Protection of Ultra-filtration Extract from Danggui Buxue Decoction (当归补血汤) on Oxidative Damage in Cardiomyocytes of Neonatal Rats and Its Mechanism

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ABSTRACT  Objective: To investigate whether the administration of the ultra-filtration extract from Danggui Buxue Decoction (当归补血汤, EDBD) was able to protect cardiomyocytes from oxidative injury of rats induced by hydrogen peroxide (H₂O₂) and its potential mechanism. Methods: Myocardial cells from 1- to 2-day-old neonatal rats were cultured in Dulbecco's modified Eagle's medium low-glucose and Ham's F12 medium (1:1), and the cellular injury was induced by H₂O₂. The ultra-filtration extract mixture from Angelica sinensis and Hedysarum polybotrys was given in three doses of 3.75, 7.5, and 15 mg/mL. Morphological changes of cardiomyocytes were observed by microscope. Survival rate of myocardial cells was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cardiomyocyte damages were estimated by detecting lactate dehydrogenase (LDH) and creatine kinase (CK) releases in the medium, superoxide dismutase (SOD) activities, and intracellular malondialdehyde (MDA) and myeloperoxidase (MPO) contents. The levels of caspase-3 and heat shock protein 70 (70 kDa) mRNA expression in cardiomyocytes were measured by reverse transcription polymerase chain reaction. Results: The EDBD could protect the cardiomyocytes from H₂O₂ injury in a dose-dependent manner (3.75, 7.50, and 15.00 mg/mL). The EDBD could significantly decrease LDH and CK leakages and intracellular MDA and MPO contents, increase SOD activity, up-regulate hsp70 expression, and down-regulate caspase-3 expression. Conclusion: The EDBD has protection on cardiomyocytes injured by H₂O₂ through improving cell antioxidant ability, up-regulating hsp70 expression, and inhibiting caspase-3 activity. KEYWORDS  ultra-filtration extract from Danggui Buxue Decoction (当归补血汤), oxidative injury, cardiomyocyte, heat shock protein 70

Danggui Buxue Decoction (当归补血汤), a famous Chinese medicine (CM) formula, has been used to help human bodies enrich blood and invigorate qi for centuries. The formula is composed of Radix Angelica sinensis and Hedysarum polybotrys with dosage ratio of 1:5. Radix Angelica sinensis (Danggui), the dried root of Angelica sinensis (Oliv.), is warm in nature, sweet and acid in taste, and mainly acts on the Liver (Gan), Heart (Xin), and Spleen (Pi). Its main components are polysaccharide, ferulic acid, and volatile oil. Radix hedysari is Hedysarum polybotrys plant root containing glucose wake acid, sticky substance, amino acids, choline, and folic acid. Pharmacological test demonstrated that the effective components of Angelica sinensis and Radix hedysari have significant effects on antioxidants scavenging free radicals, antitumor, radioresistance, and improving immune function. Ultra-filtration, as a typical membrane technology, which has an important application value regarding high efficiency, low energy consumption, simple operation, and friendly environment and exhibited the observable technological advantage in the modernization of CM production, spreads out a scene of prosperity before us. Its advantages are embodied in the separation of the CM effective components and effective sites, getting rid of impurity and heat source of medicament, medicament concentration, and solvent reclamation. Therefore, in this study, the ultra-filtration extract (100 000 molecular weights) from Danggui Buxue Decoction (EDBD) was refined, and cardiocytes of neonatal rats were performed to established cell models as...
the research objects, wherein oxidative stress was induced by hydrogen peroxide (H$_2$O$_2$). The present study's aim was to explore whether the administration of the EDBD was able to protect rat cardiomyocytes from oxidative injury and its related mechanism.

**METHODS**

**Experimental Animals**

Wistar mice with SPF grade at postnatal days 1 to 2 were obtained from the Laboratory Animal Center, Gansu College of Traditional Chinese Medicine, with a certificate serial number SCXK (Gansu) 2004-0006-0000012.

