


# Preliminary study on the anti-apoptotic mechanism of Astragaloside IV on radiation-induced brain cells

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## Abstract

With multiple targets and low cytotoxicity, natural medicines can be used as potential neuroprotective agents. The increase in oxidative stress levels and inflammatory responses in the brain caused by radiation affects cognitive function and neuronal structure, and ultimately leads to abnormal changes in neurogenesis, differentiation, and apoptosis. Astragaloside IV (AS-IV), one of the main active constituents of astragalus, is known for its antioxidant, antihypertensive, antidiabetic, anti-infarction, anti-inflammatory, anti-apoptotic and wound healing, angiogenesis, and other protective effects. In this study, the mechanism of AS-IV against radiation-induced apoptosis of brain cells in vitro and in vivo was explored by radiation modeling, which provided a theoretical basis for the development of anti-radiation Chinese herbal active molecules and brain health products. In order to study the protective mechanism of AS-IV on radiation-induced brain cell apoptosis in mice, the paper constructed a radiation-induced brain cell apoptosis model, using TUNEL staining, flow cytometry, Western blotting to analyze AS-IV resistance mechanism to radiation-induced brain cell apoptosis. The results of TUNEL staining and flow cytometry showed that the apoptosis rate of radiation group was significantly increased. The results of Western blotting indicated that the expression levels of p-JNK, p-p38, p53, Caspase-9 and Caspase-3 protein, and the ratio of Bax to Bcl-2 in radiation group were significantly increased. There was no significant difference in the expression levels of JNK and p38. After AS-IV treatment, the apoptosis was reduced and the expression of apoptosis related proteins was changed. These data suggested that AS-IV can effectively reduce radiation-induced apoptosis of brain cells, and its mechanism may be related to the phosphorylation regulation of JNK-p38.

## Keywords

apoptosis, Astragaloside IV, brain, JNK-p38, radiation

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## Introduction

With the development of society, people are becoming more and more exposed to radiation due to their treatment, diagnosis, occupation, and accidents. Therefore, the study of the effects of radiation on health has become a very important research field. Radiation causes a variety of damage to human bodies, including immune function decline, cognitive dysfunction, malignant tumors, hematopoietic dysfunction (such as anemia, leukemia, and blood abnormalities), skin ulcers,

organ fibrosis, and lens opacity.<sup>1</sup> In addition, radiation also causes multiple damages to living cells, including the loss of genetic information, mutation,

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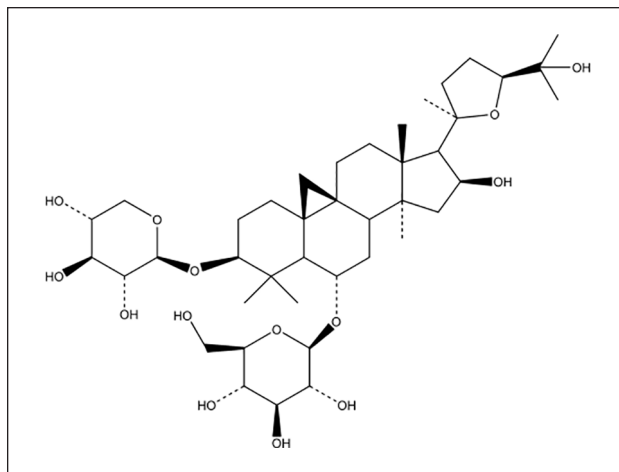
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increase genomic instability, and apoptosis. However, the anti-radiation agents currently used in clinical practice have relatively severe toxin and side effects, which limits their application in the clinical treatment of radioactive diseases. Therefore, it has become very urgent to develop a drug that can prevent radiation-induced brain damage with fewer side effects.

Natural drugs are extensively studied as potential neuroprotective agents because of their multi-target and low toxicity characteristics. Saponins, among them, are widely used due to their extensive biological activities, including anti-inflammatory, antibacterial, anti-oxidant, anti-tumor, and neuroprotective effects. Astragaloside IV (AS-IV, 3-O-b-D-xylopyranosyl-6-Ob-D-glucopyranosylcycloastragenol, Figure 1<sup>2</sup>), a cycloaltriterpenoid saponin, is one of the main active ingredients of *Astragalus*. Known for its protective effects, AS-IV is resistant to oxidation, antihypertensive, antidiabetic, anti-infarction, anti-inflammatory, anti-apoptosis, promotes wound healing and angiogenesis.<sup>3</sup> A large number of literatures show that the anti-apoptotic effect of AS-IV contributes to the improvement of various central nervous system diseases.<sup>3-8</sup> For example, AS-IV can protect primary cerebral cortex neurons exposed by oxygen and glucose deprivation by regulating the PKA/CREB signal pathway and retaining mitochondrial function. After treatment with AS-IV, the mitochondria and cell damage induced by oxygen and glucose deprivation is reversed, AS-IV significantly enhances the phosphorylation of PKA and cAMP-response element binding protein (CREB) and prevents mitochondrial dysfunction induced by oxygen and glucose deprivation, thereby protecting neurons exposed to oxygen and glucose deprivation from damage and death.<sup>9</sup> AS-IV significantly improved MPTP-induced decrease in primary astrocyte viability, increased apoptosis rate, up-regulation of p-JNK, Bax/Bcl-2 ratio and Caspase-3 activity. AS-IV inhibits Caspase-3 by inhibiting pro-apoptotic p53-mediated Bax activation and anti-apoptotic Bcl-2 activation, thereby improving early brain damage in experimental subarachnoid hemorrhage.<sup>10</sup> Besides, AS-IV can improve the hypoxia-induced damage of PC12 cells by reducing the expression of miR-124.<sup>11</sup> And AS-IV can inhibit the H<sub>2</sub>O<sub>2</sub>-induced decrease in the mitochondrial membrane potential of retinal ganglion cells, reduce the release of Cyt



**Figure 1.** Chemical structure of Astragaloside IV.

c, inhibit the expression of Bax and Caspase-3, and increase the expression of Bcl-2.<sup>12</sup> However, there are few reports on the protective mechanism of AS-IV on radiation-induced neuronal apoptosis. Therefore, this study used radiation-induced Kunming mice and PC12 cells as experimental subjects, and used AS-IV intervention, TUNEL staining, flow cytometry and western blotting to explore the mechanism of AS-IV against radiation-induced apoptosis of brain cells, which provided basic materials for innovative development of anti-radiation active molecules of traditional Chinese medicine and brain health products.

