 μM) one hour before X-ray (6 Gy) stimulation. After 24 h, cells are used for immunofluorescence.

**Nissl staining**
Mice were transcardially perfused with ice-cold saline followed by 4% PFA under deep chloral hydrate anesthesia. Brains were removed, post-fixed overnight and then coronal sections (40 mm) were cut on a vibratome (VT1200s, Leica). Every 12th section throughout the hippocampus was processed for Nissl staining. The sections were immersed in the Nissl staining solution at 37°C for 10 min. Then, the images were taken on a Zeiss Axio microscope.

**Immunocytochemistry**

The BV-2 cells were fixed using 4% PFA and blocked in PBS containing 3% normal goat serum, 0.3% (w/v) Triton X-100, and 0.1% BSA at room temperature for 1 h, followed by incubation in primary antibody at 4°C overnight. The primary antibodies were used as follows: rabbit-anti HMGB1 (1:500; ab79823, Abcam), and mouse-anti-TLR4 (1:500; ab22048, Abcam). Secondary antibodies used were goat-anti rabbit Cy3 (1:200; 115-165-003, Jackson Immuno-Research Laboratories) and goat-anti mouse Alexa-488 (1:400; 115-545-003, Jackson ImmunoResearch Laboratories). Cultures were counterstained with DAPI (D9542, Sigma) to label the nuclei. Images were captured with a fluorescence microscope (Axio Imager, Carl Zeiss).

**Cytokine assays**

The levels of IL-1β, IL-6 and TNF-α were assessed by ELISA assays kit (MEIMIAN, China) according to the manufacturer’s instructions. The final results were presented as picograms per mg protein.

**Western blotting**

Western bolt analysis was performed similar to our previous report[16]. The hippocampus of mouse was collected and homogenized in ice-cold lysis buffer containing 100 mmol/L HEPES, 200 mmol/L NaCl, 10% glycerol, 2 mmol/ L Na₄P₂O₇, 2 mmol/L dithiothreitol, 1 mmol/L EDTA, 1 mmol/L benzamidine, 0.1 mmol/L Na₃VO₄, 1 μmol/L pepstatine, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 10 μmol/L phenylmethylsulfonyl fluoride. Subsequently, the lysate was obtained with centrifugation at 12,000 rpm for 20 min in 4°C. The protein was isolated by SDS-PAGE and transferred onto PVDF membrane, which was further blocked in 5% skimmed milk for 1 h. The membranes were then incubated with primary antibodies rabbit anti-Iba-1 (1:1000; ab178846, Abcam), rabbit anti-HMGB1 (1:2000; ab79823, Abcam), rabbit anti-p-ERK (1:2000; BS4621, Biogot Technology), rabbit anti-ERK (1:4000; BS112, Biogot Technology), rabbit anti-p-p38 (1:1000, 9211, Cell Signaling Technology), rabbit anti-p38 (1:2000, 9212, Cell Signaling Technology), rabbit anti-p-p65 (1:4000; ab31481, Abcam) overnight at 4°C. Internal control was performed using β-actin antibody (1:4000; 4970, Cell Signaling Technology), or Histone H3 antibody (1:500; ab61251, Abcam). The membrane was rinsed with TBST for 5 times, and then incubated with anti-rabbit IgG HRP secondary antibodies. After washing 5 times with TBST, immunoreactivity was detected through enhanced chemiluminescence (Millipore).

**Nuclear and cytosolic extraction**
Nuclear and cytosolic protein was extracted using a Nuclear Extract Kit according to the manufacturer’s instructions (78833, Thermo Fisher Scientific). Nuclear and cytosolic extraction was further analyzed by western blotting.

**Statistical analysis**

The data were reported as the means ± s.e.m. Statistical analysis was performed using GraphPad Prism 8.0 software. Statistical significance was set at $P<0.05$. Comparisons among multiple groups were made with one-way ANOVA (one factor) followed by Turkey’s *post hoc test* or two-way repeated measures ANOVA (two factors) followed by Bonferroni’s *post hoc test*. The sample size was predetermined by analyzing pre-experimental data with PASS (power analysis and sample size) software. For animal studies, the sample size was predetermined by our prior experience.

