Breviscapine attenuates lead-induced myocardial injury by activating the Nrf2 signaling pathway

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Abstract. The present study investigated the therapeutic potential of breviscapine (Bre) in mitigating lead (Pb)-induced myocardial injury through activation of the nuclear factor erythroid-2 related factor 2 (Nrf2) pathway. Rat cardiomyocytes (H9C2 cells) were exposed to Pb to model Pb poisoning, and various parameters, including cell viability, apoptosis and reactive oxygen species (ROS) production, were assessed using Cell Counting Kit-8, flow cytometry and 2',7'-dichlorfluorescein diacetate assays, respectively. Additionally, a rat model of Pb poisoning was established in which blood Pb levels were measured using a graphite furnace atomic absorption spectrophotometer, and alterations in myocardial tissue, oxidative stress markers, inflammatory indicators, protein expression related to apoptosis and the Nrf2 pathway were evaluated via histopathology, ELISA and western blotting. The results showed that Bre treatment enhanced cell viability, decreased apoptosis, and reduced ROS production in Pb-exposed H9C2 cells. Moreover, Bre modulated oxidative stress markers and inflammatory factors while enhancing the expression of proteins in the Nrf2 pathway. In a rat model, Bre mitigated the lead-induced increase in blood Pb levels and myocardial injury biomarkers, and reversed the downregulation of Nrf2 pathway proteins. In conclusion, the current findings suggested that Bre mitigates Pb-induced myocardial injury by activating the Nrf2 signaling pathway, highlighting its potential as a therapeutic agent for protecting the heart from the harmful effects of Pb exposure. Further research is required to elucidate the exact mechanisms and explore the clinical applicability of Bre in mitigating Pb-induced myocardial damage.

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bre, Breviscapine; CAT, catalase; cTnI, cardiac troponin I; CK, creatine kinase; CK-MB, creatine kinase-MB; H&E, hematoxylin and eosin; HO-1, heme oxygenase-1; IL, interleukin; LDH, lactate dehydrogenase; MDA, malondialdehyde; Nrf2, nuclear factor erythroid-2 related factor 2; ROS, reactive oxygen species; SD, Sprague Dawley; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α

Key words: Bre, lead, myocardial injury, oxidative stress, Nrf2 signaling pathway

Introduction

Lead (Pb) is a toxicant prevalent in industrial settings and a significant contributor to environmental pollution. Advancements in industrial civilization have resulted in widespread Pb poisoning, which has emerged as a covert epidemic in the 21st century (1). In our daily lives, Pb primarily enters the organism through ingestion or inhalation from sources such as soil, food, Pb dust and other media (2). Upon entering the human body, Pb can induce various diseases that affect the neurological, respiratory, urinary and cardiovascular systems (3). Extensive research has demonstrated that exposure to Pb disrupts the delicate balance between oxidants and antioxidants, leading to vascular and cardiac damage (4-6).

Breviscapine (Bre) is a crude extract of several flavonoids from *Erigeron breviscapus (Vant.)*. It possesses a range of biological functions, including anti-inflammatory, antioxidant, anti-apoptotic and myocardial protection (7). Several studies have assessed its utility in the treatment of cardiovascular diseases, such as cardiac hypertrophy (8) and doxorubicin (DOX)-induced cardiotoxicity (9). Additionally, Bre has been shown to inhibit myocardial inflammation and apoptosis in rats with coronary embolisms (10) and exhibit protective effects against myocardial injuries induced by streptozotocin, thereby enhancing the antioxidant capacity of myocardial tissues (11). However, the effect of Bre on Pb-induced myocardial injury remains unclear.

Nuclear factor erythroid-2 related factor 2 (Nrf2) is a key transcription factor in oxidative stress regulation that augments the expression of antioxidant enzymes, thereby protecting against cell damage caused by reactive oxygen species (ROS) (12). Nrf2 maintains cellular homeostasis by regulating the expression of NAD(P)H dehydrogenase quinone 1 (NQO1) and heme oxygenase-1 (HO-1) (13,14). Furthermore, a review reported that Nrf2 protects the heart from oxidative stress damage (15). A previous study indicated that activation of the Nrf2 pathway can ameliorate Pb-induced kidney injury (16). Consequently, it was hypothesized that Bre

mitigates Pb-induced myocardial injury by activating the Nrf2 pathway.

In the present study, both animal and cellular models of Pb poisoning were established to investigate whether BRE confers myocardial protection through the Nrf2 pathway. The present study aimed to identify potential therapeutic agents for treating patients with Pb poisoning.

Materials and methods

Cell culture and treatment. Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) was used to culture rat cardiomyocyte-derived H9C2 cells (cat. no. CRL-1446; American Type Culture Collection). These cells were maintained at 37˚C under 5% CO_2. For modeling Pb poisoning, H9C2 cells were exposed to 10 μM lead acetate (PbA; cat. no. 467863; MilliporeSigma) for 24 h. Cells were pre-incubated with 1, 5 and 10 μM of Bre (cat. no. 116122362; Shanghai Yuanye Bio-Technology Co., Ltd.; high-performance liquid chromatography ≥95%) for 1 h prior to PbA exposure. To evaluate the influence of Bre on Pb-induced oxidative stress, PbA-treated cells were pretreated with 10 μM of Bre, either alone or in combination with 10 mM N-acetylcysteine (NAC; cat. no. T5518; TargetMol), a ROS scavenger, for 1 h. To elucidate the mechanistic action of Bre, PbA-exposed cells were pre-incubated with 20 μM Nrf2 inhibitor ML385 (cat. no. T4360; TargetMol) for 2 h prior to a 1 h treatment with 10 μM of Bre.

