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ORIGINAL RESEARCH

Compound Dahuang Baiji Spray Improves Acute Radiodermatitis by Down-Regulating the Expression of ALOX5

Xu Wenjing, MM; Wang Pei, MS; Ma Yueshi, MM; Liu Yong, MM; Zheng Weiqin, MB; Gao Liping, MM

ABSTRACT

Acute radiodermatitis is a type of skin injury caused by tumor radiotherapy. Compound Dahuang Baiji spray (CDBS) is a traditional Chinese medicine spray made from Dahuang and Baiji decoction. In the present study, we aimed to investigate the effects and mechanisms of CDBS on radiation dermatitis. We analyzed the main components of CDBS using High Performance Liquid Chromatography (HPLC). Through network pharmacology prediction, the target of Dahuang and Baiji was identified as arachidonate 5-lipoxygenase (ALOX5), associated with inflammation. Therefore, we constructed radiodermatitis rat models and treated them with CDBS for 14 d. Skin samples were collected from the rats' injured skin tissues, and pathological changes, oxidative stress indicators, inflammatory cytokines, and ALOX5 expression were detected using techniques such as HE staining, blood parameters analysis, ELISA, Real-time qPCR, and Western blot. The characteristic appearances of radiodermatitis were observed in different rat groups which indicated that the skin injury score in the model group was at grade II and was at grade I in the CDBS group. In addition, the HE results showed that CDBS reduced the necrosis of collagen fibers and inflammatory cell infiltration in the dermis of the radiodermatitis rats. Moreover, compared to the model group, CDBS significantly decreased leukocytes, lymphocytes, and neutrophils in the blood, as well as levels of IL-2, LTB4, 5-LO, NO, and ALOX5 expression in rat blood. Our findings suggest the therapeutic effect of CDBS on radiodermatitis by downregulating ALOX5 to inhibit inflammation, potentially serving as a radiodermatitis therapy. (*Altern Ther Health Med.* 2024;30(7):214-221).

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INTRODUCTION

Radiodermatitis is considered inflammatory skin mucosal damage caused by ionizing radiation (mainly β-rays, γ-rays, and high-energy X-rays) exposure during radiation therapy of malignant tumors. Mild radiodermatitis can heal spontaneously, while about 91.4% of radiotherapy patients have moderate or severe skin damage, which manifests as skin hair loss, atrophy, hyperpigmentation, sclerosing edema, delayed ulceration, thickening, fibrosis, and necrosis.[1-3] The skin is composed of epidermis, dermis, and subcutaneous tissues. The process of radioactive skin damage includes direct damage to basal keratin-forming cells and hair follicle stem cells, followed by free radical bursts, irreversible double-strand breaks in the DNA of the nucleus and mitochondria, and an inflammatory response, ultimately leading to skin damage.[4] Radiodermatitis seriously affects the survival quality of patients and long-term or recurrent attacks can lead to systemic infection, which endangers patients' lives, delays the treatment process, and affects the effect of tumor treatment.[5] Currently, there is no standardized treatment plan for radiation dermatitis, with topical medications being the mainstay, along with daily care. Western medicine treatment is based on chemical drugs, such as Biafine, hormone cream, and recombinant human epidermal growth factor.[6,7] There is no specific drug, and there are problems such as drug allergy and drug resistance due to long-term application. The use of Traditional Chinese Medicine (TCM) in the treatment of radiation dermatitis is gradually revealing its obvious advantages and potential and is receiving increasing recognition and attention at home and abroad.[8]

Dahuang (Rhubarb) is the rhizome of the *Polygonaceae* family plants *Rheum palmatum L.*, *R. tanguticum* Maxim. ex Balf, and *R. officinale* Baill.[9] In the *Compendium of Materia Medica* compiled by Li Shizhen of the Ming Dynasty, it is written that ground Dahuang mixed with honey can not only

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relieve pain but also eliminate scar of scald. The Dahuang powder mixed with vinegar is useful for treating carbuncle.[10] The *Anthology of Chinese Herb of Whole Nation* mentions that Dahuang is used for external treatment of burns and scalds, suppurative skin diseases, carbuncles, and swollen sores.[11] It can be seen that TCM has a full understanding of Dahuang for the treatment of burn and infection. 153 chemical components have been isolated and identified from medicinal plants of the *Rheum L*. genus to date, which are mainly grouped into seven categories, anthraquinone, anthrone, stilbene, tannic acid, phenylbutazone, chromogenic ketone, and others (sugars, organic acids, volatile components and trace elements).[12] The main pharmacological activities of Dahuang include anti-tumor,[13] antibacterial,[14] anti-inflammatory,[15] anti-fibrosis, and regulation of intestinal flora.[16] Most of the pharmacological effects are the result of the combined action of anthraquinones and stilbenes in Dahuang, except for rhubarbin and sennosides, which are the main components of antifibrosis. Dahuang has now been reported in the treatment of various types of dermatitis. Dahuang Zhechong Pill (Rhubarb and Eupolyphaga Pill) combined with topical fire needling can significantly relieve itching and reduce the recurrence of recurrent neurodermatitis.[17] It is reported that rats with atopic dermatitis were fed with 30-300 mg/kg/d Dahuang (70% ethanolic extract of *R. tanguticum*) and the skin severity score decreased significantly after 5 weeks.[18]

