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Cardioprotective and antioxidant activities of a polysaccharide from the root bark of *Aralia elata* (Miq.) Seem

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ABSTRACT

A water-soluble polysaccharide (AEP-w1) was isolated from the root bark of *Aralia elata* and its molecular weight was about 4.5×10^4 Da. Monosaccharide component analysis indicated that AEP-w1 appeared to be arabinogalactan, consisting of arabinose, galactose and trace glucose with molar ratios of 6.3:3.5:0.2. The antioxidant and cardioprotective potential of AEP-w1 were evaluated in vitro for the first time. AEP-w1 showed potent superoxide and hydroxyl radical scavenging activities and reducing power in vitro. Treatment with H_2O_2 resulted in the death of H9c2 cells, whereas pretreatment with 50–400 μg/ml AEP-w1 for 24 h prior to H_2O_2 exposure significantly increased cell viability. Furthermore, AEP-w1 evidently suppressed cardiomyocyte apoptosis, the mitochondrial membrane potential change and cytochrome c release in H_2O_2-treated H9c2 cells. In addition, intracellular reduced glutathione (GSH) reduction caused by H_2O_2 in H9c2 cells was also restored by AEP-w1 pretreatment. Taken together, these date provided the first evidence that the cardioprotective effects of AEP-w1 in H9c2 cells were at least in part associated with its antioxidant activity and inhibition effect on mitochondrial dysfunction.

1. Introduction

Aralia elata Seem (Araliaceae) is widely distributed in Oriental countries, such as Korea, Japan, and China. The young shoots of *A. elata* are a popular edible plant, especially in the spring. Its barks and root cortexes are widely used in folk medicine for the treatment of diabetes, gastric ulcer, hepatitis, and rheumatoid arthritis (Lee, Jeong, Lee, & Jeong, 2009; Nhiem et al., 2011). Several bioactive constituents from root bark of *A. elata* were reported to have various biological activities, such as cytotoxic (Tomatsu, Kameyama, & Shibamoto, 2003), anti-inflammatory (Suh, Jin, & Kim, 2007), liver-protecting (Saito et al., 1993), antioxidative (Zhang et al., 2006), antiviral (Li, Tian, & Shi, 1994), hypolipidemic (Yoshikawa, Matsuda, Harada, & Elatoside, 1994) and antidiabetic properties (Kim, 1993). The effective components of *A. elata* included saponins, alkaloids, glycosides, palmitic acid, linoleic acid, etc. (Lee, Kim, Kang, & Jeong, 2005). However, the pharmacological action and the chemical components of the polysaccharides have rarely been reported. To expand and protect the resources of this valuable medicinal plant, we initiated chemical investigations on the polysaccharide constituents from the root bark of *A. elata* and evaluated its antioxidant and cardioprotective activities in vitro.

2. Materials and methods

2.1. Materials and reagents

The root barks of *A. elata* were harvested from the Northern countryside region of Shaanxi province, China. The samples were thoroughly washed with water, air-dried and finely pulverized into a powder.

DEAE-52 cellulose, Sephadex G-200 and Dextrans of different molecular weights (T-130, T-80, T-50, T-25 and T-10) were purchased from Amersham Co. (Sweden). Dulbecco's modified essential medium (DMEM), fetal bovine serum (FBS) and other tissue culture reagents were obtained from Life Technologies Inc. (Gaithersburg, MD, USA). Nitro blue tetrazolium (NBT), vitamin C, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), phenazine methosulfate (PMS), hydrogen peroxide (H_2O_2), ferrozine, nicotinamide adenine dinucleotide-reduced (NADH), trifluoroacetic acid (TFA) and trichloroacetic acid (TCA) were from Sigma Chemical Co. (St. Louis, MO, USA). All of other reagents were analytical grade from Peking Chemical Co. (Peking, China).

2.2. Extraction, separation and purification of the polysaccharide

After the dried root bark powder of *A. elata* (200 g) were cleaned, dried, and ground, the powders were extracted with 80% ethanol for 24 h to remove the interference components such as monosaccharide, disaccharide, and oligosaccharide in the samples at 80 °C.

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Then the residue was extracted thrice with 2500 ml of distilled water at 70–80 °C for 6 h. The filtrate of the combined extract was condensed to a 1/5 original volume, to which cold 95% ethanol (approximately 1500 ml) was added at 4 °C overnight. The crude polysaccharides precipitated by ethanol were repeatedly washed sequentially with possibly less amounts of ethanol, acetone and ether in turn, and collected by centrifugation, and then dried under reduce pressure. The refined crude polysaccharide was re-dissolved in distilled water (240 ml) at a concentration of 1% (w/v) followed by filtration, and then was frozen at −20 °C, and thawed at room temperature and centrifuged to remove insoluble materials. The supernatant was deproteinated by Sevag method (Staub, 1965), and then crude water soluble polysaccharides (AEP, 8.5 g) was obtained by centrifugation at 12,000 × *g* for 10 min followed precipitation by anhydrous ethanol overnight at 4 °C.