Cell viability was assessed using the Cell Counting Kit-8 (Beyotime Institute of Biotechnology). The assay was performed with a cell density of $5x10^3$ cells/well, using 10 μl CCK-8 reagent per well. The plates were then incubated at 37˚C for 2 h, and absorbance was detected at a wavelength of 450 nm.

Cell apoptosis. After trypsinization, H9C2 cells ($1x10^5$) were resuspended in binding buffer (195 μl) and incubated with Annexin V-FITC (5 μl) and propidium iodide (PI, 10 μl) for 20 min at 25˚C in the dark. Apoptosis was analyzed using a BD LSRFortessa X-20 flow cytometry (BD Biosciences). Data analysis was conducted using FlowJo software version 7.6 (FlowJo LLC).

ROS detection. Collected cells ($2x10^5$) were incubated with 2',7'-dichlorfluoresceindiacetate (DCFH-DA) at 37˚C for 20 min. ROS levels were quantitatively measured using flow cytometry at a wavelength of 488 nm and analyzed using FlowJo software version 7.6 (FlowJo LLC).

Establishment and processing of animal models. Six-week-old Sprague Dawley (SD) rats (male; weight, 185±25 g) were purchased from GemPharmatech Co. Ltd. (Nanjing, China) and housed in a controlled environment at a temperature of 22±2˚C, under a 12/12-h light/dark cycle, 50-60% humidity, and *ad libitum* access to food and distilled water. A total of 12 SD rats were randomly assigned to two groups (n=6): control and model. Subsequently, 24 SD rats were randomly assigned to four groups (n=6): model, model + 10 mg/kg Bre, model + 20 mg/kg Bre, and model + 40 mg/kg Bre. The drinking water of the rats in the model group contained PbA (0.5 g/l). In the subgroup treated with Bre, PbA was also included in the drinking water in conjunction with daily administration of Bre through gavage at doses of 10, 20 and 40 mg/kg. After 56 days of continuous consumption, blood samples were collected from the abdominal arteries under ether anesthesia. During the administration of ether anesthesia, the following parameters were rigorously monitored to ensure the animals were solely anesthetized and not euthanized:

i) Respiratory rate: Maintained within the range of 66-114 breaths per min.
i) Heart rate: Monitored via electrocardiogram or a dedicated heart rate monitor, and kept within the range of 370-580 beats per min.
iii) Body temperature: Controlled within a range of 37.8-38.7˚C.
iv) Anesthetic dosage: Administered at 30-50 mg/kg (3-5%), adjusted according to individual animal weight and response to anesthesia.

Following euthanasia through cervical dislocation, the heart tissues were collected for follow-up experiments. All animal procedures were conducted in compliance with the protocols approved by the Institutional Animal Care and Use Committee of The Third People's Hospital of Yunnan (approval no. 2020-029; Kunming, China).

Measurement of blood Pb levels. In accordance with a previous study (17), blood Pb levels were measured using a graphite furnace atomic absorption spectrophotometer (TAS-990; Beijing General Analytical Instrument) operating at a wavelength of 283.3 nm.

Histopathology. After 4% formaldehyde fixation for 24 h at 4˚C, ethanol dehydration, and paraffin embedding, the collected myocardial tissues were sliced into 5-μm sections. For hematoxylin and eosin (H&E) staining, the sections were incubated with hematoxylin for 5 min at room temperature, followed by incubation with eosin for 2 min at room temperature. For immunohistochemical staining, the underwent antigen retrieval in a microwave oven and were soaked in 3% H_2O_2 for 20 min. Following incubation with goat serum (Beijing Solarbio Science & Technology Co., Ltd.) for 10 min, slices were incubated with B-cell lymphoma 2 (Bcl-2; 1:200; cat. no. ab196495; Abcam), Bcl-2-associated X protein (Bax; 1:250; cat. no. ab32503; Abcam), and cleaved caspase-3 (1:400; cat. no. 9661; Cell Signaling Technology, Inc.) overnight at 4˚C, then incubated with the goat anti-rabbit IgG HRP-conjugated secondary antibody (1:2,000; cat. no. ab205718; Abcam) for 15 min at room temperature. After staining with 3,3-diaminobenzidine (DAB), the tissues were counterstained with hematoxylin for 3 min, and images were captured using a light microscope (Olympus Corporation).

