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# Protective effect of *Dendrobium officinale* polysaccharides on $H_2O_2$-induced injury in H9c2 cardiomyocytes

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ABSTRACT

The preliminary studies have shown that *Dendrobium officinale* possessed therapeutic effects on hypertension and atherosclerosis. Studies also reported that *Dendrobium officinale* polysaccharides showed antioxidant capabilities. However, little is known about its effects on myocardial cells under oxidative stress. The present study was designed to study the protective effect of *Dendrobium officinale* polysaccharides against $H_2O_2$-induced oxidative stress in H9c2 cells. MTT assay was carried out to determine the cell viability of H9c2 cells when pretreated with *Dendrobium officinale* polysaccharides. Fluorescent microscopy measurements were performed for evaluating the apoptosis in H9c2 cells. Furthermore, effects of *Dendrobium officinale* polysaccharides on the activities of antioxidative indicators (malondialdehyde, superoxide dismutase), reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) levels were analyzed. *Dendrobium officinale* polysaccharides attenuated $H_2O_2$-induced cell death, as determined by the MTT assay. *Dendrobium officinale* polysaccharides decreased malondialdehyde levels, increased superoxide dismutase activities, and inhibited the generation of intracellular ROS. Moreover, pretreatment with *Dendrobium officinale* polysaccharides also inhibited apoptosis and increased the MMP levels in H9c2 cells. These results suggested the protective effects of *Dendrobium officinale* polysaccharides against $H_2O_2$-induced injury in H9c2 cells. The results also indicated the anti-oxidative capability of *Dendrobium officinale* polysaccharides.


## 1. Introduction

Ischemic heart disease, a leading cause of morbidity and mortality, is one of the most prevalent health problems worldwide [1]. Myocardial ischemia is one of the ischemic heart diseases, it occurs when myocardial oxygen demand exceeds supply, or in the context of coronary artery thrombosis which causes the developments of the acute myocardial infarction [2]. Increasing evidence suggested that myocardial ischemia is a major risk factor of myocardial infarction, which induces myocardial remodeling [3]. Myocardial infarction remains one of the leading causes of death and disability in cardiovascular disease all over the world [4]. Myocardial ischemia is a pathological process that results in extensive cell death. Cardiomyocytes death involves apoptotic and necrotic cell death, and apoptosis is one of the main causes of ischemia injury [5]. The mechanism of myocardial ischemia injury is apparently a multifactorial process [6]. Considerable evidences have indicated that oxidative stress and the generation of reactive oxygen species (ROS) are important contributors to the pathophysiology of myocardial ischemia injury [7]. The increase of reactive oxygen species (ROS) production is one of the key events in myocardial

*Abbreviations:* ROS, reactive oxygen species; DOE, *Dendrobium officinale* extracts; MMP, mitochondrial membrane potential; T-SOD, total superoxide dismutase; MDA, malondialdehyde; DMSO, dimethyl sulfoxide; MTT, 3-[4,5-dimethyl-2-thiazolyl]-2 5-diphenyl-2-tetrazolium bromide; AO, acridine orange; CVDs, cardiovascular diseases; MI, myocardial infarction; TCM, traditional Chinese medicine.

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ischemia injury [3]. Excessive ROS generation leads to mitochondrial injury, including loss of mitochondrial membrane potential (ΔYm) and results in apoptosis [8].

In recent years, traditional Chinese medicine has gained increased attention for the treatment of various diseases. It has been demonstrated that traditional Chinese medicine can affect the occurrence and development of ventricular remodeling in a number of aspects [3]. *Dendrobium officinale Kimura et Migo* [9] is one of the traditional Chinese medicinal herbs and has been used as herbal medicine in many Asian countries for centuries [10]. Due to its various pharmacological effects, *Dendrobium officinale* is recognized as a high quality health food in China and other south-east Asian countries [11]. Increasing evidence showed that *Dendrobium officinale* possesses various potential effects, such as anti-oxidative [12], anti-cancer [13,14] and immunomodulation effects [15]. In addition, *Dendrobium officinale* could also inhibit pro-inflammatory cytokines and attenuate inflammation response [16]. On basis of these findings, we hypothesized that *Dendrobium officinale* might possess the protective effect against oxidative stress and apoptosis induced by $H_2O_2$ in H9c2 cells in vitro.

In the present study, we have investigated the effects of *Dendrobium officinale* exacts (DOE) in $H_2O_2$ induced oxidative stress in H9c2 cells. Our results showed that *Dendrobium officinale* possesses the protective effects against $H_2O_2$ induced oxidative stress and apoptosis in H9c2 cells, and the mechanism is possibly associated with the inhibition of oxidative stress and the reduction of apoptosis by increasing mitochondrial membrane potential.