The crude polysaccharide AEP (8.5 g) was re-dissolved in 50 ml distilled water, filtered through 4.5×10^{-4} mm filters, loaded on a DEAE-52 cellulose column (2.6 × 30 cm) and eluted with distilled water and different concentrations of stepwise NaCl solution (0.3, 0.6 and 1.0 M NaCl), respectively, at a flow rate of 1.0 ml/min. Fraction was collected and monitored with the phenol–sulfuric acid method. One main fraction eluted by distilled water was collected, dialyzed, lyophilized, and was further purified on a Sephadex G-200 column (2.6 × 70 cm) column eluted with 0.15 M NaCl. The main fraction, codes as AEP-w1, was collected, dialyzed and lyophilized for further analysis.

2.3. Physicochemical property of the polysaccharide

2.3.1. Measurement of carbohydrate, protein and uronic acid contents

Total carbohydrate content of AEP-w1 was determined by phenol–sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was quantified according to the Bradford's method (Bradford, 1976). Total uronic acid content was measured by mhydroxydiphenyl method (Filisetti-Cozzi & Carpita, 1991).

2.3.2. Molecular weight determination

The homogeneity and molecular weight of AEP-w1 were evaluated and determined by high performance gel permeation chromatography (HPGPC). The samples were dissolved in 0.1 mol/l Na_2SO_4 to reach a final concentration of 5 mg/ml and filtered through 0.45 μm filter membrane before injection. The filtrate was performed on a Shimadzu LC-10ATvp HPLC system fitted with a TSK-GEL G3000 SWXL column (7.8 mm × 300 mm) and a Shimadzu RID-10A detector set at 40 °C, and eluted with Na_2SO_4 (0.1 mol/l) at a flow rate of 0.5 ml/min. The column calibration was performed with various standard dextrans (T-130, T-80, T-50, T-25 and T-10) and then the elution volumes were plotted against the logarithm of their respective molecular weights. The molecular weight of AEP-w1 was estimated by reference to the calibration curve made above.

2.3.3. Monosaccharide composition

GC–MS was used for identification and quantification of the monosaccharide composition (Li, Yuan, & Rashid, 2009). Twenty milligrams of polysaccharides were hydrolyzed with 2 M TFA (4 ml) at 120 °C for 6 h in a sealed tube. After removal of TFA, the hydrolyzates were dissolved in H_2O (2 ml) and reduced with $NaBH_4$ (40 mg) at room temperature for 2 h, followed by acidification with acetic acid. It was then co-distilled with methanol to remove the excess boric acid. Thereafter, the resulting alditols were treated with 1:1 pyridine–acetic anhydride to convert into their alditol acetates and analyzed by gas chromatography (GC). Their corresponding alditol acetates were subjected to gas GC on an Agilent 4890D system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and a DB-35 capillary column (30.0 m × 0.32 nm × 0.25 μm). The operation was performed in the following conditions: The temperature of the injector and detector was 280 °C; column temperature programmed: from 110 °C to 190 °C at 5 °C/min, holding for 5 min at 190 °C, then increasing to 250 °C at 10 °C/min and finally holding for 5 min at 230 °C. Nitrogen was used as the carrier gas. Quantitation was calculated from the peak area using response factors.

2.4. Assay for antioxidant activity

2.4.1. Superoxide radical assay

A method as described by Wang et al. (2009) was used to measure superoxide anion scavenging ability of AEP-w1. Superoxide radicals were generated in a PMS/NADH system containing varying concentrations of AEP-w1 (25, 50, 100, 200 and 400 μg/ml), Tris–HCl (16 mM, pH 8.0), NADH (338 μm), NBT (72 μm) and PMS (30 μm). The mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm. The deionized water was used as the blank control and vitamin C was used as positive control. The scavenging activity of superoxide radicals (%) was calculated according to the following equation:

$$\text{Scavenging effect}(\%) = \frac{A_{560}(\text{blank}) - A_{560}(\text{sample})}{A_{560}(\text{blank})} \times 100$$

where $A_{560(blank)}$ was the absorbance of the control (deionized water, instead of sample); $A_{560(sample)}$ was the absorbance of the test sample mixed with reaction solution.