Enzyme-linked immunosorbent assay (ELISA). Levels of the myocardial injury biomarkers aspartate aminotransferase (AST; cat. no. Y120639; Shanghai Jining Industrial), alanine aminotransferase (ALT; cat no. Y122657; Shanghai Jining Industrial), lactate dehydrogenase (LDH; cat. no. ml059178; Shanghai Enzyme-linked Biotechnology Co., Ltd.), cardiac troponin I (cTnI; cat. no. Y120631; Shanghai Jining

Industrial), creatine kinase (CK; cat. no. ml026272; Shanghai Enzyme-linked Biotechnology Co., Ltd.), and creatine kinase-MB (CK-MB; cat no. Y118621; Shanghai Jining Industrial) in serum and cells were measured using the corresponding ELISA kits, in accordance with the manufacturer's instructions. Levels of the inflammatory cytokines interleukin (IL)-1β (cat. no. ml037361), IL-6 (cat. no. ml064292), and tumor necrosis factor-α (TNF-α; cat. no. ml002859) in cells were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.). Oxidative stress factors, including the ratio of glutathione (GSH) to oxidized glutathione (GSSG; cat. no. ab138881; Abcam), as well as the content of malondialdehyde (MDA; cat. no. E-00579; Shanghai Jining Industrial), superoxide dismutase (SOD) and catalase (CAT; cat. no. BC0205; Solarbio, Beijing, China) in the cells and myocardial tissues were detected using commercial kits.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Myocardial tissue apoptosis was measured using a TUNEL kit (cat. no. C1086; Beyotime Institute of Biotechnology). Briefly, myocardial tissue slices were incubated with proteinase K at 37˚C for 30 min, followed by incubation with TUNEL reaction solution in the dark at 37˚C for 1 h. Finally, images were captured using a fluorescence microscope (Olympus Corporation). A total of five random fields of view per tissue section were examined for each sample to ensure robust statistical analysis. Fields of view were selected randomly to minimize bias.

Western blotting. Total protein from myocardial tissue or cells was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). The protein sample concentration was determined using a bicinchoninic acid (BCA) kit (Beyotime Institute of Biotechnology). After gel electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel, protein samples (25 μg) were transferred onto polyvinylidene difluoride membranes and blocked with fat-free milk for 1 h at 25˚C. Membranes were incubated overnight with primary antibodies at 4˚C, followed by incubation with the goat anti-rabbit IgG HRP-conjugated secondary antibody (1:2,000; cat. no. ab205718; Abcam) for 1 h at 25˚C. Protein bands were visualized using the ChemiDoc XRS System (Bio-Rad Laboratories, Inc.). Densitometric analysis was performed using Image Lab software (Version 6.1; Bio-Rad Laboratories, Inc.). The primary antibodies used in the present study included Bcl-2 (1:1,000), Bax (1:1,000), cleaved caspase-3 (1:1,000), Nrf2 (1:1,000; cat. no. ab92946; Abcam), heme oxygenase 1 (HO-1; 1:10,000; ab68477; Abcam), NAD(P)H quinone dehydrogenase 1 (NQO1; 1:10,000; cat. no. ab80588; Abcam), glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:10,000; cat. no. ab181602; Abcam), and lamin B1 (0.1 μg/ml; cat. no. ab16048; Abcam).

Statistical analysis. The SPSS 25.0 software (IBM Corp.) was used for experimental data analysis, and the experimental data are displayed as the mean ± standard deviation (SD). Prior to inferential analyses, a test for normality of data distribution was conducted using the Shapiro-Wilk test. The unpaired t-test was used for comparison between groups, whereas one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for the comparison of multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Bre reduces Pb-induced rat cardiomyocyte injury. To ascertain the effect of Bre on apoptosis in rat cardiomyocytes after Pb poisoning, H9C2 cells were pretreated with 1, 5 and 10 μM Bre for 1 h before exposure to PbA. As shown in Fig. 1A, compared with the control group, PbA stimulation significantly reduced the viability of H9C2 cells. Bre treatment significantly promoted H9C2 cell viability in a dose-dependent manner (P<0.01). Moreover, PbA induction significantly increased apoptosis compared with that of normal cells, whereas 5 and 10 μM Bre administration decreased apoptosis (P<0.01; Fig. 1B). The expression levels of the pro-apoptotic protein Bax and the downstream protein caspase-3 were higher, and that of the anti-apoptotic protein Bcl-2 was lower in the model group than in the control group. These trends were mitigated by Bre administration (P<0.01; Fig. 1C). LDH and CK-MB levels are indicators of cardiomyocyte injury (18). Bre dose-dependently attenuated the PbA-induced upregulation of LDH and CK-MB levels (P<0.01; Fig. 1D). Consequently, 10 μM Bre was selected for subsequent experiments.

Bre alleviates Pb-induced oxidative stress in rat cardiomyocytes. To elucidate the effect of Bre on oxidative stress in Pb-induced rat cardiomyocytes, H9C2 cells were pretreated with NAC (a ROS scavenger) with or without Bre for 1 h before PbA treatment. ROS production was significantly increased after PbA exposure in H9C2 cells, and this effect was significantly decreased by NAC or Bre treatment (P<0.01). The NAC-induced inhibition of ROS production was further amplified by Bre treatment (P<0.01; Fig. 2A). Simultaneously, GSH, SOD and CAT levels, as well as the GSH/GSSG ratio, were lower, and MDA levels were higher in the model group than in the control group (P<0.01). Bre administration effectively reversed these changes, and this effect was further potentiated by NAC (P<0.01; Fig. 2B). Additionally, Bre administration substantially lowered the levels of inflammatory cytokines IL-1β, IL-6 and TNF-α induced by PbA (P<0.01; Fig. 2C).