Baiji (Rhizoma Bletillae) is the tube of *Bletilla striata* and has a long history of medicinal use.[19] The Compendium of Materia Medica records that it can treat furuncles, swollen sores, chapped hands and feet, and burns. The main components of Baiji are bibenzyl, dihydrophenes, biphenyls, ethers, steroids, and triterpenes.[19] Its pharmacological activities include antibacterial,[20] antioxidant,[21] antiviral,[22] promote wound healing,[23] hemostasis,[24] and anti-tumor effects.[25] Likewise, Baiji can be used clinically in dermatitis therapy. The 50% ethanol extract of Baiji inhibited both 2, 4-dinitrofluorobenzene (DNFB) and picryl-chloride-induced contact dermatitis in mice.[26] Patients with seborrheic dermatitis had significantly lower pruritus scores and skin injury scores than the control group (oral vitamin B6) when Baiji powder was applied to the wounded areas for 28 d at 30 min once daily.[27]

Compound Dahuang Baiji Spray (CDBS) is a TCM spray made from Dahuang (Rhubarb) and Baiji (Rhizoma Bletillae) decoction. Li et al. found that self-made CDBS significantly improves the pain and skin conditions of patients with acute radiodermatitis caused by nasopharyngeal cancer radiotherapy, as well as reducing the inflammatory levels of patients.[28] Our previous study have shown that the use of CDBS in radiotherapy patients can significantly delay the onset of radiation-induced skin damage, reduce the severity of damage, shorten the duration, and enhance the tolerance dose of radiation.[29] Currently, the clinical application of Dahuang Baiji soup or powder is mainly limited to the treatment of hemorrhagic diseases,[30,31] and there are only a few studies on the use of Dahuang Baiji soup in the treatment of radiodermatitis. In our clinical practice, a small number of patients with severe acute radiodermatitis have experienced significant relief of pain, improvement of erosion, and reduction of pigmentation with the external use of Dahuang Baiji soup. Therefore, this research was to investigate the role of Dahuang Baiji soup in the prevention and treatment of acute radiodermatitis and its specific mechanism and to provide a potential therapy approach.

METHODS

Preparation of Compound Dahuang Baiji Spray (CDBS)

The CDBS was manufactured in the Pharmacy Center of Chongqing Hospital of Traditional Chinese Medicine. 10 g of Dahuang (Taiji Group, China), 20 g of Baiji (Taiji Group, China), and 500 mL of distilled water were mixed together for 1.5 h decoction. Next, the decoction was concentrated to 150 mL and filtered, and the filtrate was collected using 0.1% potassium sorbate (T25295, Shanghai Yuanye Bio-Technology, China) as a preservative. Finally, the filtrate was made into spray (pH = 6~7).[28]

Prediction of CDBS target

The Dahuang and Baiji were used as search terms to predict their main components in the BATMAN-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/). The predicted candidate target score for each component was no less than 20 points, with Dahuang selecting the top 9 compounds in the network and Baiji selecting the top 2 compounds in the network that interacted with the target for mapping. All adjusted *P*-value cutoff was 0.05.[32]

Main components analysis of CDBS

Determination of total and free anthraquinone. 8.58 mg of Aloe emodin (as standard substrate) (B20772, Shanghai Yuanye Bio-Technology, China) was taken into a 50 mL volumetric bottle, methanol (201809, Chuandong Chemical, China) was added for ultrasonic dissolution, and the volume was fixed to the scale. After shaking well, solutions of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL were respectively taken into a 10 mL volumetric bottle. The methanol was held at the scale, shaken well and filtered, and the filtrate was collected for High Performance Liquid Chromatograph (HPLC) (Agilent 1260, Agilent Technology, USA) detection.

5 g of CDBS sample was taken into a round-bottomed flask, 15 mL of methanol was added and weighed. After heating reflux extraction for 1 h, the weight of reflux liquid was weighed. The weight loss was made up with methanol. The mixture was shaken well and filtered, and the filtrate was collected to obtain the free anthraquinone sample to be measured (sample I).