## Materials and methods

### Animals

One hundred 30-day-old male Kunming mice (20–25 g) were purchased from the Medical and Laboratory Animal Center of Lanzhou University, and randomly divided into five groups, with 20 in each: blank control group, solvent group (DMSO), DMSO (Solarbio, Beijing, China) + radiation group (DMSO + R), low concentration AS-IV (Dalian Meilun Biotechnology Co. LTD, Dalian, China,  $\geq 98\%$ , BR, CAS NO. MB1955) + radiation group (AS-IV-L + R), high concentration AS-IV + radiation group (AS-IV-H + R). The test was administered by intraperitoneal injection once a day. The control group was given normal saline, both the DMSO group and the DMSO + R group were given DMSO with the final concentration less than 0.01%. The doses of AS-IV-L + R and AS-IV-H + R groups were 20 mg/kg and 40 mg/

kg, respectively. After 1 month of administration,  $^{60}\text{Co}$  radiation (Lanzhou Weite radiation Co., LTD, Lanzhou, China) was used in vivo, and the cumulative radiation dose was 8 Gy.<sup>13–15</sup>

### Cell culture

PC12 cells were derived from Lanzhou University School of Basic Medicine and cultured in a humidified incubator containing 5%  $\text{CO}_2$  in high glucose medium (Cell Max, Hyclone, Logan City, UT, USA). The high glucose medium contained 5% fetal bovine serum (Gibco, Grand Island, NY, USA), 10% horse serum (Gibco), and 100 U/mL of penicillin with 100 mg/mL of streptomycin (Hyclone). PC12 cells in logarithmic growth phase were collected and divided into five groups: control group (Control), solvent group (DMSO), DMSO + Radiation group (DMSO + R), low concentration AS-IV + radiation group (AS-IV-L + R), high concentration AS-IV + radiation group (AS-IV-H + R). No treatment was performed in the Control group, DMSO was administered to both the DMSO group and the DMSO + R group, with the final concentration less than 0.01%. The doses of AS-IV-L + R group and AS-IV-H + R group were 25  $\mu\text{g}/\text{mL}$  and 50  $\mu\text{g}/\text{mL}$ ,<sup>16–19</sup> respectively. After attaching to 70%–80% of the wall, the cells were irradiated at a vertical distance of 15 cm from the UVA light as radiation source (the cell radiation dose was about 6.5  $\text{J}/\text{cm}^2$ ), the irradiation time was 45 min, then treated with the drugs.<sup>13,20–22</sup>

### TUNEL staining

The tissue sections were dehydrated gradiently, and after adding the proteinase K diluted with PBS (Solarbio, Beijing, China) dropwise to the sections, the tissues were covered and digested at 37°C for 10 min. Wash PBS three times for 5 min each time. The sections covered with tissue, onto which the labeling buffer added with TdT and DIG-d-UTP and mixed were dropped, were placed in a wet box, labeled and set at 37°C for 2 h. Wash PBS three times for 5 min each time. Adding blocking solution and setting at room temperature for 30 min, the sections were mashed off but it not washed. Add digoxin antibody diluted with antibody dilution dropwise to the sections until the tissues were covered, so they can be left overnight at 4°C. Wash

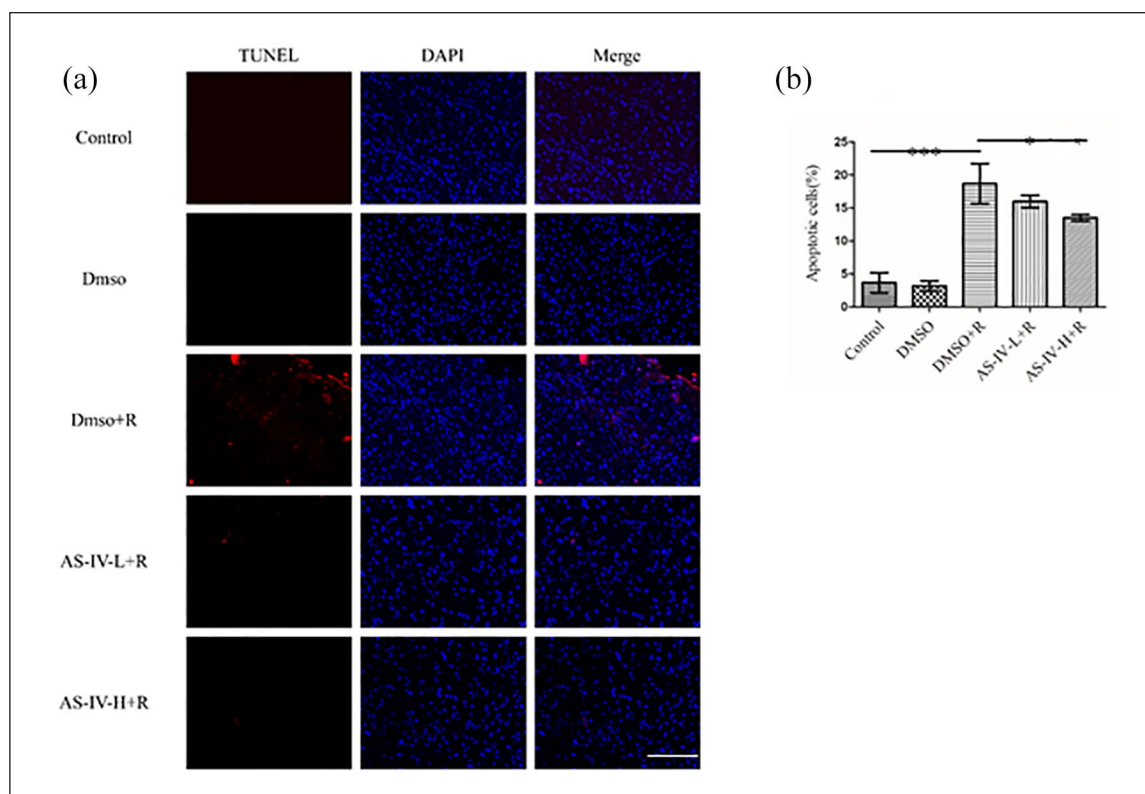
PBS three times for 5 min each time. Add SABC diluted with antibody dilution dropwise to the sections until the tissues were covered so they can be and incubated at room temperature for 2 h in the dark. Wash PBS three times for 5 min each time. Add DAPI staining solution dropwise to the sections for counterstaining and set at room temperature for 10 min. Wash PBS three times for 5 min each time. The plate was mounted with an anti-fluorescence quenching tablet and observed under a fluorescence microscope (Olympus BX53, Tokyo, Japan).

### Flow cytometry

PC12 cells were seeded at  $2 \times 10^5$  cells/well in a 6-well plate. After adhered, the cells were administered in groups and irradiated according to the above method. After the irradiation, the culture was continued for 24 h at 37°C in an incubator with 5%  $\text{CO}_2$ . Each group of cells was collected in a centrifuge tube, centrifuged at 3000 rpm (960 g) for 5 min, washed twice with PBS, and the supernatant of which was removed. Dealt with gentle actions to avoid damage, the cells, were then resuspended in PBS, counted about  $1 \times 10^5$ , stained by Annexin V-FITC/PI apoptosis detection kit, incubated at room temperature for 10 min in the dark, and then immediately tested on the flow cytometer (BD Biosciences, New York, USA).