Bre attenuates Pb-induced myocardial injury by activating the Nrf2 pathway. Activation of the Nrf2 pathway attenuates Pb-induced kidney injury (16). To investigate whether Bre alleviates Pb-induced myocardial injury by activating the Nrf2 pathway, H9C2 cells were pretreated with Bre and ML385 (an Nrf2 inhibitor) prior to Pb exposure. Compared with the model group, ML385 impaired the viability of PbA-treated H9C2 cells, whereas Bre enhanced cell viability (P<0.05). ML385 also negated the Bre-induced enhancement of cell viability (P<0.01; Fig. 3A). Similarly, ML385 and Bre enhanced and attenuated the apoptotic ratio of rat cardiomyocytes exposed to Pb, respectively (P<0.01). ML385 treatment resulted in the Bre-induced inhibition of apoptosis (P<0.01; Fig. 3B). Furthermore, western blotting demonstrated that the expression of Nrf2, nuclear Nrf2, HO-1 and NQO1 was downregulated in PbA-induced H9C2 cells (P<0.01). ML385 significantly reduced the expression of Nrf2, HO-1 and NQO1 in PbA-induced rat cardiomyocytes (P<0.01). Bre

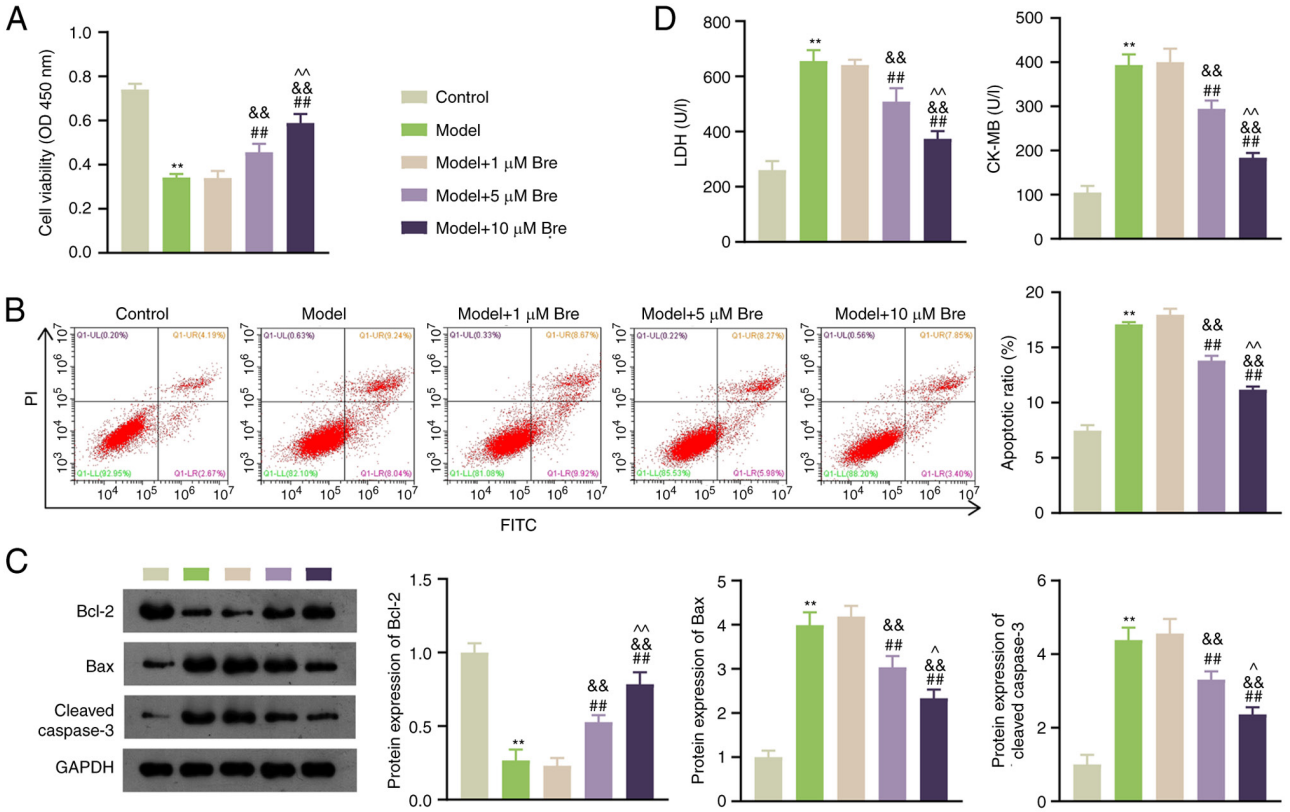

Figure 1. Bre reduces Pb-induced rat cardiomyocyte injury. (A) Cell Counting Kit-8 was used to determine cell viability. (B) Apoptosis was analyzed using flow cytometry. (C) Bcl-2, Bax and cleaved caspase-3 expression was assessed using western blotting. (D) LDH and CK-MB levels were assessed using ELISA kits. H9C2 cells were pretreated with 1, 5 and 10 μM Bre for 1 h before exposure to lead acetate. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. model group; $^{\&\&}$P<0.01 vs. model + 1 μM Bre group; ^P<0.05 and ^^P<0.01 vs. model + 5 μM Bre group. Post-hoc analyses were conducted using Tukey's test following ANOVA. Bre, Breviscapine; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB.

administration significantly increased the expression of Nrf2, nuclear Nrf2, HO-1 and NQO1 in PbA-induced rat cardiomyocytes, which was impaired by the addition of ML385 (P<0.05; Fig. 3C).