Next, 5 mL of the sample I was transferred into a flask, 10 mL of 8% hydrochloric acid solution was added, and ultrasonic processing was carried out for 2 min. Then 10 mL of trichloromethane (T108192, Aladdin biochemical technology, China) was added for 1 h heating reflux. The

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cooled reflux liquid was placed in the separator funnel to collect the trichloromethane layer. The filter residue was recovered by vacuum filtration, dissolved in methanol, and transferred to a 10 mL measuring bottle. Methanol was further added to the scale line, shaken well, and filtered, and the filtrate was collected to obtain the total anthraquinone sample to be measured (sample II). 10 μL of samples I and II were taken separately for the HPLC detection. The chromatographic column was Phenomenex Beta C18 LC Column (5 μm, 250 × 4.6 mm), the mobile phase was methanol: 0.1% PA = 60: 40, flow rate was 1.0 mL/min, column temperature was 35 °C, and the detection wavelength of 254 nm was used.[33]

Determination of gallic acid and batatasin III. 8.91 mg of gallic acid (B20851, Shanghai Yuanye Bio-Technology, China) and 8.74 mg of batatasin III (B23201, Shanghai Yuanye Bio-Technology, China) (as standard substrate) were placed in the 50 mL volumetric bottle respectively, then dissolved with 50% methanol by ultrasound and fixed to the scale line. After shaking well, solutions of 0.2, 0.6, 1.0, 1.4, 1.8, and 2.2 mL were added to the 10 mL volumetric bottles, fixed to the scale with 50% methanol, and filtrated. The filtrate was collected for HPLC detection.

2 g of the CDBS sample was placed in a conical flask, 25 mL of 50% methanol was added, weighed, and ultrasonic processing was performed for 30 min. Next, the weight of the sample was measured again and the weight loss was made up with 50% methanol. Finally, the filtrate was collected and 10 μL of it was used for HPLC detection.

The chromatographic column was Phenomenex Agela Venusil AQ C18 Column (5 μm, 250×4.6 mm) (Phenomenex, USA), the mobile phase was acetonitrile: 0.1%PA = 3: 97 (at 1-10 min), 45:55 (at 12-27 min), and 90:10 (at 30 min), the flow rate was 1.0 mL/min, column temperature was 35 °C, and detection wavelength of 270 nm was used.[34]

Establishment of acute radiodermatitis model in rats

A total of thirty-two 7-8 weeks old female SD rats (Hunan Silaike Laboratory Animal Co., Ltd., license No. SCXK(Xiang) 2019-0004) weighing 180-200 g were selected. Rats were fed in the SPF-grade animal house of Chongqing Hospital of Traditional Chinese Medicine at an ambient temperature of 25 °C and humidity of 50%-55% and were given pure water and special rat sterilized feed. The rats were divided into control, model, CDBS, and radiotherapy protection spray (RPS) groups. The rat skin of the right thigh was shaved 1 day before radiotherapy to facilitate later observation of the skin status. Except for the control group, rats were anesthetized with pentobarbital intraperitoneally, and fixed in the prone position on a plate. Then the animals were irradiated using the linear accelerator (Elekta Synergy, Elekta, Sweden) with 4 MeV-β electron wire, maintaining a source-skin distance of 100 cm, radiation field of 3 cm × 3 cm, and a single exposure of 20 Gy to establish the acute radiodermatitis model on the right thigh.

Treatments

From the second day after irradiation, the control and model groups were given saline on the right leg, the CDBS group was given CDBS, and the RPS group was given RPS in the irradiated area. All treatments were applied twice a day for 10 minutes each time for 14 days, and the skin changes of the rats were observed.

Skin injury score (SIS) evaluation

The skin injury score evaluation of rats was based on the human acute radiation response score criteria (RTOG/EOTRC) which has 5 levels. Grade 0 indicates no change, Grade 1 indicates dotted and flaky erythema with dry peeling, Grade 2 indicates obvious area of erythema, small amount of wet peeling and mild edema, Grade 3 indicates moderate edema and large area of wet peeling, Grade 4 indicates large area of skin ulceration, bleeding and necrosis, and Grade 5 indicates death due to radiodermatitis.[35]

HE staining

The skin tissues of the right thigh of rats were collected and fixed with 4% paraformaldehyde (G0002, Wuhan Sevier Biotechnology Co., Ltd., China). The tissues were soaked successively in the increasing concentration of ethanol (75%-85%-95%-100%-100%) for dehydration, for 2 h in each concentration. Then the tissues were immersed in 1/2 xylene (xylene:ethanol = 1:1), xylene (I) and xylene (II) for 10 min, respectively, and embedded in the melted paraffins. Next, the solidified paraffins were made into sections and immersed in xylene (II), xylene (I), and 1/2 xylene for 10 min, and decreasing concentration of ethanol (100%-100%-95%-85%-75%) for 5 min, respectively. The sections were washed with distilled water and stained with hematoxylin (G1004, Wuhan Sevier Biotechnology Co., Ltd., China) for 6 min and eosin (G1002, Wuhan Sevier Biotechnology Co., Ltd., China) for 2 min. After washing the excess staining solution away, the sections were immersed in 95% and 100% ethanol separately for 2 min and xylene (III) and xylene (IV) for 5 min. Ultimately, the sections were mounted with neutral gum (10004160, China Pharmaceutical Group Co., Ltd.), and the photographs were captured using a microscope (MF53, Guangzhou Mingmei Optoelectronic Technology, China).[36]