### Western blotting

About 100 mg of brain tissue was placed in a homogenizer, to which a cell lysate containing phenylmethylsulfonyl fluoride (PMSF) (generally 990  $\mu\text{L}$  RIPA plus 10  $\mu\text{L}$  PMSF) was added to the homogenizer, and the tissue samples were placed on ice for rapid homogenization. After ultrasonic crushing, centrifugation was performed at 12,000 rpm for 15 min. Supernatant was extracted and the sample was mixed with 5 $\times$  protein loading buffer at a ratio of 4:1. Protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Boston, MA, USA). The PVDF membranes were incubated at 4°C overnight with the antibodies. After washing and incubating with an HRP-conjugated secondary antibody (Bioss, Beijing, China), the membrane was visualized on hypersensitive chemical fluorescence luminometer by using an ECL reagent.



**Figure 2.** TUNEL staining showed the effect of AS-IV on apoptosis of brain cells in mice induced by radiation. (a) TUNEL staining maps of mice brain sections (scale 50  $\mu$ m). (b) Statistical results of apoptosis rate of mice brain cells.  $n=3$ , \*\*\* $P < 0.001$ , \* $P < 0.05$ .

### Statistical analysis

Protein data were analyzed by EvolutionCapt for gray value of the bands. GraphPad Prism 5.0 (GraphPad software, San Diego, CA, USA) was used for mapping, using one-way analysis of variance. All data were subjected to standard error and significance analysis. Data were analyzed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Measurement data were expressed by mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), analyzed by paired  $t$ -test. Counting data was expressed as percentage (%), analyzed by paired  $\chi^2$ -test.  $P < 0.05$  indicated a statistically significant difference. The data are the means of three independent experiments.

## Results

### AS-IV attenuated radiation-induced apoptosis of brain cells of mice

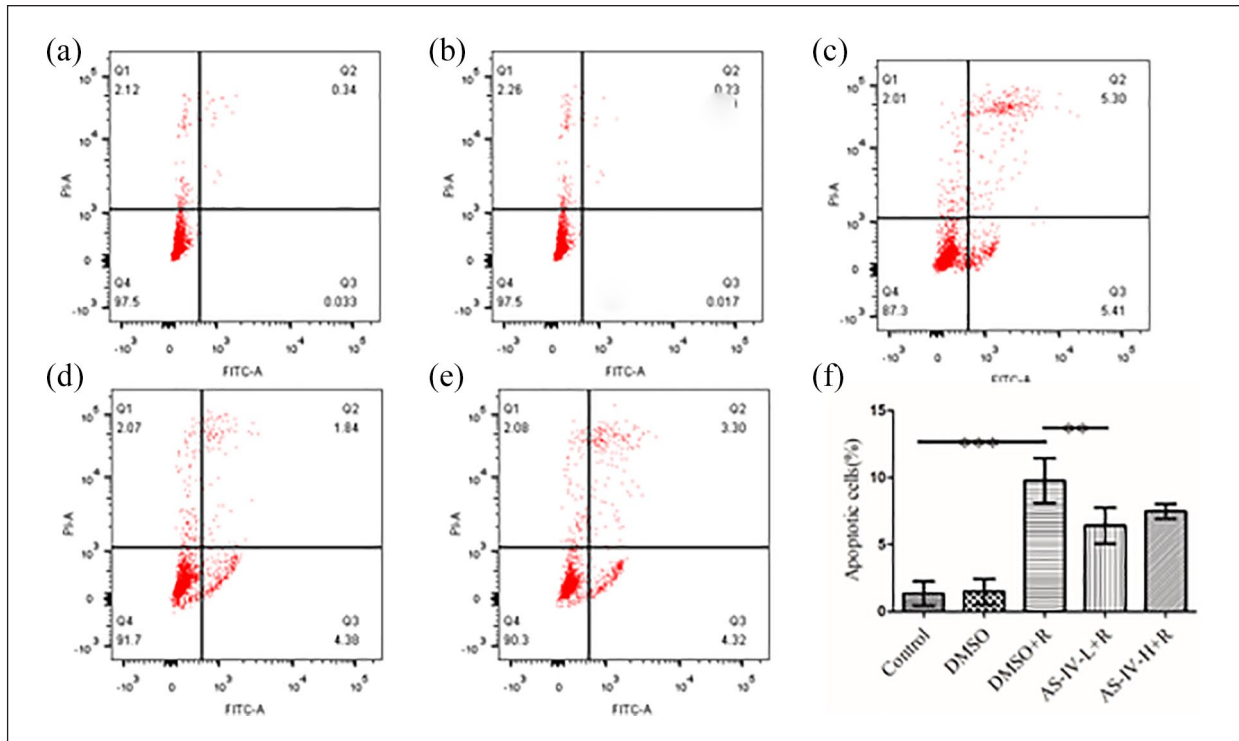
The results of TUNEL staining are shown in Figure 2(a). There were almost no TUNEL-positive cells in the Control group. In the DMSO

+ R group, a large number of TUNEL-positive cells appeared, while in the AS-IV-H + R group, TUNEL-positive cells were decreased. As shown in Figure 2(b), by counting the apoptotic rate, the apoptosis rate in the DMSO + R group was significantly higher than that in the Control group ( $P < 0.001$ ). Compared with the DMSO + R group, the AS-IV-H + R group ( $P < 0.05$ ) showed a significant decrease of the mortality rate.

### AS-IV attenuated radiation-induced apoptosis of PC12 cells

To investigate the effect of AS-IV on apoptosis of PC12 cells induced by radiation, the handle cells were subjected to flow cytometry for apoptosis detection of Annexin V-FITC/PI cells. As shown in Figure 3(c), showed that compared with the Control group, the DMSO + R group ( $P < 0.001$ ) presented a significantly higher apoptosis rate. As shown in Figure 3(d), compared with the DMSO + R group, the AS-IV-L + R group ( $P < 0.01$ ) had a decrease apoptosis rate.





**Figure 3.** Effect of AS-IV on radiation-induced apoptosis in PC12 cells. (a–e) Scatter plots of PC12 cells apoptosis in Control group, DMSO group, DMSO + R group, AS-IV-L + R group and AS-IV-H + R group. (f) Statistical results of apoptosis in each group of PC12 cells.  $n > 3$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ .

#### Effect of AS-IV on expression of apoptosis-related proteins in brain cells of mice induced by radiation

As shown in Figure 4(a), the specific conditions of AS-IV for apoptosis-induced protein expression in brain cells of mice are as follows.