**Reagents, Drugs, and Instruments**

Dulbecco's modified Eagle's medium low-glucose (LG-DMEM) and Ham's F12 medium (1:1 comparison), collagenase II, and 5'-bromodeoxyuridine were purchased from Sigma Co. (USA). Newborn bovine calf serum (NBCS) was obtained from Hangzhou Sijiqing Biological Pharmacy Co. (Hangzhou, China). Trypsin solutions (Invitrogen) were purchased from Sangon Biotech Co. (Shanghai, China). 30% H$_2$O$_2$ was purchased from Tianjing Baishi Chemical Industry Co. (Tianjin, China). Malondialdehyde (MDA), superoxide dismutase (SOD), lactate dehydrogenase (LDH), creatine kinase (CK), and myeloperoxidase (MPO) assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Beyotime Institute of Biotechnology (Haimen, China). Reverse transcription polymerase chain reaction (RT-PCR) assay kit was bought from Fermentas Co. (Canada). Trizol, Invitrogen and Primer were purchased from Takara Biotechnology Co. (Dalian, China). All other chemical reagents used were of the highest grade commercially available in China.

The test drug, consisting of *Angelica sinensis* and *Hedysarum polybotrys*, was provided by the Chinese Herb Extraction Room of the Medical Experimental Center, Gansu College of Traditional Chinese Medicine, and the crude drugs were purchased from Minxian County and Yanchang County, Gansu Province, China. It was prepared into a solution containing 1 g crude drugs in each milliliter, by extraction, concentration, high-pressure sterilization, and preservation in 4 °C refrigerator, ready for final molecular weight of 100 000 to produce the ultra-filtration($^{10,11}$) extract from *Angelica sinensis* and *Hedysarum polybotrys*.

The CO$_2$ constant temperature incubator (BB16UV) was purchased from Heraeus Co. (Germany). IX71 inverse-phase microscope was purchased from Olympus Optical Co. (Japan). Gene amplifying instrument was purchased from FE Co. (USA).

**Cell Culture and Grouping**

Referring to literature($^{12}$) and with slight improvements, cardiomyocytes were prepared by the following procedure: commercial frozen cardiomyocytes harvested from ventricles of 1- to 2-day-old neonatal rats were thawed and cultured according to the provider's manual. Briefly, the frozen tube was thawed in a 37 °C water bath, and the solution ($5 \times 10^5$ cells/mL) was mixed with the appropriate volume of cell culture medium kept at 37 °C to reach the concentration of $5 \times 10^5$ cells/mL. Glucose was included in the medium and cardiomyocytes were spontaneously actuated using glucose as energy source. Next, all of the solution was poured over the dish containing the microchip, which was then incubated at 37 °C in a humidified atmosphere with 5% CO$_2$. One day after seeding, the entire medium was replaced with fresh medium. Cardiomyocytes required 3 days to reach confluence, at which point they pulsed visibly, synchronously, and spontaneously in culture. Although it was difficult to observe all cells on a diagonal thin membrane, the synchronous beating was confirmed using phase-contrast microscopy.

Cultured primary neonatal rat cardiac myocyte cells were randomly divided into 5 groups as follows: (1) Normal group: the normal group without any inducer, phosphate-buffered saline (PBS) was taken as reagent. (2) Model group: the group was treated with H$_2$O$_2$ as inducer at the concentration of 400 μ mol/L.$^{(13)}$ (3) Low-dose EDBD (LEDDB) group: myocardial cells were pretreated 24 h with 3.75 mg/mL EDBD and then treated with 400 μ mol/L H$_2$O$_2$ for 6 h. (4) Medium-dose EDBD (MEDBD) group: myocardial cells were pretreated 24 h with 7.5 mg/mL EDBD and then treated with 400 μ mol/L H$_2$O$_2$ for 6 h. (5) High-dose EDBD (HEDBD): myocardial cells were pretreated with 15 mg/mL EDBD for 24 h and then treated with 400 μ mol/L H$_2$O$_2$ for 6 h.