Determination of blood parameters

Carotid blood was collected and the number of leukocytes, lymphocytes, and neutrophils in the anticoagulant blood was measured by an automatic blood cell analyzer (BC6800, Mindray Medical, China).[37]

ELISA

The blood and skin tissue lapping liquid were centrifuged at 3000 rpm for 10 min and the supernatant was collected. The levels of leukotriene B4 (LTB4), 5-lipoxygenase (5-LO), nitric oxide (NO), interleukin-2 (IL-2) in rat skin tissues, and serum IL-2 were measured according to the ELISA kit instructions. Rat TNF-α ELISA Kit (RX302058R), IL-2

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ELISA Kit (RX302867R), IL-6 ELISA Kit (RX302856R), NO ELISA Kit (RXWB0246-96), 5-LO ELISA Kit (RX101151R) and LTB4 ELISA Kit (RX203065R) were purchased from Quanzhou Ruixin Biotechnology Co., Ltd (China).[38]

Real-time qPCR

Total RNA extraction from skin tissues was performed according to the instructions of the TransZol Up Plus RNA Kit (ER501, Beijing TransGen Biotech, China), and the RNA concentration was measured using an OD1000 microspectrophotometer (NanoDrop One/One C, Thermofisher, USA). The real-time qPCR reaction system was formulated based on the 2 × TransStarTM Top Green qPCR SuperMix Kit (AQ131-01, Beijing TransGen Biotech, China). Reaction conditions were also optimized to determine the optimal template concentration and annealing temperature to ensure the specificity of primers and the efficiency of amplification.[39]

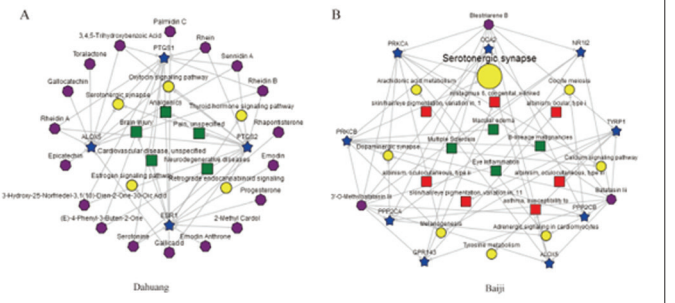
Western blot

Total protein extraction was performed using RIPA lysis buffer (contains PMSF P408676, Aladdin biochemical technology, China) and protease inhibitor cocktail (P1045, Beyotime Biotechnology, China). Protein concentration was measured using a TU-1901 double-beam UV-visible spectrophotometer (TU-1901, Shanghai Analytical instrument Co., Ltd., China). Electrophoresis was performed using 10% SDS-PAGE (200 V, 45 min) (PG112, Shanghai Epizyme Biology, China). The gel containing the target protein was cut off, the transfer cassette current was set at 200 mA, and the target protein was transferred to the PVDF membrane (10600023, Amersham, Germany). After 2 h, the membranes were blocked at room temperature for 2 h using 3% BSA (diluted by 0.5% TBST) (ST023, Beyotime Biotechnology, China) and incubated overnight with GAPDH (A19056, ABclonal Technology, China) and ALOX5 Rabbit mAb (A2158, ABclonal Technology, China) separately in a shaker at 4°C. The membranes were washed 3 times with TBST (PS103, Shanghai Epizyme Biology, China) and incubated with HRP Goat Anti-Rabbit IgG antibody (AS014, ABclonal Technology, China) for 1 h. After washing the membranes 3 times with TBST, they were exposed for detection in a western blot apparatus (Universal Hood II, Bio-Rad, USA).[40]

Data statistics

Data were analyzed using SPSS 26.0 statistical software (IBM Corp., USA) and expressed as mean ± standard deviation. One-way ANOVA was used for comparison of means between multiple groups. If the Levene test variance was equal, the LSD method was used for comparison, and if the Levene test variance was not equal, the Dunnett T3 test was used for comparison. Rank sum test (Kruskal-Wallis) was used for rank data, Steel-Dwass test was used for pairwise comparison. Differences were considered statistically significant at $P < .05$.

Figure 1. Predicted Targets of the Main Components of Dahuang (A) and Baiji (B) Based on Network Pharmacology. The Purple Represents Constituent Absorbed into Blood, the Blue Represents Drug Target, the Yellow Represents Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway, the Red Represents Online Mendelian Inheritance in Man (OMIM) Disease, and the Green Represents Comparative Toxicogenomics Database (CTD) Disease.