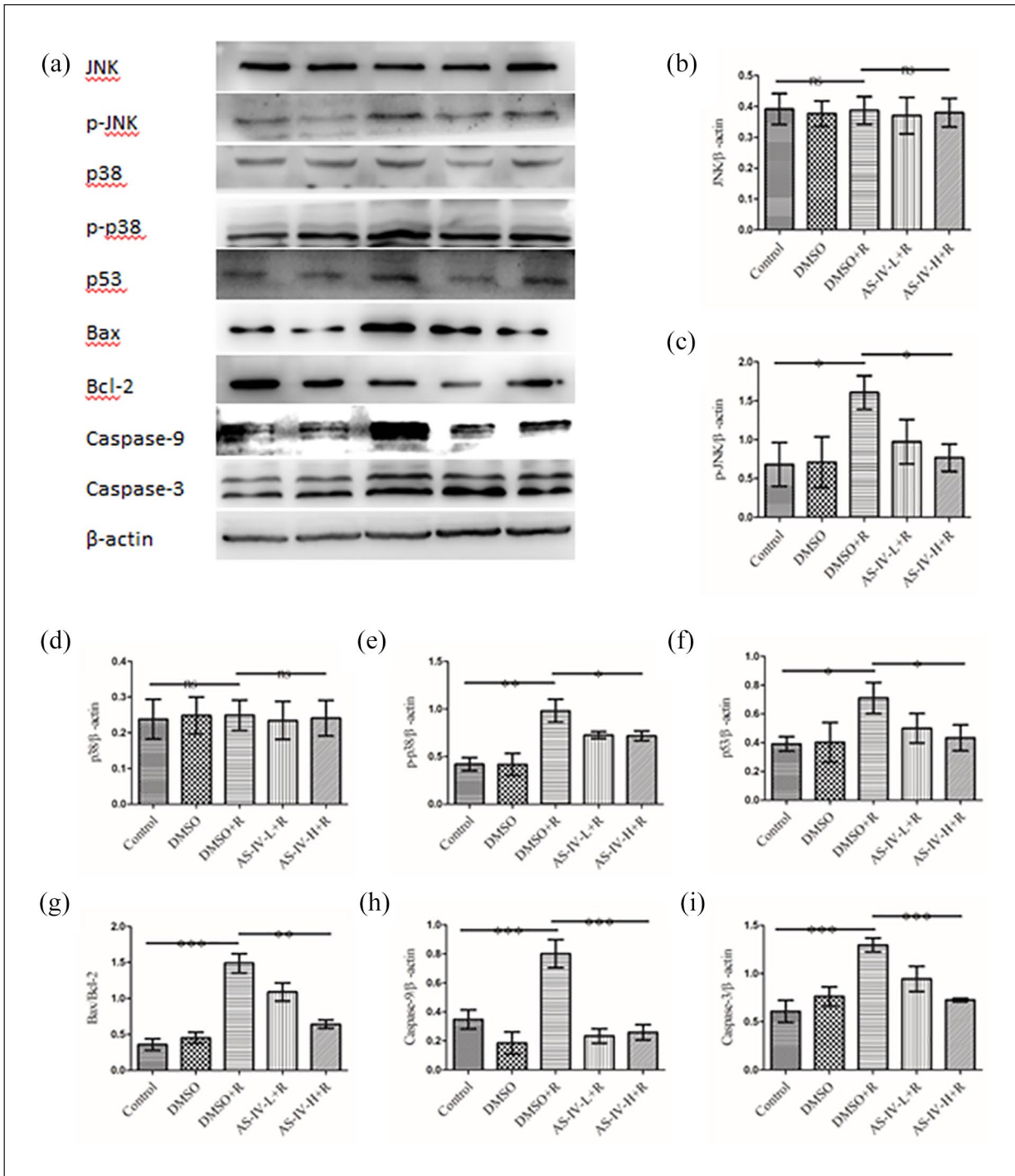
Compared with the Control group, expression of phosphorylated JNK and p38 in the DMSO + R group showed a significant upward trend (Figure 4(c),  $P < 0.05$ , Figure 4(e),  $P < 0.01$ ). Similarly, expression of p53, Caspase-9, Caspase-3, and the ratio of Bax and Bcl-2 both showed a significant upward trend (Figure 4(f),  $P < 0.05$ , Figure 4(h),  $P < 0.001$ , Figure 4(i),  $P < 0.001$ , Figure 4(g),  $P < 0.001$ ). However, there was no significant difference in protein expression levels between JNK and p38 (Figure 4(b),  $P > 0.05$ , Figure 4(d),  $P > 0.05$ ). Compared with the DMSO + R group, expression of phosphorylated JNK and p38 in the AS-IV-H + R group showed a significant downward trend (Figure 4(c),  $P < 0.05$ , Figure 4(e),  $P < 0.05$ ). Expression of p53, Caspase-9, Caspase-3, and the ratio of Bax and Bcl-2 showed a significant downward trend (Figure 4(f),  $P < 0.05$ ,

Figure 4(h),  $P < 0.001$ , Figure 4(i),  $P < 0.001$ , Figure 4(g),  $P < 0.01$ ). There was no significant difference in protein expression levels of JNK and p38 (Figure 4(b),  $P > 0.05$ , Figure 4(d),  $P > 0.05$ ).

#### Effect of AS-IV on expression of apoptosis-related proteins in PC12 cells induced by radiation

The results of the PC12 cells were shown in Figure 5(a). The specific conditions of AS-IV for apoptosis-induced protein expression in PC12 cells are as follows.