**Results**

**Hesperidin reverses X-ray-induced cognitive deficits in mice**

It is reported that hesperidin (Fig. 1A), a bioflavonoid extracted from citrus fruits, exerts anti-inflammatory effects. To test whether hesperidin improves radiation-induced cognitive impairment, we performed step-down test and Morris water maze (MWM) test according to schematic diagram (Fig. 1B-H). The escape latency to find the hidden platform was observed to decrease progressively in mice during the training session (Fig. 1C). Compared to mice in Control group, the mice receiving radiation displayed longer escape latency (Fig. 1C, Group, $F_{2,35}=30.64, P<0.001$; Time, $F_{4,140}=53.91, P<0.001$; Group×Time, $F_{8,140}=2.63, P=0.010; ***P<0.001$; Fig. 1D, $F_{2,35}=22.18, ***P<0.001$), less time in the target quadrant (Fig. 1E, $F_{2,35}=14.60, ***P<0.001$) and less target crossing times (Fig. 1F, $F_{2,35}=13.60, ***P<0.001$) in MWM test. Importantly, hesperidin substantially reversed the radiation-induced these behavioral changes (Fig. 1C-F, for C, $$P=0.004$; for D, $$P=0.004$; for E, $^P=0.037$; for F, $^P=0.042$), suggesting that hesperidin improves cognitive function. Moreover, in the step-down test, the mice exposed to radiation showed a significant decrease in the latency to step down from the platform and a notable increase in the number of times stepped down from the platform, compared to the mice in Control group (Fig. 1G-H, for G, $F_{2,29}=15.66, ***P<0.001$; for H, $F_{2,29}=6.48, **P=0.006$). Treatment with hesperidin robustly increased the step-down latency, and markedly decreased the error counts (Fig. 1G-H, for G, $^P=0.037$; for H, $^P=0.029$). Thus, hesperidin obviously improves radiation-induced cognitive impairment.

**Hesperidin attenuates neuronal damage in mice exposed to X-ray**

To explore whether hesperidin exerts neuroprotective effect against radiation damage in hippocampal neurons, we performed Nissl staining analysis and found that the number of Nissl-positive cells in the CA1 region of hippocampus was significantly reduced in mice subjected to X-rays (Fig. 2A-B, $F_{2.9}=24.56, ***P<0.001$), while treatment with hesperidin remarkably increased the number of nissl-positive cells
(Fig. 2A-B, \#P = 0.018), indicating a substantial neuroprotective effect of hesperidin against radiation-related brain injury.

**Hesperidin decreases the levels of pro-inflammatory cytokines in the hippocampus**

Inflammation plays important roles in various physiological and pathological processes. Thus, neuroprotective effect of hesperidin may result from inhibition of inflammation. To test this notion, we detected the levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α using the method of ELISA and found that hesperidin substantially reversed the radiation-induced elevation of inflammatory cytokines (Fig. 2A, C-E, for C, \(F_{2,12} = 14.41, ***P < 0.001, \#P = 0.019\); for D, \(F_{2,12} = 21.90, ***P < 0.001, \#P = 0.027\); for E, \(F_{2,12} = 21.48, ***P < 0.001, ##P = 0.006\)). Together, these results confirm that hesperidin could inhibit X-ray-induced neuroinflammation.

**Hesperidin regulates HMGB1-TLR4 signaling in BV-2 cells**

Given the critical roles of microglia activation in radiation damage [16–17], we detected the protein ionized calcium binding adaptor 1 (Iba-1), a microglia marker, in the hippocampus, and found that X-ray stimulation increased the expression of Iba-1, suggesting microglia activation after radiation (Fig. 3A, B, \(F_{2,12} = 43.57, ***P < 0.001\)). As expected, hesperidin contributed to a drastic reduction in the protein expression of Iba-1 (Fig. 3A, B, ##P = 0.002), which may indicate that hesperidin suppresses microglia activation and microglia-related inflammation.

HMGB1-TLR4 signaling is reported to participate in microglia related neuroinflammation[18]. Accordingly, we test whether hesperidin attenuates the radiation damage via the regulation of HMGB1-TLR4 signaling. Considering the fact that nucleocytoplasmic shuttling of HMGB1 is of great importance in regulating neuroinflammation, we detected the total HMGB1 expression as well as its levels in cytoplasm and nucleus in the hippocampus (Fig. 3A, C-E). The results showed that radiation increased total HMGB1 expression, and hesperidin reversed radiation-induced upregulation of total HMGB1 (Fig. 3C, \(F_{2,12} = 18.97, ***P < 0.001, ##P = 0.002\)). Intriguingly, cytosolic HMGB1 level was higher in Radiation group than that in Control group (Fig. 3D, \(F_{2,12} = 27.31, ***P < 0.001\), while nuclear HMGB1 lower (Fig. 3E, \(F_{2,12} = 13.91, ***P < 0.001\)), implying that radiation induces HMGB1 translocation from nucleus to cytoplasm. Treatment with hesperidin significantly reversed the changes in radiation-induced HMGB1 redistribution between nucleus and cytoplasm (Fig. 3D-E, for D, \#P = 0.011, for E, \#P = 0.012). To further directly observe the phenomenon of nucleocytoplasmic shuttling of HMGB1, the BV-2 cells were exposed to X-ray (6 Gy) after being pre-treated with hesperidin for an hour, and 24 hour later, immunocytochemistry analysis was performed with the antibody against HMGB1. Consistent with the above experiments in vivo, immunocytochemistry analysis revealed that hesperidin relieved the increase in the expression of total HMGB1 caused by radiation and reversed the radiation-induced increase in HMGB1 in the cytosolic fraction and decrease in the nuclear fraction (Fig. 3F). Moreover, immunocytochemistry analysis also showed that radiation elevated the TLR-4 expression, and hesperidin reversed radiation-induced upregulation of TLR-4 (Fig. 3G).
These results demonstrated that HMGB1-TLR4 signaling in BV-2 cells participates in the improvement effect of hesperidin on radiation related cognitive impairment.