## 2. Materials and methods

### 2.1. Chemicals and materials

Total superoxide dismutase (T-SOD) and malondialdehyde (MDA) diagnostic kits were obtained from Jiancheng Bioengineering institute (Nanjing, China). Dimethyl sulfoxide (DMSO), 3-[4,5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2-tetrazolium bromide (MTT) and acridine orange (AO) were purchased from sigma chemicals (St Louis, Mo, USA). DMEM were purchased from HyClone (USA). The fetal bovine serum was obtained from PAN Biotech (Germany). All other reagents used were of commercial analytical grade.

### 2.2. DOE preparation

The dry stems of *Dendrobium officinale* were purchased from Beijing Tongrentang. The voucher specimens are deposited in Southwest University. The dry stems were grinded into fine powder through 100-mesh. The powdered materials (100 g) were pre-extracted by petroleum ether. The residues were extracted 3 times with hot distilled. The crude extracts were filtered, concentrated and then dried by lyophilization and then 23.5 g powder extracts were produced. The water extracts mainly consist of polysaccharide and the concentration of polysaccharide determined by phenol-sulfuric acid method was 92.8%.

### 2.3. Cell culture

Rat-derived H9c2 cardiomyocytes were purchased from ATCC. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin at 37 °C in 5% $CO_2$. Cells were passaged and subcultured to 90% confluence with 0.25% trypsin (w/v) every 2–4 days.

### 2.4. Oxidative stress injury induced by $H_2O_2$

H9c2 cells were collected with trypsin and re-suspended and then seeded in 96-well multiplates with a final density of $1 \times 10^5$ cells per well. After incubation for 24 h, H9c2 cells were exposed with (0, 50, 100, 150, 200, 250, 300, 400 μM) $H_2O_2$ for 6 h. Then the MTT assay was performed to detect the cell viability as described previously [17]. Briefly, 10 μl of the MTT (5 mg/mL) was added to each well, and the final concentration of MTT was 0.5 mg/mL. After incubation for 4 h under standard condition, the cell supernatants were removed. The DMSO (100 μl) was added into every well to dissolve the MTT crystals (formanzan) and the absorbance was measured with a BIO RAD microplate reader (model 630, USA) at 490 nm. The relative cell viability was expressed as the percentage of control well (not treated with drugs)

### 2.5. Cell viability assay

Cardiomyocytes were pretreatment with 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL polysaccharides followed by exposure to $H_2O_2$ (200 μM). Then the cell viability was determined by the MTT assay.

### 2.6. Determination of superoxide dismutase (SOD) and malondialdehyde (MDA) activity

The SOD and MDA activities in cultured supernatant were determined by commercially available kits in accordance with the manufacturer's instructions.

### 2.7. Mitochondria membrane potential (MMP)

MMP was measured as previously reported [18]. Briefly, following drug treatment, cells were washed twice with PBS. The cells were further incubated with 10 μg/mL of Rhodamine-123 for 1 h at 37 °C in dark. The fluorescence intensity was measured with spectrofluorometer at 485 nm excitation and 530 nm emission wavelengths.

### 2.8. Fluorescent microscopy measurements

Acridine orange (AO) staining was used for detecting the cell apoptosis [19]. In the present study, H9c2 cells were seeded in 6 well plates at a density of $1 \times 10^6$ cells per well. Then cells were treated with $H_2O_2$ or different concentrations of polysaccharides (0.1, 0.2, 0.4 mg/mL) for 12 h. 10 μl of prepared AO working solution (100 μg/mL in PBS) was added into each well. The cells were immediately examined with a fluorescence microscope (Olympus U-RFLT50, Tokyo, Japan). Morphologically apoptotic and necrotic cells were counted from 10 visual fields of 5 different areas for each group.

### 2.9. Measurement of ROS production

DCFH-DA (2′, 7′-dichlorofluorescin-diacetate) fluorescent labeling was used to detect intracellular ROS accumulation in H9c2 cells. H9c2 cells were exposed to $H_2O_2$ (200 μM) or polysaccharides (0.1, 0.2, 0.4 mg/mL). Then the cell supernatants were removed and continually incubated with 10 μM DCFH-DA for 20 min at 37 °C in dark. After DCFH-DA was removed, the cells were washed twice with PBS and maintained in 1 mL serum-free medium. The fluorescence analysis were performed under a fluorescence microscope (OLYMPUS U-RFLT50, Japan) under × 200 magnification with the filter (excitation at 470–490 nm and emission at 510–550 nm)[20].