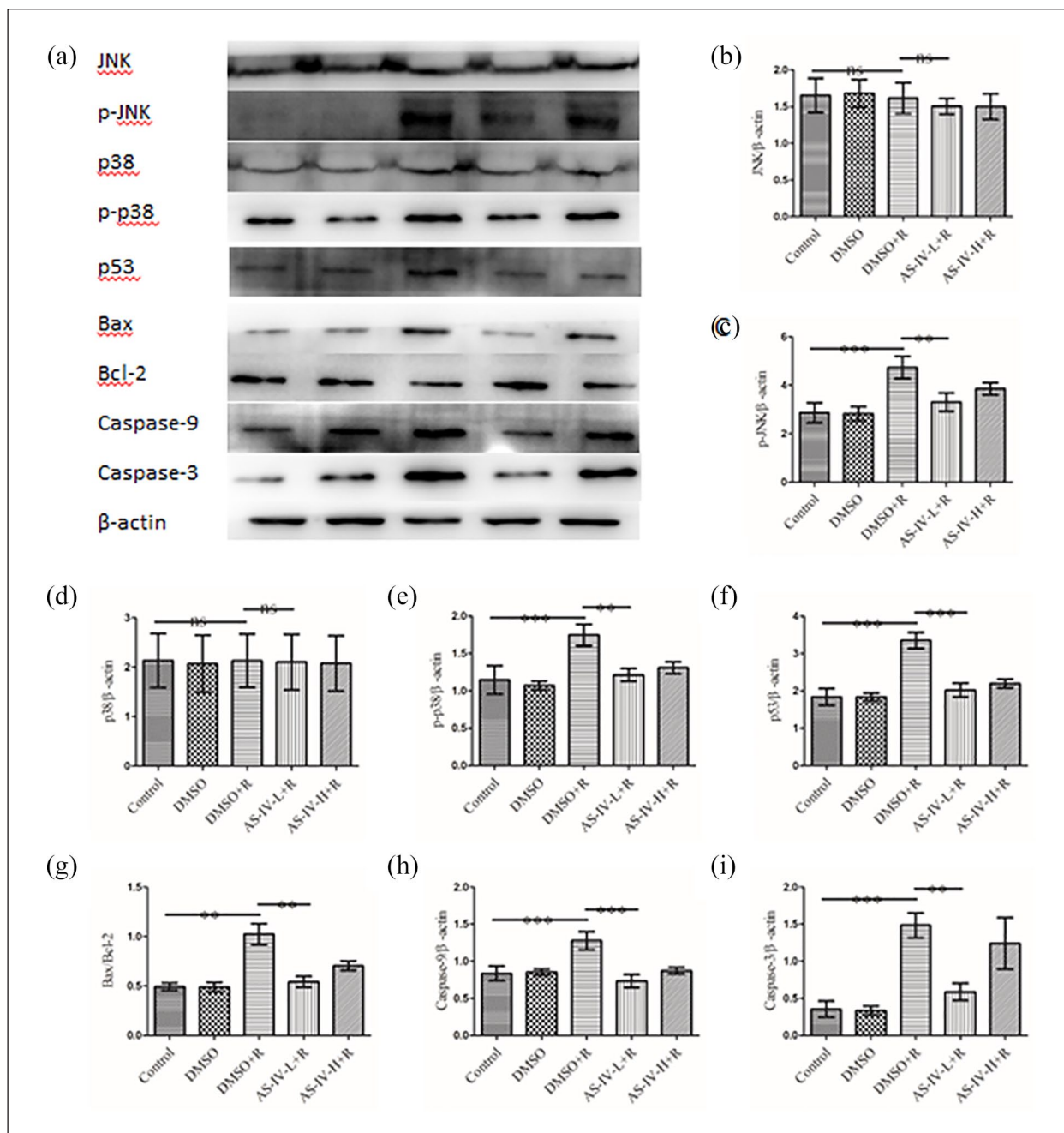
Compared with the Control group, expression of phosphorylated JNK and p38 showed a significant upward trend in the DMSO + R group (Figure 5(c),  $P < 0.001$ , Figure 5(e),  $P < 0.001$ ). Similarly, expression of p53, Caspase-9 and Caspase-3, as well as the ratio of Bax and Bcl-2 proteins both showed a significant upward trend (Figure 5(f),  $P < 0.001$ , Figure 5(h),  $P < 0.001$ , Figure 5(i),  $P < 0.001$ , Figure 5(g),  $P < 0.01$ ). However, there was no significant difference in protein expression levels between JNK and p38 (Figure 5(b),  $P > 0.05$ , Figure 5(d),  $P > 0.05$ ). Compared with the DMSO + R group, expression of



**Figure 4.** Effect of AS-IV on radiation-induced apoptosis-related protein expression and phosphorylation in mice brain cells. (a) Western blotting assay for apoptosis-related proteins and their phosphorylation levels. (b-i) Statistical plots of relative gray values of JNK, p-JNK, p38, p-p38, p53, Bax/Bcl-2, Caspase-9 and Caspase-3 proteins in animal model, respectively.  $n > 3$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .

phosphorylated JNK and p38 in the AS-IV-L + R group showed a significant downward trend (Figure 5(c),  $P < 0.01$ , Figure 5(e),  $P < 0.01$ ). Expression of p53, Caspase-9 and Caspase-3, and

the ratio of Bax and Bcl-2 showed a significant downward trend (Figure 5(f),  $P < 0.001$ , Figure 5(h),  $P < 0.001$ , Figure 5(i),  $P < 0.01$ , Figure 5(g),  $P < 0.01$ ). There was no significant difference in



**Figure 5.** Effect of AS-IV on apoptosis-related protein expression and phosphorylation in PC12 cells induced by radiation. (a) Western blotting assay for apoptosis-related proteins and their phosphorylation levels. (b–i) Statistical plots of relative gray values of JNK, p-JNK, p38, p-p38, p53, Bax/Bcl-2, Caspase-9 and Caspase-3 proteins in PC12 cells, respectively.  $n > 3$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ .

protein expression levels of JNK and p38 (Figure 5(b),  $P > 0.05$ , Figure 5(d),  $P > 0.05$ ).

## Discussion

With the rapid increase of the global nuclear power plant construction, the use of radiation therapy in

clinical medicine and diagnostic radiology equipment, food sterilization, agricultural research breeding and many other fields, the improper application of which can lead to tissue or organ damage. Therefore, the study of the effect of radiation exposure on our health has become a very important area of research. The central nervous

system is particularly sensitive to radiation that a single large dose of radiation (10 Gy) may significantly impair brain cell in the nervous system.<sup>23</sup> When exposed to radiation, proliferating nerve cells are highly sensitive to ionizing radiation that may lead to mitosis termination, interruption of the update, and further damage of the nervous system. Radiation can directly induce chromosomal aberrations, DNA base damage, thereby blocking DNA replication. In addition, it can also trigger the production of oxidative metabolites, ionizations, free radicals and ROS reactions of water molecules, and damage the structure and function of DNA, lipids and proteins, leading to metabolic and functional changes and ultimately cell apoptosis and affecting neurogenesis and causing cognitive impairment.<sup>24</sup> Therefore, radiation protection has gradually become a hot spot in scientific research. Although the existing radiation protection agents have good radiation protection effects, they also have severe toxin and side effects, that's why it is very important to develop natural drugs with anti-radiation effects and explore their mechanism.

Astragalus, one of the most commonly used Chinese herbs, is derived from the roots of *astragalus membranaceus*. According to Chinese herbs, astragalus can be used as an immune enhancer, liver protection agent, antiperspirant, diuretic and tonic.<sup>25</sup> AS-IV, a kind of cyclopentane triterpenoid saponin, is one of the main active constituents of *Astragalus*. It is known for its antioxidant, anti-inflammatory, anti-apoptotic, wound healing, vascular regeneration, and other protective effects.<sup>26</sup> However, few studies have been reported on the anti-radiation effects of AS-IV on brain cells. In this study, we used AS-IV to intervene in mice and cells, and then irradiate. Through constructing a model of brain cell apoptosis induced by radiation in vivo and in vitro, the AS-IV resistance mechanism of apoptosis induced by radiation was explored.