HMGB1-TLR4 regulates MAPK and NF-κB signaling in various diseases. To explore the role of MAPK and NF-κB signaling in radiation-induced neuroinflammation, we detected the phosphorylated levels of p38, ERK, p65 in the hippocampus using Western blotting. As shown in Fig. 4, radiation led to a drastic increase in expression of p-ERK, p-p38, p-p65 (for p-ERK, $F_{2,12}=69.04$, ***$P<0.001$; for p-p38, $F_{2,12}=13.13$, ***$P<0.001$; for p-p65, $F_{2,12}=24.81$, ***$P<0.001$). Treatment with hesperidin alleviated the increase in the phosphorylated levels of these proteins (Fig. 4, for p-ERK, ###$P<0.001$; for p-p38, #P = 0.020; for p-p65, ##P = 0.001). These data suggested that hesperidin may improve cognitive impairment induced by x-ray via deactivation of HMGB1-TLR4-MAPK/NF-κB signaling pathway.

**Discussion**

The present study showed the mitigation of hesperidin on radiation-induced cognitive dysfunction via the inhibition of microglia-mediated neuroinflammation in vivo. Neuroinflammation induced by X-ray stimulation has been observed in our work by examining the release of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α. Microglial activation is a key feature of neurological immune responses which may underlie much of the pathology[19]. And the aberrant immune activation is implicated in CNS diseases[20]. Our work showed microglia were activated after radiation, indicative of higher expression of Iba-1 in CA1 region of hippocampus. The phenomena about microglial activation, pro-inflammatory cytokine release and neuronal apoptosis can be regulated by the TLR4/NFκB pathway[21–22]. Reportedly, TLR4 has close relation to MAPK signaling pathway. Available evidence suggested that TLR4 positively regulates ERK signaling activation[23]. There is also a report that the activation of p38 MAPK was completely blunted in TLR4 knocked out mice [24]. Intrathecal injection of TLR4 siRNA alleviated inflammation-induced nociceptive hypersensitivity via p38 MAPK signaling in rats with bone pain[25]. In our study, the increased TLR4 expression was shown in BV-2 cells exposed to X-ray. We also found that radiation elevated the phosphorylated levels of ERK, p38 and p65, suggesting activation of MAPK and NF-κB signaling. NF-κB was a pleiotropic regulator of various genes which involved in neuroinflammation and was mediated by TLR4 and MAPK signaling[26]. Consistently, we also observed that radiation upregulated the levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, probably resulting from enhanced TLR-4 expression and thereby activated MAPK and NF-κB signaling after radiation. Thus, balancing TLR4/MAPK/NF-κB signaling might be a potential therapeutic approach for neuroinflammation-induced cognitive dysfunction.

Hesperidin, a bioavonoid, was extracted from citrus fruits with substantial anti-inflammatory effect. Hesperidin was shown to alleviate inflammatory cell infiltration and cell death in the lung tissues through regulating NF-κB signaling axis [27]. Hesperidin inhibited neuronal apoptosis and cognitive impairments in the sevoflurane anesthetized rat via the PI3/Akt/PTEN and NF-κB signaling pathways [28]. There is also another study that the effects of hesperidin on chronic restraint stress and lipopolysaccharide-induced hippocampus and frontal cortex damage were related to the role of TLR4/NF-κB, p38
MAPK/JNK, and Nrf2/ARE signaling [29]. The studies indicate that hesperidin exerts anti-inflammatory and neuroprotective effects, perhaps by regulating MAPK and NF-κB signaling. Accordingly, in this study, we investigated whether hesperidin could attenuate radiation-induced cognitive impairments through TLR4/MAPK/NF-κB signaling pathways. As expected, hesperidin significantly reversed radiation-produced upregulations in the levels of TLR4, p-ERK, p-38, and p-p65 and decreased the levels of IL-1β, IL-6 and TNF-α in vivo or in vitro. More importantly, radiation-induced cognitive impairments were largely recused by hesperidin treatment. These results indicated that hesperidin could mitigate neuroinflammation-induced brain injury by inhibiting the activation of TLR4/MAPK/ NF-κB signaling pathways.