RESULTS

The targets of CDBS

The major targets corresponding to the main components of Dahuang and Baiji were predicted using the BATMAN-TCM database. Dahuang has binding interactions with arachidonate 5-lipoxygenase (ALOX5) in the arachidonic acid pathway (associated with inflammation), with prostaglandin-endoperoxide synthase 1 (PTGS1) and prostaglandin-endoperoxide synthase 1 (PTGS2) on the prostaglandin synthesis pathway, and with estrogen receptor 1 (ESR1) and progesterone receptor (PGR) on the cell proliferation pathway (Figure 1A). Baiji has binding interactions with protein phosphatase 2 catalytic subunit alpha (PPP2CA), protein phosphatase 2 catalytic subunit beta (PPP2CB) (involved in skin cell proliferation and differentiation), protein kinase C-beta (PRKCB), protein kinase C-alpha (PRKCA) (involved in cell proliferation), G protein-coupled 143 receptors (GPR143), oculocutaneous albinism type II (OCA2), tyrosinase-related protein 1 (TYRP1) (involved in pigment production metabolism), and Nuclear Receptor Subfamily 1 Group I Member 2 (NR1I2) (involved in antioxidant and detoxification function) (Figure 1B).

Results of CDBS main components analysis

Aloe emodin, gallic acid, and batatasin III were used as the standard substrates to determine anthraquinone, gallic acid, and batatasin III contents in CDBS. A standard curve was established with the concentration of the standard substrate (mg/mL) as the horizontal coordinate and the peak area as the vertical coordinate. The regression equation was $y = 44049x + 23.475$ ($R^2 = 0.9993$). The peaks of total anthraquinone and free total anthraquinone were observed from the chromatogram (Figure 2A). The calculated average content of total and free anthraquinone in CDBS was 0.041 and 0.013 mg/mL, respectively (n = 3). The regression equation of the gallic acid was $y = 30652x + 11.694$ ($R^2 = 0.9984$). The peaks of gallic acid could be observed from the

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chromatogram, and its average content is 0.16 mg/mL (n = 3) (Figure 2B). However, no batatasin III was detected in CDBS.

Effect of CDBS on acute radiodermatitis in rats

The appearances of radiodermatitis in rats of each group are shown in Figure 3A, and the SIS results are shown in Table 1. No abnormalities were observed in the right thigh skin of the control group, and the hair grew well. The SIS of rats in model group was at grade II-III, mainly grade II, with poor hair growth and still a lot of hair loss. The SIS in the CDBS group was at grade I-II, with grade I predominant. The rats' hair growth resumed, with localized hair loss still evident. The SIS in the RPS group was at grade I-II, with grade I predominant. And there was still a small amount of hair loss of the rats. Pairwise comparison showed that the differences between the control group and all other groups were statistically significant. The differences between model group and CDBS group, or between RPS group were statistically significant, and the difference between the CDBS group and between RPS was not statistically significant.

HE staining was used to observe the pathology of the injured skin tissue in rats. As shown in Figure 3B, the epidermal structure of the skin tissue in the control group was intact and clear, the keratinized layer was distinct, the stratified squamous epithelium layer was thin, and the cells were neatly and closely arranged. The collagen fibers in the dermis were staggered and dense, and the cytoplasm was red-stained and uniform, and the air follicles and sebaceous glands were intact. In the model group, the keratinized layer of skin tissue was significantly thickened, the collagen fibers in the dermis were looser, a small number of cells in the epidermis were necrotic, and the nuclei disintegrated or disappeared. Furthermore, the number of hair follicles, sebaceous glands, and other appendage structures was significantly reduced. More hair follicles were necrotic, and the internal structure was destroyed or disappeared, accompanied by neutrophil and lymphocyte infiltration. In the CDBS group, the epidermal keratinized layer was obvious, the stratified squamous epithelium layer was thickened, a small number of collagen fibers in the dermis was necrotic, and the nuclei were atrophied. In addition, the number of hair follicles and sebaceous glands was reduced, and a few band forms of neutrophil granulocytes were infiltrated. In the RPS group, the epidermal structure was intact, the keratinized layer was observed, the stratified squamous epithelium layer was slightly thickened, and the collagen fibers in the dermis were mostly arranged in a staggered pattern. The hair follicles and sebaceous glands were more numerous, a few neutrophil granulocytes were infiltrated, and the subcutaneous structures were clearly visible.