The mice were intraperitoneally administered daily, after 30 days, 8 Gy <sup>60</sup>Co gamma rays were subjected to uniform radiation whole body at one time. It has been reported that radiation can directly damage neuronal progenitor cells, reduce their proliferative capacity and induce apoptosis.<sup>27</sup> Stem cells and neuronal precursor cells that are proliferating are very sensitive to radiation therapy. Ionizing radiation can induce apoptosis, and cause apoptosis as low as 0.25 Gy.<sup>28</sup> In vivo experiments,

TUNEL immunofluorescence staining showed that compared with that of the Control group, the apoptosis rate was significantly increased in the DMSO + R group, indicating that the radiation induced apoptosis of brain cells of mice. Compared with the DMSO + R group, the apoptosis rate of the AS-IV pretreatment re-radiation group at a concentration of 40 mg/kg was significantly decreased, suggesting that AS-IV at a concentration of 40 mg/kg can effectively inhibit radiation-induced brain cell apoptosis. This is consistent with the result that AS-IV can effectively inhibit the apoptosis of hematopoietic cells induced by radiation.<sup>29</sup>

The clonal line, PC12 originally derived from a solid rat adrenal medulla tumor, has been widely used as a dopaminergic neuronal model for in vitro studies of neuronal cell differentiation.<sup>30</sup> When exposed to nerve growth factor (NGF), PC12 cells stop dividing, neural network began and cells became electrically excited to obtain the characteristics of adrenergic neuron phenotype. Therefore, they are similar to mature sympathetic neurons.<sup>31</sup> PC12 cells serve as a principal dopaminergic model in molecular neuroscience for investigating NGF mechanisms of action under normal or after various insults.<sup>32</sup> In addition, the ability to grow PC12 cells in continuous culture with a well-defined secretory cell phenotype has been advantageous for studying secretory pathway mechanisms.<sup>33</sup> In addition to revealing the differentiation into neuronal phenotypes, PC12 cells are also excellent in vitro tools for studying some aspects of various neurological diseases (e.g. glutamate excitotoxicity,<sup>34</sup> Parkinson's disease,<sup>35</sup> Alzheimer's disease,<sup>36</sup> and epilepsy<sup>37</sup>). And effect of oxidative stress-related result on neuronal cell survival.<sup>38</sup> Studies using PC12 cells have also solved some problems, such as the impact of serum starvation,<sup>39</sup> NGF deprivation<sup>40</sup> and drug cytotoxicity.<sup>41</sup> So the test in vitro was performed on the neuron-like PC12 cells. After attaching to 70%–80% of the wall, and AS-IV was added, the cells were then subjected to UVA radiation at a dose of 6.5 J/cm<sup>2</sup> for 45 min. In the early stage of apoptosis, phosphatidylserine is evanescent from the inside of the cell membrane and used as a marker. Flow cytometry combined with FITC-Annexin V/PI fluorescence staining was used to detect radiation-induced PC12 cells. The results showed that compared with that in the Control group, the apoptosis rate was significantly increased in the DMSO + R group, indicating that



radiation induced PC12 cells apoptosis, and the radiation model was successfully established in vitro. Compared with that in the DMSO + R group, the apoptotic rate of the AS-IV pretreatment re-irradiation group at 25  $\mu\text{g}/\text{mL}$  was significantly decreased, suggesting that AS-IV at concentration of 25  $\mu\text{g}/\text{mL}$  can effectively inhibit radiation-induced brain cell apoptosis. This is consistent with the results in vivo. In vitro and in vivo radiation models showed that AS-IV can effectively inhibit radiation-induced brain cell apoptosis. Subsequently, we examined the expression of proteins on the apoptosis-related signaling pathway and found that AS-IV at concentration of 40 mg/kg or 25  $\mu\text{g}/\text{mL}$  can effectively inhibit the phosphorylation of JNK and p38, and the content of p53, Caspase-9 and Caspase-3 and Bax/Bcl-2 in brain tissue, and PC12 cells induced by radiation.

MAPK is present in many cells and belongs to the serine/threonine kinase. Many downstream targets of MAPK signaling are involved in neuron development, cell differentiation, cell migration, cancer, cardiovascular dysfunction and inflammation through its role in promoting apoptosis, cell vitality and regulating the functions of various cytokines. MAPK is capable of responding to specific physiological responses caused by a variety of extracellular signals or stimuli, such as radiation, ischemia/reperfusion and inflammation. In mammalian cells, the MAPK family mainly includes three regulatory pathways, namely ERK pathway, JNK pathway and p38 pathway. Studies have found that lipopolysaccharide can induce apoptosis of primary cultured hippocampal neurons through JNK and p38 signaling pathways, and the apoptosis is significantly inhibited by JNK inhibitor SP600125 and p38 inhibitor SB202190. Activated by upstream kinase, p38 plays a signal transduction role by acting on a specific substrate. In this experiment, we first observed the effects of radiation on the phosphorylation levels of JNK and p38 in brain tissue and PC12 cells. The results showed that radiation induced the increase of phosphorylated JNK and p38, indicating that radiation can activate JNK signaling pathway. This result is consistent with the expression of JNK and p38 in UV-induced mouse skin.<sup>42</sup> It has also been found that phosphorylation of JNK and p38 is activated in hematopoietic stem cells after irradiation. The phosphorylation levels of JNK and p38 caused by irradiation were effectively inhibited after using

AS-IV at concentration of 40 mg/kg or 25  $\mu\text{g}/\text{mL}$  in vivo and vitro. Thus, it was concluded that JNK and p38 signaling pathways are involved in the regulation of AS-IV antagonistic of radiation-induced apoptosis in brain tissue and PC12 cells. Study has shown that AS-IV improves apoptosis induced by  $\text{A}\beta_{25-35}$ -induced endoplasmic reticulum stress by inhibiting p38 signaling pathway in PC12 cells.<sup>43</sup> AS-IV also protects LPS-induced endometritis by inhibiting the activation of p38 and JNK signaling pathways in mouse.<sup>44</sup>

JNK and p38 can directly activate pro-apoptotic Bcl-2 protein to enhance mitochondrial apoptosis pathway, but the phosphorylation of p53 by JNK and p38 is the most important factor in ultraviolet-mediated apoptosis, which delays the degradation of p53 by proteasome and increases the half-life of p53. When exposed to radiation damage, p53 is stabilized and activated by phosphorylation at the Ser15 and Ser20 sites to regulate cell cycle checkpoints and DNA repair. Because p53 can transcribe both pro-survival and pro-apoptotic genes, p53 can initially protect cells from DNA damage after irradiation. If the injury is not repaired, p53 will promote apoptosis, thereby activating the JNK and p38 signaling pathways for a long time, and finally activating the apoptosis pathway. In addition, studies had shown that low doses of radiation induced up-regulation of p53 in embryonic rat brain and induced apoptosis through endogenous pathways.<sup>45</sup> Hematopoietic stem cells (HSCs) of p53-deficient mice were less sensitive to radiation than that of wild-type mice, and p53 inhibitors protected from radiation-induced lethality by inhibiting p53-dependent apoptosis.<sup>46</sup> The Bcl-2 gene is one of the most important anti-apoptotic genes that inhibits apoptosis by regulating the function of the mitochondrial membrane.<sup>47</sup> Bax, another member of the Bcl-2 family, has broad amino acid homology with Bcl-2 but functions differently. Bax has an inhibitory effect on apoptosis.<sup>48</sup> Previous reports have shown that the ratio of Bax to Bcl-2 determines, at least to some extent, the sensitivity of cells to death signals.<sup>49</sup> Radiation activates p53, which up-regulates Bax expression, and Bax transfers to the mitochondrial outer membrane, forming a pore across the mitochondrial outer membrane, resulting in a decrease in membrane potential and an outflow of Cyt c and AIF.<sup>45</sup> The change of mitochondrial