Bre relieves myocardial injury in Pb-poisoned rats. To further elucidate whether Bre can ameliorate Pb-induced myocardial damage, a rat model of Pb poisoning was constructed and the pathological changes and levels of Nrf2 pathway-related proteins in rat myocardial tissues were recorded. As revealed in Fig. S1A, rats in the control group survived and showed enhanced grasping power, whereas rats in the model group exhibited signs of depression, weaker grasping power, discoloration and hair loss. Importantly, the administration of 20 and 40 mg/kg Bre significantly attenuated these adverse effects (Fig. 4A). Blood Pb levels in the model rats were substantially elevated compared with those in the control rats, and this elevation was mitigated by the addition of 20 and 40 mg/kg Bre (P<0.01; Figs. S1B and 4B). H&E staining showed orderly arranged myocardial cells with minimal inflammatory infiltration in the control rats, whereas the model rats displayed partial myogenic fiber lysis, myocardial fiber breakage and disorganized myocardial fiber arrangement, in addition to increased inflammatory infiltration (Fig. S1C). However, this damage was relieved by Bre administration (Fig. 4C). ELISA results showed that the serum levels of the myocardial injury biomarkers AST, ALT, LDH, cTnI, CK and CK-MB increased following PbA exposure compared with those in the controls, which were weakened by Bre (P<0.01; Figs. S1D and 4D).

Further investigations were conducted to assess the effects of Bre on oxidative stress and apoptosis of myocardial histiocytes in Pb-exposed rats. The results demonstrated that the levels of GSH, SOD and CAT, as well as the GSH/GSSSG ratio, decreased in the model group. By contrast, MDA levels were elevated compared with those in the controls (P<0.01; Fig. S1E). These phenomena were reversed by Bre treatment (P<0.01; Fig. 4E). Additionally, the apoptosis-associated green fluorescence signal increased in the model group compared with the control group and was attenuated by Bre in a dose-dependent manner (Figs. S1F and 4F). Immunochemical staining revealed that Bre administration reversed PbA-induced upregulation of Bax and cleaved caspase-3 and downregulation of Bcl-2 (Figs. S1G and 4G). Western blotting corroborated these findings (P<0.05; Figs. S1H and 4H). Furthermore, Bre addition reversed the PbA-induced downregulation of Nrf2, nuclear Nrf2, HO-1 and NQO1 (P<0.05; Figs. S1H and 4H).

Discussion

Early studies have delineated the toxic effects of chronic Pb exposure on the cardiovascular system (19). In the present study, it was determined that Bre ameliorates Pb-induced myocardial injury. The underlying mechanism may involve