HMGB1, as a critical ligand of TLR4, activates TLR4 on the target cells during inflammasome activation, and contributes to tissue dysfunction and cell damage upon its extracellular release provoked by acute injuries[22,30]. It is well known that HMGB1 can be released by neurons and glia in the inflammatory response and the release of HMGB1 contributes to physiological functions such as neuritic outgrowth and cell migration during normal brain development. In our work, the role of HMGB1 in the radiation-associated neuroinflammation and cognitive deficits was investigated in the rodent radiation brain injury model. In X-ray-induced brain injury, HMGB1 expression in hippocampus of the mice was elevated, which could be significantly decreased by hesperidin. In response to radiation, HMGB1 can translocate from nucleus to cytoplasm, and then be released out of the cells to act as an early initiator of inflammation. The results obtained from both mice and BV-2 cells showed that the total and cytosolic HMGB1 protein levels were increased, while the nuclear HMGB1 protein level was decreased under condition of X-ray stimuli. Treatment with hesperidin significantly reversed these changes in hippocampus of mice and BV-2 cells, as well as recovered behavioral changes. These results were supported by the previous study that inhibiting the translocation of HMGB1 from nucleus to cytoplasm improved learning and memory capabilities and protected against neuroinflammation[31]. Thus, hesperidin regulated nucleocytoplasmic shuttling of HMGB1.

Conclusion

In summary, we found that hesperidin alleviated radiation-induced cognitive dysfunction. The underlying mechanism is associated with neuroinflammation induced by activation of HMGB1/TLR4 signaling pathway in microglia. Radiation induced translocation of HMGB1 from nucleus to cytoplasm and aggravated the extracellular secretion of HMGB1. Hesperidin had great therapeutic efficacy by inhibiting radiation-induced translocation of HMGB1, reducing neuroinflammation, and thus improved cognitive function. These findings indicates that hesperidin could serve as a candidate for treatment of radiation-induced cognitive impairments.

Abbreviations

HMGB1: High mobility group box1; IR: ionizing radiation; CNS: the central nervous system; BBB: blood brain barrier; RBI: radiation-induced brain injury; TLR4: toll-like receptor 4; AD: Alzheimer's Disease; iNOS:
Inducible Nitric Oxide Synthase; TNF-α: tumor necrosis factor α; LPS: lipopolysaccharide; FBS: foetal bovine serum; PFA: Paraformaldehyde; PBS: Phosphate Buffer Saline; BSA: Bull Serum Albumin; DAPI: 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride; IL-1β: interleukin 1β; IL-6: interleukin 6; ELISA: enzyme-linked immunosorbent assay; HEPES: N’-a-hydroxyethylpiperazine-N’-ethanesulfonic acid; EDTA: Ethylene Diamine Tetraacetic Acid; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF: Polyvinylidene Fluoride; TBST: Tris Buffered Saline Tween; IgG: immunoglobulin G; HRP: Horseradish peroxidase; MWM: Morris water maze; Iba-1: ionized calcium binding adaptor 1; MAPK: Mitogen-activated protein kinase.

Declarations

Funding

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Author contributions

WH and LX were involved in drafting the manuscript and revising it critically. They were also involved in substantial contributions to conception and study design. WH, LX and JM carried out the experimental work, conducted the investigation and visualization. HL and LC helped with analysis and interpretation of data. DY and CY were involved in software, supervision and funding acquisition. All authors have read and approved the final manuscript.

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Conflict of interests
The authors declare that there is no conflict of interest.

Data availability

The data presented in this study are available on request from the corresponding authors.

References


Figures
Figure 1

Effects of hesperidin on X-ray-induced cognitive impairment. (A) Chemical structure of hesperidin. (B) Experimental design. Mice were pretreated with hesperidin (100 mg/kg, i.p.) 2 hours before X-ray stimulation (40 Gy), and then hesperidin was intraperitoneally administrated daily to the mice for 13 consecutive days. Step-down test and Morris water maze (MWM) test were tested at days 14-15 and days 16-21 after X-ray stimulation, respectively. (C) The latency of mice finding a hidden platform over 5
consecutive training days in MWM test. ***$P < 0.001$, ##$P = 0.004$. (D) The latency of mice finding a hidden platform on the 5th day of the spatial acquisition session in MWM test. ***$P < 0.001$, #$P = 0.037$. 