membrane permeability leads to the release of Cyt C from mitochondria to cytoplasm and the activation of Caspase-9, which eventually leads to the activation of apoptotic enzyme Caspase-3. Activated Caspase-3 reacts with Caspase-9 proenzyme to form a positive feedback pathway. Caspase-3 is the major hydrolase of the apoptotic process and causes apoptosis by hydrolyzing specific protein substrates.<sup>50</sup> Our results show that radiation can increase the ratio of Bax to Bcl-2 and up-regulate the expression of p53, Caspase-9 and Caspase-3 in mouse brain and PC12 cells, which is consistent with the results of endogenous induction of sperm apoptosis in rats by electromagnetic radiation.<sup>51</sup> However, the treatment of AS-IV at concentration of 40 mg/kg or 25 µg/mL can effectively inhibit the increase of p53 in mouse brain tissue and PC12 cells after radiation. Studies have shown that AS-IV can inhibit the expression of apoptosis-related factors (p53, Bax, Caspase-9 and Caspase-3) in retinal ganglion cells induced by oxygen glucose deprivation.<sup>52</sup> This indicated that AS-IV can effectively inhibit radiation-induced apoptosis of brain cells, thereby further preventing radiation damage. However, the specific anti-apoptotic mechanism of AS-IV remains to be further studied.

## Conclusion

In summary, AS-IV can effectively reduce the apoptosis of brain cells induced by radiation. The mechanism of AS-IV on radiation-induced apoptosis of brain cells may be related to the phosphorylated regulation of JNK-p38.

## Author contributions

X.L. and B.S. designed the study and wrote the manuscript. X.L., Y.D. and B.S. performed the experiments and data analysis. W.C., S.S., L.M. and C.J. participated in the collection of experimental samples. J.W. and S.Z. revised and endorsed the final draft. All authors approved the final manuscript.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethics approval

This study was carried out in accordance with the regulations of Lanzhou University and the ARRIVE guidelines. All experimental procedures and protocols were approved by the Ethics Committee of Lanzhou University.

## Animal welfare

All animal studies were approved by the Lanzhou University Institutional Care and Use of Animals.

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## Data sharing statement

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

## References

1. Kim JS, Yang M, Kim SH, et al. (2013) Neurobiological toxicity of radiation in hippocampal cells. *Histology and Histopathology* 28(3): 301–310.
2. Lia L, Houb X, Xub R, et al. (2017) Research review on the pharmacological effects of astragaloside IV. *Fundamental & Clinical Pharmacology* 31(1): 17–36.
3. Chan WS, Durairajan SSK, Lu JH, et al. (2009) Neuroprotective effects of Astragaloside IV in 6-hydroxydopamine-treated primary nigral cell culture. *Neurochemistry International* 55(6): 414–422.
4. Xi C, Hao W, Chen H, et al. (2018) Astragaloside VI promotes neural stem cell proliferation and enhances neurological function recovery in transient cerebral ischemic injury via activating EGFR/MAPK signaling cascades. *Molecular Neurobiology* 56(4): 3053–3067.
5. Pan YF, Jia XT, Song EF, et al. (2018) Astragaloside IV Protects Against Aβ<sub>1-42</sub>-induced oxidative stress, neuroinflammation and cognitive impairment in rats. *Chinese Medical Sciences Journal* 33(1): 29–37.
6. Chang CP, Liu YF, Lin HJ, et al. (2016) Beneficial effect of astragaloside on Alzheimer's disease condition using cultured primary cortical cells under beta-amyloid exposure. *Molecular Neurobiology* 53(10): 7329–7340.
7. Sun Q, Ning J, Wang W, et al. (2014) Protective effects of astragaloside IV against amyloid beta<sub>1-42</sub> Neurotoxicity by inhibiting the mitochondrial permeability transition pore opening. *Plos One* 9(6): e98866.
8. Xia L, Guo D and Chen B (2017) Neuroprotective effects of astragaloside IV on Parkinson disease