Results of blood parameters and ELISA

The leukocytes, lymphocytes, and neutrophils in the blood of the model, CDBS, and RPS groups were significantly reduced compared with the control group (Figure 4). In addition, the IL-2 levels in serum and LTB4, 5-LO, NO, and IL-2 levels in tissue showed a significant increase in the model

Figure 2. Determination of Anthraquinone (A) and Gallic Acid (B) in CDBS by HPLC

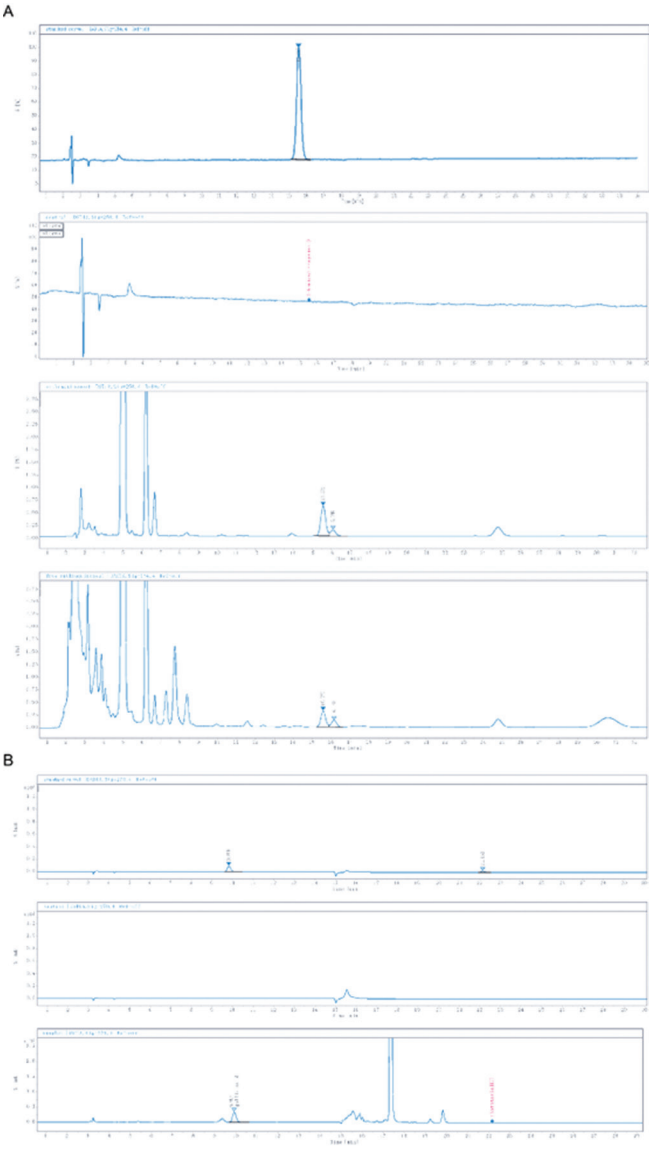

Table 1. The SIS Evaluation Based on RTOG/EORTC

Group	n	Grade			χ^2	*P* value
		I	II	III		
Control	8	0	0	0	19.28	.000
Model	8	2	4	2		
CDBS	8	5	3	0		
RPS	8	4	4	0		

Figure 3. Evaluation of Radiodermatitis Reaction in the Right Thigh Skin (A) and Pathological Observation of Skin Tissue by HE Staining (B). Magnification, 100×.

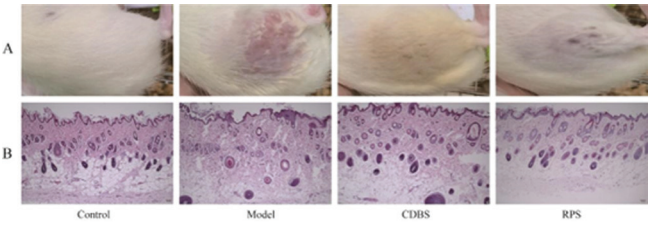

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Figure 4. The Number of Leukocytes, Neutrophils, and Lymphocytes were Measured in Rats Blood

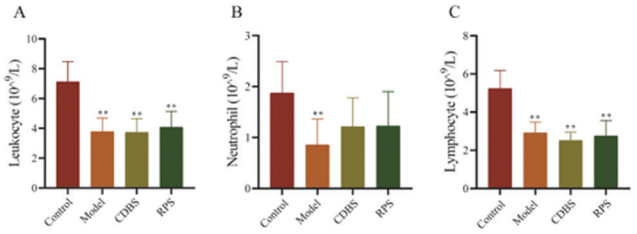


Figure 5. Determination of the Levels of NO, IL-2, 5-LO, and LTB5 in Rats by ELISA

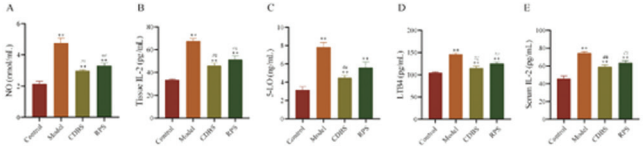
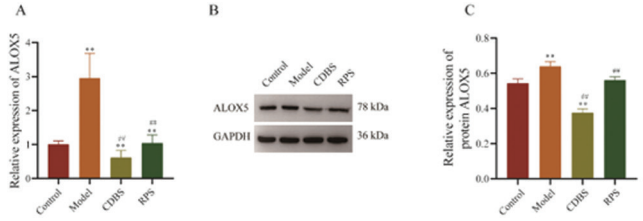


Figure 6. Real-Time qPCR Assay (A) and Western Blot Assay (B-C) for the Relative Expression of ALOX5 in Rat Skin Tissues



group (Figure 5), suggesting a radiation-induced inflammatory response. However, the inflammatory cytokines in CDBS and RPS groups were significantly reduced compared to the model group. These results indicated that CDBS could significantly decrease the contents of 5-LO and LTB4.