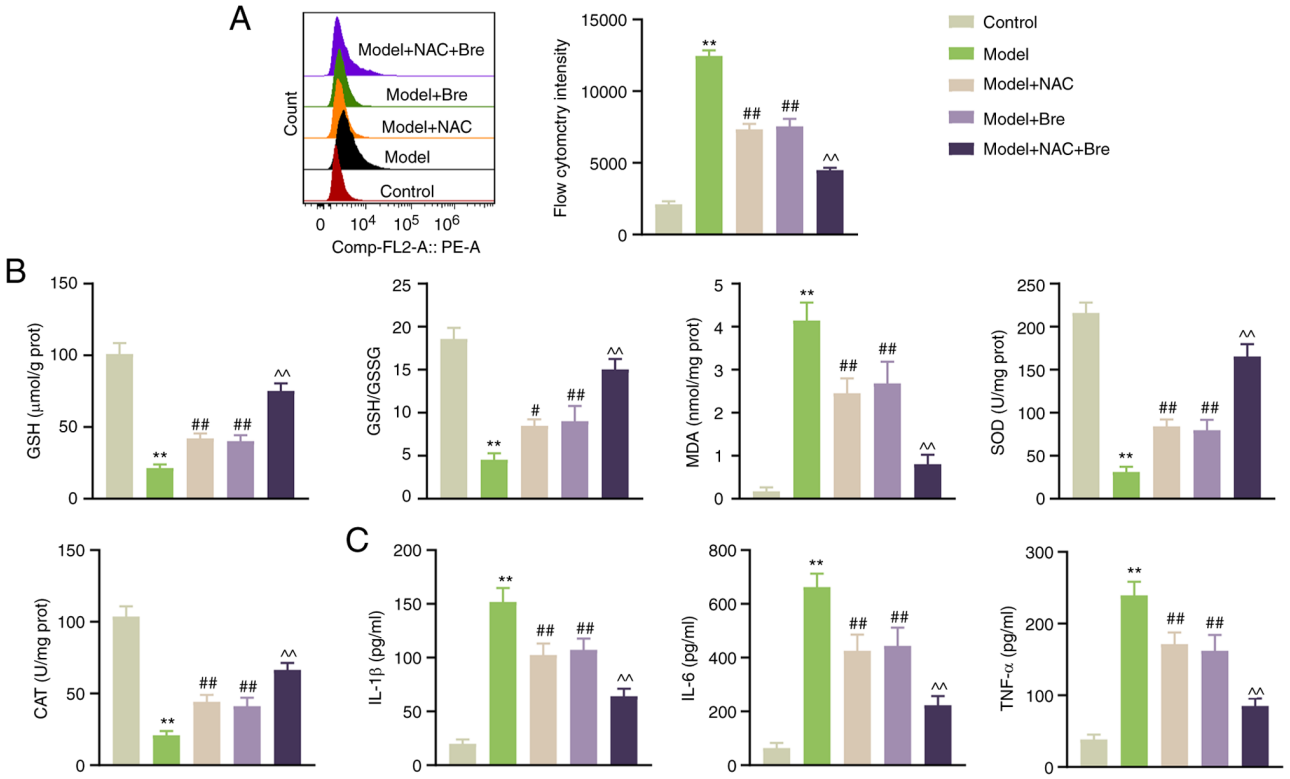

Figure 2. Bre alleviates Pb-induced oxidative stress in rat cardiomyocytes. (A) Flow cytometry was used for detection of reactive oxygen species. (B) GSH/GSSG ratio as well as GSH, MDA, SOD and CAT content in H9C2 cells were measured using ELISA. (C) IL-1β, IL-6 and TNF-α levels in H9C2 cells were determined using ELISA. H9C2 cells were pretreated with 10 mM NAC with or without 10 μM Bre for 1 h before lead acetate treatment. **P<0.01 vs. control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. Model; ^^P<0.01 vs. model + Bre group. Post-hoc analyses were conducted using Tukey's test following one-way ANOVA. Bre, Breviscapine; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; NAC, N-acetylcysteine.

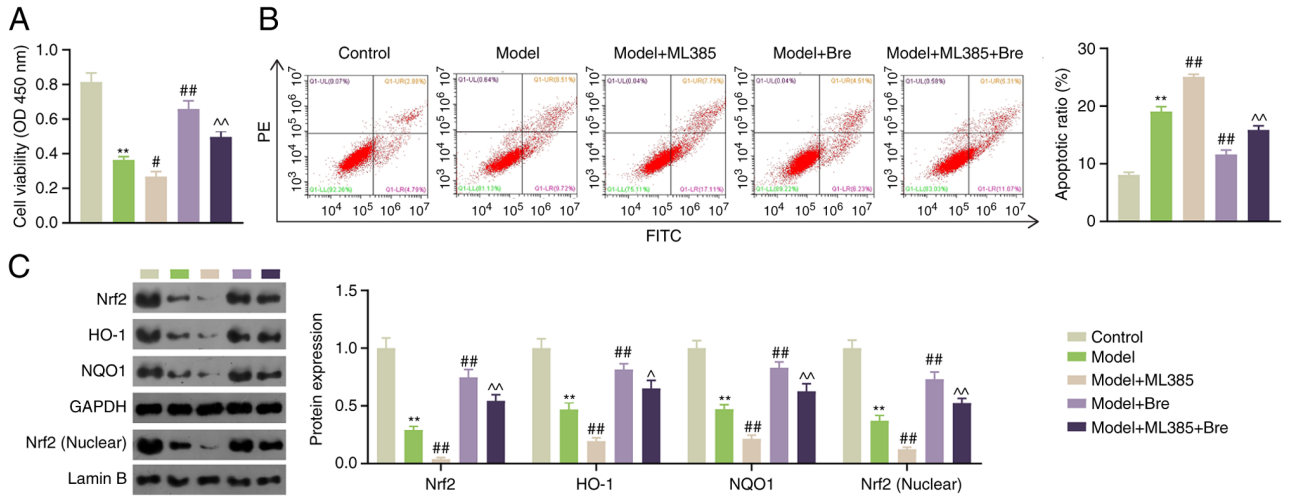

Figure 3. Bre attenuates Pb-induced myocardial injury by activating the Nrf2 pathway. (A) Cell Counting Kit-8 was used to measure cell viability. (B) Apoptosis was analyzed using flow cytometry. (C) Nrf2, HO-1, NQO1 and nuclear Nrf2 expression was assessed using western blotting. H9C2 cells were pretreated with 10 μM Bre and 20 μM ML385 prior to lead acetate exposure. **P<0.01 vs. control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. model group; ^P<0.05 and ^^P<0.01 vs. model + Bre group. Post-hoc analyses were conducted using Tukey's test following one-way ANOVA. Bre, Breviscapine; Nrf2, Nuclear factor erythroid-2 related factor 2; HO-1, heme oxygenase-1; NQO1, NAD(P)H dehydrogenase quinone 1.

activation of the Nrf2 pathway, which mitigates Pb-induced oxidative stress.