(E) The percentage of time spent in the target quadrant during the probe trial. ***$P < 0.001$, #$P = 0.037$. 

(F) The average crossing number over the platform-site during the probe trial. ***$P < 0.001$, #$P = 0.042$. 

(G) The step-down latency of mice during the test session. ***$P < 0.001$, #$P = 0.037$. 

(H) The error counts of mice during the test session in step-down test. ***$P < 0.001$, #$P = 0.029$. In C-F, n = 12, 13, 13 for Control, Radiation and Radiation+Hes, respectively. In G-H, n = 10, 11, 11 for Control, Radiation and Radiation+Hes, respectively. In C, two-way repeated measures ANOVA followed by Bonferroni’s post hoc test. In D-H, one-way ANOVA followed by Turkey’s post hoc test.
Neuroprotective and anti-inflammatory effects of hesperidin against radiation-induced brain injury. (A) Experimental design. Mice were pretreated with hesperidin (100 mg/kg, i.p.) 2 hours before X-ray stimulation (40 Gy), and then hesperidin was intraperitoneally administrated daily to the mice for 13 consecutive days. Nissl staining and ELISA for detecting the concentrations of IL-1β, IL-6 and TNF-α were performed at day 14 after X-ray stimulation. (B) Representative images of Nissl-stained brain slices. Bar
graph showing the number of Nissl-stained neurons in CA1 region of the hippocampus. n = 4. ***P < 0.001, #P = 0.018. Scale bar, 50 μm. (C) Bar graph showing the contents of IL-1β in the hippocampus. n = 5. ***P < 0.001, #P = 0.019. (D) Bar graph showing the contents of IL-6 in the hippocampus. n = 5. ***P < 0.001, #P = 0.027. (E) Bar graph showing the contents of TNF-α in the hippocampus. n = 5. ***P < 0.001, ##P = 0.006. In B-E, one-way ANOVA followed by Turkey’s post hoc test.
Hesperidin regulated HMGB1-TLR4 signaling pathway in BV-2 cells. (A) Experimental design for B-E. Mice were pretreated with hesperidin (100 mg/kg, i.p.) 2 hours before X-ray stimulation (40 Gy), and then hesperidin was intraperitoneally administrated daily to the mice for 13 consecutive days. Western blotting for Iba-1, total HMGB1, cytosolic and nuclear HMGB1 was performed at day 14 after X-ray stimulation. (B) Immunoblots (upper) and bar graph (lower) showing the expression of Iba-1 in the hippocampus. n = 5, ***P < 0.001, ##P = 0.002. (C) Immunoblots (upper) and bar graph (lower) showing the expression of total HMGB1 in the hippocampus. n = 5, ***P < 0.001, #P = 0.011. (D) Immunoblots (upper) and bar graph (lower) showing the expression of cytosolic HMGB1 in the hippocampus. n = 5, ***P < 0.001, #P = 0.012. (E) Immunoblots (upper) and bar graph (lower) showing the expression of nuclear HMGB1 in the hippocampus. n = 5, ***P < 0.001, #P = 0.012. (F) Representative images showing HMGB1 expression in BV 2 cells. n = 3. (G) Representative images showing TLR-4 expression in BV 2 cells. n = 3. In B-E, one-way ANOVA followed by Turkey’s post hoc test.

Figure 4

Hesperidin regulated MAPK and NF-κB signaling in the hippocampus. (A) Experimental design for F. Mice were pretreated with hesperidin (100 mg/kg, i.p.) 2 hours before X-ray stimulation (40 Gy), and then hesperidin was intraperitoneally administrated daily to the mice for 13 consecutive days. Western blotting for p-ERK, p-p38 and p-p65 was performed at day 14 after X-ray stimulation. (B) Immunoblots showing the levels of p-ERK, p-p38 and p-p65 in the hippocampus. (C) Bar graph showing the levels of p-ERK in the hippocampus. n = 5, ***P < 0.001, ###P < 0.001. (D) Bar graph showing the levels of p-p38 in the hippocampus. n = 5, ***P < 0.001, #P = 0.020. (E) Bar graph showing the levels of p-p65 in the hippocampus. n = 5, ***P < 0.001, ##P = 0.001.
Figure 5

A model showing radiation induced HMGB1 cytoplasmic translocation activated HMGB1-TLR4-MAPK/NF-κB signaling, and thereby produced cognitive impairments, while hesperidin reversed these changes and improved radiation-induced cognitive dysfunction.