CDBS decreased the expression of ALOX5

After using radiation therapy, ALOX5 mRNA and protein expression was significantly elevated of rats in the model group, while they were significantly reduced after treatment with CDBS (Figure 6). It is suggested that the components in CDBS could regulate the ALOX5 expression to suppress the inflammatory response and relieve radiomatitis.

DISCUSSION

Radiation therapy is an important tool in the treatment process of malignant tumors. Together with oncologic surgery and chemotherapy, it is an important method of oncologic treatment. It is reported that about 95% of patients receiving radiation therapy will exhibit some form of skin toxicity,[41] and nearly 47% of patients will have a radiological skin reaction of grade II.[42] Radiodermatitis causes redness and pain in the patient's skin and can easily get complicated with erosions and infections, or even ulcers and necrosis. Severe radiodermatitis can even interrupt the radiation therapy or prolong the treatment process, thereby affecting the tumor control rates and significantly decreasing the patients' life quality.[42] The current treatment strategy in Western medicine focuses on improving the distribution of radiation to reduce skin dosage for prevention. Medicinal treatment primarily involves using hormones and steroid ointments, radiation-protective creams containing superoxide dismutase, compound vitamin B12 mixtures, high molecular weight material ointments, growth factor ointments, etc., but these has certain limitations.[43,44]

TCM can effectively regulate skin damage in a multi-targeted and multi-directional manner, thus providing good therapy results. In recent years, reports on the treatment of radiodermatitis with TCM have gradually increased. A randomized phase III clinical trial showed that Moist Exposed Burn Ointment, made from *Phellodendri Chinensis* Cortex, *Coptidis* Rhizoma, *Scutellariae* Radix, *Pheretima*, and *Papaveris* Pericarpium, reduced the itching and pain of acute dermatitis caused by radiation therapy for breast cancer and improved patient comfort.[45] Another meta-analysis reported that TCM could prevent the occurrence of radiodermatitis, reduce its severity, and promote the healing of skin lesions.[46] In our preliminary clinical practice, we have discovered that Dahuang and Baiji, regarded in traditional medicine for their potential to clear heat, detoxify, promote blood circulation, and aid tissue regeneration, have demonstrated effective results in relieving pain, ulceration, and bleeding in the skin and mucous membranes. Additionally, CDBS has been observed to alleviate pain and reduce the severity of skin ulceration in some patients with severe acute radiodermatitis. Through HPLC analysis, we identified the main components of CDBS as anthraquinone, free anthraquinone and gallic acid. Research indicates that Dahuang is a versatile medicinal herb, containing free anthraquinone compounds with antibacterial effects, Anthraquinone glycosides and dianthrones have laxative properties, while tannin compounds offer hemostatic and astringent effects.[47] Further examination using HE staining revealed noticeable improvement in the damaged skin tissues of rats treated with CDBS for 14 days. This observation is consistent with the previous findings.[29] Thus, exploring the therapeutic effects and mechanisms of CDBS could offer new strategies for the prevention and treatment of radiation dermatitis.

Theoretically, due to the ionizing effect of radioactive rays in radiation therapy, water molecules form reactive oxygen species (ROS) with superoxide anions, there causing peroxidative cellular damage.[48] In addition, the reduced expression of GTP-cyclohydrolase-1 and the reduced synthesis and utilization of 5,6,7,8-tetrahydrobiopterin in skin tissues exposed to radiation lead to uncoupling of nitric oxide synthase and an imbalance of NO and ROS. These events promote the oxidative stress and further aggravate radiation-induced cellular damage.[49] More specifically, a cascade of cytokines and chemokines is triggered immediately after the irradiation of tissues and organs, and mediators released in the irradiated tissues remain for a long time, thus enhancing and prolonging the inflammatory response. This leads to tissue inflammatory cell infiltration and elevated IL-2, IL-6, IL-10 and TNF-α.[50] These inflammatory factors

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have a strong chemoattractant and activating effect on T lymphocytes and neutrophils, thereby contributing to local inflammation.[51] Our results indicate that CDBS can reduce IL-2 levels in rat serum and skin tissues, as well as NO in skin tissues, along with decreased leukocytes, lymphocytes, and neutrophils in the blood. This suggests that CDBS can alleviate oxidative stress and inflammatory reactions to some extent, aligning with previous research findings.[52]