Apoptosis is the primary cause of tissue damage (20). The anti-apoptotic protein Bcl-2, pro-apoptotic protein Bax, and downstream protein caspase-3 play important roles in the apoptotic process (21). Yuan *et al* (22) reported that Bre promotes cardiomyocyte proliferation and alleviates apoptosis in hypoxic environments. In the present study, Pb exposure reduced H9C2 cell viability, decreased Bcl-2 expression, and increased Bax and cleaved caspase-3 expression relative to

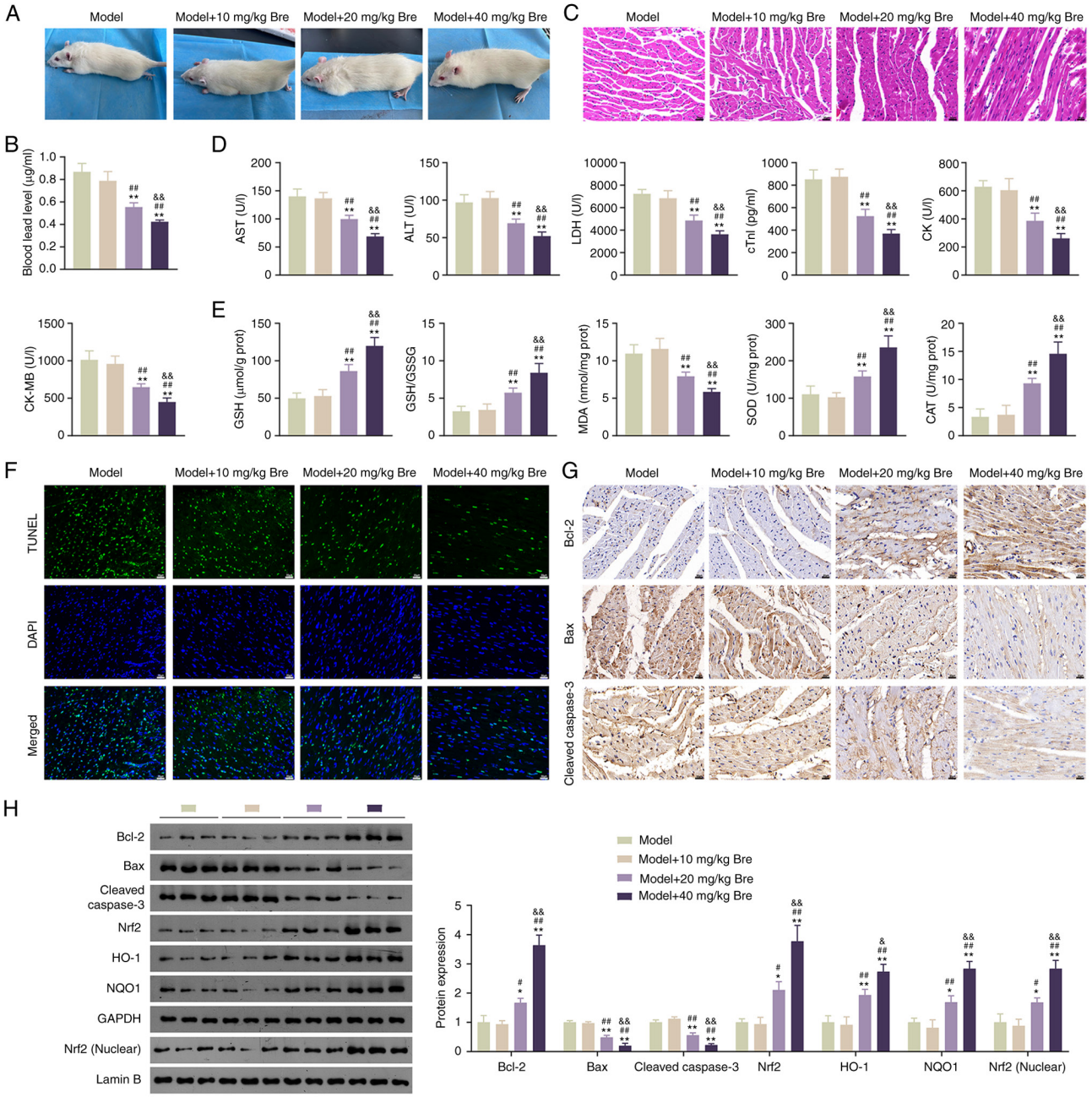

Figure 4. Bre relieves myocardial injury by activating the Nrf2 pathway in Pb-poisoned rats. (A) The appearance of Pb-poisoned rats. (B) Determination of Pb content in blood. (C) H&E staining was used to assess pathological changes in rat myocardial tissues. Scale bar, 20 μm. (D) ELISA was used to determine AST, ALT, LDH, cTnI, CK and CK-MB levels in rat myocardial tissues. (E) ELISA was used to determine GSH/GSSG as well as MDA, SOD and CAT content in rat myocardial tissues. (F) TUNEL assay was used to assess apoptosis of rat myocardial tissues. Scale bar, 20 μm. (G) Immunohistochemistry was used to analyze Bcl-2, Bax and cleaved caspase-3 expression in rat myocardial tissues. Scale bar, 20 μm. (H) Western blotting was used to determine Bcl-2, Bax, cleaved caspase-3, Nrf2, HO-1, NQO1 and nuclear Nrf2 expression in rat myocardial tissues. *P<0.05 and **P<0.01 vs. model group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. model + 10 mg/kg Bre group; $^{\&\&}$P<0.01 vs. model + 20 mg/kg Bre group. Post-hoc analyses were conducted using Tukey's test following one-way ANOVA. Bre, Breviscapine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; cTnI, cardiac troponin I; CK, creatine kinase; CK-MB, creatine kinase-MB; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; Nrf2, Nuclear factor erythroid-2 related factor 2; HO-1, heme oxygenase-1; NQO1, NAD(P)H dehydrogenase quinone 1.