- models of mice and primary astrocytes. *Experimental and Therapeutic Medicine* 14(6): 5569–5575.
9. Xue B, Huang J, Ma B, et al. (2019) Astragaloside IV protects primary cerebral cortical neurons from oxygen and glucose deprivation/reoxygenation by activating the PKA/CREB pathway. *Neuroscience* 404: 326–337.
  10. Shao A, Guo S, Tu S, et al. (2014) Astragaloside IV alleviates early brain injury following experimental subarachnoid hemorrhage in rats. *International Journal of Medical Sciences* 11(10): 1073–1081.
  11. Yu W, Lv Z, Zhang L, et al. (2018) Astragaloside IV reduces the hypoxia-induced injury in PC-12 cells by inhibiting expression of miR-124. *Biomedicine & Pharmacotherapy* 106: 419–425.
  12. Hao M, Liu Y, Chen P, et al. (2018) Astragaloside IV protects RGC-5 cells against oxidative stress. *Neural Regeneration Research* 13(6): 1081–1086.
  13. Shao S, Yi J, Regenstein JM, et al. (2018) Protective effects on  $^{60}\text{Co}$ - $\gamma$  radiation damage of pine cone polyphenols from *Pinus koraiensis*-loaded chitosan microspheres in vivo. *Molecules* 23(6): 1392.
  14. Chen TT, Hua W, Zhang XZ, et al. (2017) The effects of pprf gene of *Deinococcus radiodurans* R1 on acute radiation injury of mice exposed to  $^{60}\text{Co}$   $\gamma$ -ray radiation. *Oncotarget* 8(2): 2008–2019.
  15. Wang Z, Zhao Z, Lu J, et al. (2015) A comparison of the biological effects of  $^{125}\text{I}$  seeds continuous low-dose-rate radiation and  $^{60}\text{Co}$  high-dose-rate gamma radiation on non-small cell lung cancer cells. *PLoS One* 10(8): e0133728.
  16. Hu JY, Han J, Chu ZG, et al. (2009) Astragaloside IV attenuates hypoxia-induced cardiomyocyte damage in rats by upregulating superoxide dismutase-1 levels. *Clinical and Experimental Pharmacology and Physiology* 36(4): 351–357.
  17. Zheng R, Deng Y, Chen Y, et al. (2012) Astragaloside IV attenuates complement membranous attack complex induced podocyte injury through the MAPK pathway. *Phytotherapy Research* 26(6): 892–898.
  18. Kim MH, Kim SH and Yang WM (2014) Beneficial effects of Astragaloside IV for hair loss via inhibition of Fas/Fas L-mediated apoptotic signaling. *PLoS One* 9(3): e92984.
  19. Chen X, Peng LH, Li N, et al. (2012) The healing and anti-scar effects of astragaloside IV on the wound repair in vitro and in vivo. *Journal of Ethnopharmacology* 139(3): 721–727.
  20. Kamenisch Y, Baban TSA, Schuller W, et al. (2016) UVA-Irradiation induces melanoma invasion via the enhanced Warburg effect. *Journal of Investigative Dermatology* 136(9): 1866–1875.
  21. Komatsu T, Sasaki S, Manabe Y, et al. (2017) Preventive effect of dietary astaxanthin on UVA-induced skin photoaging in hairless mice. *PLoS One* 12(2): e0171178.
  22. Chairprasongsuk A, Onkokoosong T, Pluemsamran T, et al. (2016) Photoprotection by dietary phenolics against melanogenesis induced by UVA through Nrf2-dependent antioxidant responses. *Redox Biology* 8: 79–90.
  23. Lee WH, Cho HJ, Sonntag WE, et al. (2011) Radiation attenuates physiological angiogenesis by differential expression of VEGF, Ang-1, tie-2 and Ang-2 in rat brain. *Radiation Research* 176(6): 753–760.
  24. Mukherjee PK, Gunasekharan R, Subburaju T, et al. (1999) Studies on the antibacterial potential of *Cryptostegia grandiflora* R. BR. (Asclepiadaceae) extract. *Phytotherapy Research* 13(1): 70–72.
  25. Sinclair S (1998) Chinese herbs: A clinical review of Astragalus, Ligusticum and Schizandrae. *Alternative Medicine Review* 3: 338–344.
  26. Guclu-Ustundag O and Mazza G (2007) Saponins: Properties, applications and processing. *Critical Reviews in Food Science and Nutrition* 47(3): 231–258.
  27. Tada E, Parent JM, Lowenstein DH, et al. (2000) X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. *Neuroscience* 99(1): 33–41.
  28. Fritsch P, Richard-Le Naour H, Denis S, et al. (1994) Kinetics of radiation-induced apoptosis in the cerebellum of 14-day-old rats after acute or during continuous exposure. *International Journal of Radiation Biology* 66(1): 111–117.
  29. Li YR, Cao W, Guo J, et al. (2011) Comparative investigations on the protective effects of rhodioid, ciwujianoside-B and astragaloside IV on radiation injuries of the hematopoietic system in mice. *Phytotherapy Research* 25(5): 644–653.
  30. Tischler GAS (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc Natl Acad Sci USA* 73(7): 2424–2428.
  31. Anderson DJ (1993) Molecular control of cell fate in the neural crest: The sympathoadrenal lineage. *Annual Review of Neuroscience* 16(1): 129–158.
  32. Vaudry D, Stork PJ, Lazarovici P, et al. (2002) Signaling pathways for PC12 cell differentiation: Making the right connections. *Science* 296(5573): 1648–1649.
  33. Lahiani A, Brand-Yavin A, Yavin E, et al. (2018) Neuroprotective effects of bioactive compounds and MAPK pathway modulation in “ischemia”-stressed PC12 pheochromocytoma Cells. *Brain Sciences* 8(2): 32.
  34. Kritis AA, Stamoula EG, Paniskaki KA, et al. (2015) Researching glutamate-induced cytotoxicity in different cell lines: A comparative/collective analysis/study. *Frontiers in Cellular Neuroscience* 9: 91.
  35. Shimoke K and Chiba H (2001) Nerve growth factor prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced cell death via the Akt pathway by

- suppressing caspase-3-like activity using PC12 cells: Relevance to therapeutical application for parkinson's disease. *Journal of Neuroscience Research* 63(5): 402–409.
36. Troy CM, Rabacchi SA, Xu Z, et al. (2001)  $\beta$ -Amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. *Journal of Neurochemistry* 77(1): 157–164.
37. Menei P, Pean JM, Nerrière-Daguin V, et al. (2000) Intracerebral implantation of NGF-releasing biodegradable microspheres protects striatum against excitotoxic damage. *Experimental Neurology* 161(1): 259–272.
38. Pan Z, Sampath D, Jackson G, et al. (1997) Nerve growth factor and oxidative stress in the nervous system. *Advances in Experimental Medicine & Biology* 429: 173–193.
39. Rukenstein A, Rydel R and Greene L (1991) Multiple agents rescue PC12 cells from serum-free cell death by translation- and transcription-independent mechanisms. *Journal of Neuroscience* 11: 2552–2563.
40. Batistatou A and Greene LA (1991) Aurintricarboxylic acid rescues PC12 cells and sympathetic neurons from cell death caused by nerve growth factor deprivation: Correlation with suppression of endonuclease activity. *The Journal of Cell Biology* 115(2): 461–471.
41. Fischer SJ, Podratz JL and Windebank AJ (2001) Nerve growth factor rescue of cisplatin neurotoxicity is mediated through the high affinity receptor: Studies in PC12 cells and p75 null mouse dorsal root ganglia. *Neuroscience Letters* 308(1): 1–4.
42. Choi KS, Kundu JK, Chun KS, et al. (2014) Rutin inhibits UVB radiation-induced expression of COX-2 and iNOS in hairless mouse skin: p38 MAP kinase and JNK as potential targets. *Archives of Biochemistry and Biophysics* 559: 38–45.
43. Ma Y and Xiong L (2019) Astragaloside IV ameliorates endoplasmic reticulum stress-induced apoptosis of A $\beta$ 25-35-treated PC12 cells by inhibiting the p38 MAPK signaling pathway. *Molecular Medicine Reports* 19(3): 2005–2012.
44. Wang F, Chen S, Deng L, et al. (2019) Protective effects of astragaloside IV against LPS-induced endometritis in mice through inhibiting activation of the NF- $\kappa$ B, p38 and JNK signaling pathways. *Molecules* 24: 373.
45. Bolaris S, Bozas E, Benekou A, et al. (2001) In utero radiation-induced apoptosis and p53 gene expression in the developing rat brain. *International Journal of Radiation Biology* 77(1): 71–81.
46. Komarov PG, Komarova EA, Kondratov RV, et al. (1999) A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. *Science* 285(5434): 1733–1737.
47. Matsuyama S, Xu Q, Velours J, et al. (1998) The mitochondrial F<sub>0</sub>F<sub>1</sub>-ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells. *Molecular Cell* 1(3): 327–336.
48. Brady HJ and Gil-Gómez G (1998) Molecules in focus Bax. The pro-apoptotic Bcl-2 family member, Bax. *The International Journal of Biochemistry & Cell Biology* 30(6): 647–650.
49. Oltvai ZN, Milliman CL and Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74(4): 609–619.
50. Bratton SB, MacFarlane M, Cain K, et al. (2000) Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Experimental Cell Research* 256: 27–33.
51. Liu Q, Si T, Xu X, et al. (2015) Electromagnetic radiation at 900 MHz induces sperm apoptosis through bcl-2, bax and caspase-3 signaling pathways in rats. *Reproductive Health* 12: 65–65.
52. Bao H, Sun D, Qi P, et al. (2019) Astragaloside protects oxygen and glucose deprivation induced injury by regulation of microRNA-21 in retinal ganglion cell line RGC-5. *Biomedicine & Pharmacotherapy* 109: 1826–1833.