We used the BATMAN-TCM database to predict that Dahuang binds to the binding sites of inflammatory mediators in pathways such as PTGS1, PTGS2, ESR1, and PGR. PTGS comprises two isoforms: constitutive PTGS1 and inducible PTGS2, regulated by specific stimuli, suggesting their involvement in inflammation and cell division.[53] ESR1 encodes an estrogen receptor, serving as a ligand-activated transcription factor, possibly forming homodimers or heterodimers with estrogen receptor 2, influencing cell proliferation and differentiation in target tissues.[54] PGR gene encodes a member of the steroid receptor superfamily that can inhibit hormone-dependent cell proliferation in endometrial and breast cancer cells.[55]

The skin is a terminal organ affected by estrogen and progesterone, and changes in hormone levels can impact skin structure and function.[56] These pathways are involved in inflammation and skin cell proliferation, suggesting a close association between Dahuang and anti-inflammatory and analgesic effect pathways, as well as pathways involved in maintaining the skin function. Baiji binds to PRKCB, PRKCA, GPR143, OCA2, TYRP1, and NR1I2. Proteins encoded by PPP2CA and PPP2CB are subunits of protein phosphatase 2A (PP2A), which participates in the MAPK signaling pathway in the body, contributing to processes like skin cell proliferation, differentiation, and wound healing.[57] GPR143, also known as G-protein coupled receptor 143, plays a crucial role in melanosome synthesis.[58] *OCA2* gene encodes a transmembrane protein primarily expressed in melanocytes.[59] *YRP1* belongs to the tyrosinase gene family and directly regulates melanin production, influencing melanocyte development, survival, and function.[60] *NR1I2* gene encodes SXR (Steroid X Receptor), which plays a role in downregulating the expression of the cytochrome P450 family, thereby influencing the antioxidant and detoxification capabilities of cells.[61] These findings suggest that Baiji is also closely related to anti-inflammatory effects and is involved in maintaining the skin function.

Furthermore, we found that Dahuang, Baiji, and several high-ranking weighted components (such as aloesin, ellagic acid, Diosgenin III, etc.) can all selectively target and mediate the arachidonic acid (AA) metabolic signaling pathway of ALOX5. Arachidonic acid is one of the most abundant, widely distributed and active essential fatty acids in the body, and its metabolic network is a key component of the inflammatory network.[62] ALOX5 is a non-heme iron-containing dioxygenase that encodes arachidonate 5-lipoxygenase, an initiating catalase involved in bypassing the production of inflammatory mediators such as leukotriene (LTs) and lipoxins (LXs). After activation, ALOX5 in the cytoplasm or nucleus can be transferred to the nuclear membrane and interact with 5-flipoxygenase-activating protein (5-FLAP), allowing ALOX5 to bind to arachidonic acid to induce an enzymatic reaction. Thus, the arachidonic acid will be converted into leukotrienes and 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which is metabolized to LTs subtypes (LTA4, LTB4, LTC4, and LTIM) by enzymatic catalysis.[63] The activity of 5-LO and its expression are usually elevated in inflammation, Alzheimer's disease, tumors, and anaphylaxis, resulting in increased biosynthesis of LT metabolites. LTs have various pro-inflammatory effects, among which LTB4 can activate leukocytes to produce chemotaxis, promote leukocyte adhesion to the vascular endothelium, release myeloperoxidase-like lysosomal enzymes, and produce peroxide anions.[64] These events mentioned above lead to oxidative stress and inflammation response, thereby aggravating the radiodermatitis symptoms. Our results indicate that CDBS significantly inhibits the expression activity of ALOX5 in rat skin tissues, reducing the levels of 5-LO and LTB4. This suggests that CDBS can notably alleviate inflammation and improve acute radiodermatitis caused by radiotherapy.

CONCLUSION

The study found that CDBS, composed mainly of anthraquinones, free anthraquinones, and gallic acid, significantly improves damaged rat skin tissues after 14 days of treatment. Our research demonstrates that CDBS can inhibit the expression activity of ALOX5, downregulate the production of downstream LTB4, and reduce levels of ROS, NO, and IL-2, thereby improving acute radiodermatitis induced by radiotherapy. Therefore, CDBS can be considered for the clinical treatment of radiation-induced dermatitis caused by radiotherapy.

ETHICS APPROVAL

This study was reviewed and approved by Chongqing Traditional Chinese Medicine Hospital (No. 2022-DWSY-XWJ). All animals used in the study were treated humanely.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Xu Wenjing performed most of the experiments, analyzed the data, and drafted the manuscript. Wang Pei and Ma Yueshi were mainly involved in the acquisition of data and article writing. Liu Yong and Zheng Weiqin interpreted the data and participated in the article revision. Gao Liping participated in the project design and critically revised the manuscript. All authors contributed to and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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