controls. These changes were reversed by Bre supplementation. The findings of the present study extend the current understanding of the effects of BRE on cell viability and proliferation. Moreover, AST, ALT, cTnI, LDH, CK and CK-MB have been identified as reliable biomarkers of myocardial injury (23). The present results corroborated that Bre treatment attenuates Pb-induced upregulation of AST, ALT, cTnI, LDH, CK and CK-MB in myocardial tissues, which is consistent with the findings of a previous study (24). The results suggested that Bre ameliorated Pb-induced myocardial injury.

Toxic metals induce excessive ROS production in the tissues, thereby triggering oxidative stress (3). This is a hallmark phenomenon in the development of various diseases, such as cardiovascular diseases (25). Endogenous antioxidant enzyme systems act as defense mechanisms against internal oxidative stress (26). The GSH/GSSG ratio maintains redox

homeostasis in cardiomyocytes by reducing excess ROS production (27,28). The present study revealed that PbA exposure in rats significantly decreased antioxidant parameters, including GSH, CAT and SOD levels, as well as the GSH/GSSG ratio in myocardial tissues, while concomitantly elevating oxidative parameters and MDA content. These findings were consistent with a previous study (29). Excessive ROS production is responsible for DOX-induced endothelial toxicity and cardiotoxicity (30,31). Bre treatment increased the contents of GSH, CAT and SOD, as well as the GSH/GSSG ratio, and decreased the MDA content. In addition, *in vitro* experiments showed that NAC, acting as a ROS scavenger, diminished ROS and MDA levels while augmenting GSH and SOD levels and the GSH/GSSG ratio. Bre treatment exhibited an antioxidant effect comparable to that of NAC, and their combination provided synergistic benefits. These results indicated that Bre can attenuate oxidative stress in Pb-exposed cells and rat models.

ROS also facilitates inflammation and cytokine production. The inflammatory response participates in essential physiological processes, including the elimination of injured cells and tissues and the facilitation of cell and tissue repair (32). The ELISA results of the present study showed that PbA treatment increased the levels of inflammatory cytokines in H9C2 cells, which were alleviated by Bre administration. Bre reduced inflammatory cell infiltration in the myocardial tissue of Pb-exposed rats. Collectively, these findings suggested that Bre can inhibit the inflammatory response in the myocardial tissues, thereby mitigating myocardial damage. This supports the growing interest in BRE as a promising anti-inflammatory agent.

Nrf2 is a central regulator of oxidative stress (33). Numerous studies have reported that Nrf2 maintains redox homeostasis and prevents tissue damage caused by oxidative stress following exposure to heavy metals (34-37). In the present study, Bre treatment significantly enhanced the expression of Nrf2 pathway-related proteins (Nrf2, HO-1 and NQO1), corroborating the results of a previous study (16). Interestingly, the addition of the Nrf2 inhibitor ML385 increased apoptosis, which was suppressed by Bre treatment. These findings suggested that Bre attenuated Pb-induced myocardial injury by activating the Nrf2 pathway.

A notable contribution of the present study is the examination of the potentially multifaceted pharmacological roles of Bre. The current results suggested that Bre functions as a multifunctional agent, implementing the activation of specific signaling pathways such as the Nrf2 pathway, in addition to its known anti-inflammatory and antioxidant effects. These interactions between Bre and these signaling pathways require further investigation to elucidate novel therapeutic applications.

In conclusion, the present study showed that Bre alleviated Pb-induced myocardial injury by activating the Nrf2 pathway. This insight enriches the understanding of the mechanisms by which Bre mitigates oxidative stress and highlights its potential use in therapeutic interventions for Pb poisoning. However, certain limitations exist in the present study, such as that the focus was solely on the Nrf2 pathway as a mechanism for alleviating Pb-induced myocardial injury, without exploring alternative pathways. This introduces specific scientific gaps, which should be addressed in future studies. The present study serves as a foundational framework for future Pb poisoning treatment approaches.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DL, ZX and YL were responsible for study conceptualization. DL, ZX, YH, JY, HL and BZ contributed to data curation, formal analysis, investigation and methodology. YL and YH were responsible for project administration, resources and supervision. DL analyzed and interpreted the software results. ZX, YH, JY and BZ repeated the experiments and confirm the authenticity of all the raw data. DL and ZX contributed to visualization and writing of the original draft. YL reviewed and edited the manuscript. DL was responsible for funding acquisition. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Research related to animal use complied with all relevant national regulations and institutional policies for animal care and use. The present study was approved (approval no. 2020-029) by the Institutional Animal Care and Use Committee of the Third People's Hospital of Yunnan (Kunming, China).

Patient consent for publication.

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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