2.4.2. Hydroxyl radical assay

Assessment of the scavenging ability of AEP-w1 on hydroxyl radicals was performed by the method previously described by Wang et al. (1994), with some modification. In brief, different concentration (25, 50, 100, 200 and 400 μg/ml) samples was prepared in deionized water, and 100 μl of each sample solution was sequentially mixed with EDTA-$Fe2^+$ (2 mM), saffron (360 μg/ml), and H_2O_2 (3%) in potassium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 °C. Subsequently the mixture was detected by monitoring the absorbance at 520 nm. Deionized water and vitamin C served as blank and positive control, respectively. Solutions of ferrous sulfate and ascorbic acid were made immediately before use. Hydroxyl radical bleached the saffron, so decreased absorbance of the reaction mixture indicated a decrease in hydroxyl radical-scavenging ability. The hydroxyl radical scavenging effect was calculated as follows:

Scavenging effect(%) $= \frac{A_{520(\text{sample})}}{A_{520(\text{blank})}} \times 100$where $A_{520(blank)}$ was the absorbance of the control (deionized water, instead of sample); $A_{520(sample)}$ was the absorbance of the test sample mixed with reaction solution.

2.4.3. Reducing power assay

The reducing power of AEP-w1 was measured following the method described earlier by Yen and Chen (1995) with minor modification. Different concentrations of samples (25, 50, 100, 200 and 400 μg/ml, 2.5 ml) were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated for 20 min at 50 °C. Distilled water was used as a control, and vitamin C was used for comparison. The reaction terminated by adding TCA solution (10%, w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1%, w/v) solution and the absorbance was measured at 700 nm against blank. A higher absorbance indicated a higher reducing power.

2.5. Cell and cell culture

A H9c2 myoblast cell line (Catalo No. CRL-1446), a clonal line derived from embryonic rat heart, was purchased from American Type Culture Collection (ATCC, Manassas, VA). The cells were routinely maintained in DMEM with 10% (v/v) heat-inactivated FBS, penicillin G (100 U/ml), streptomycin (100 mg/ml) and L-glutamine (2 mM), in a humidified incubator at 37 °C under 5% CO_2/air prior to use. The medium was changed every 2 days and when confluence was reached, the cells were subcultured by detaching with 0.25% trypsin–EDTA solution (Sigma) and re-seeding into new plates at a ratio of 1:5, then incubated in DMEM containing 2% FBS. Cells were kept at 37 °C in a humidified 5% CO_2/95% air incubator.

2.6. Measurement of cell death response to oxidative stress.

To determine the injury induced by H_2O_2, the H9c2 cells were seeded into a 96-well cultivation plate at 10^5 cells/well and cultured with different concentrations of H_2O_2 ranging from 50 to 400 μM in DMEM media under an atmosphere of 5% CO_2 at 37 °C for 12 and 24 h, respectively. The cell viability was determined by a colorimetric MTT assay, as described previously (Ren, Zhao, Yang, & Fu, 2008). Briefly, following exposure to H_2O_2, 20 μl of the MTT solution (5 mg/ml) was added into each well with the final concentration at 0.5 mg/ml, and then the plates were incubated for an additional 4 h at 37 °C and measured by absorbance at 490 nm in a microplate reader. Cell viability (%) was calculated using the following equation:

$$\text{Cell viability}(\%) = \frac{A_{\text{treatment}}}{A_{\text{control}}} \times 100\%$$

The OD of formazan formed in the control cells was taken as 100% viability. All determinations were conducted in triplicate.

In order to evaluated the protective effect of AEP-w1 on H_2O_2-induced oxidative stress to H9c2 cells, cells were pretreated with AEP-w1 (100, 200, 400 μg/ml) for 24 h in DMEM media. After two wash with phosphate buffered saline (PBS, pH 7.4), the cells were exposed to 200 μM H_2O_2 for 24 h, and control cells were also incubated under the same conditions. Cell viability was determined by MTT assay as described above.

2.7. Flow cytometry analysis for apoptosis

Flow cytometric analysis of apoptotic cell death was performed by both FITC-Annexin V and propidiumiodide (PI) double labeling as described elsewhere (Zamal, Falcieri, Marhejka, & VitaleI, 1996). H9c2 cells (10^6 cells/well) treated with H_2O_2 in the presence or absence of AEP-w1 (100, 200, 400 μg/ml) for 24 h were harvested, washed with PBS, and then stained with FITC-Annexin V and PI followed by flow cytometry analysis with a Beckton–Dickinson flow cytometer (FACS).

2.8. Measurement of mitochondrial membrane potential

Mitochondrial membrane potential ($\Delta\Psi m$) was measured by the incorporation of cationic fluorescent dye, rhodamine 123 (Rh123) (Wu et al., 2012). After 24 h incubation in normal medium with or without AEP-w1 at the concentrations of 100, 200 and 400 μg/ml, the H_2O_2-challenged cells were harvested and incubated for 15 min at 37 °C with 5 μM Rh123 in the dark. The cells were then collected and the fluorescence intensity was analyzed within 15 min by a spectrophotofluorimeter (BD FACScanTM, USA, 490 nm excitation and 515 nm emission).

2.9. Measurement of mitochondrial cytochrome c release

Cytochrome c ELISA kits were used to detect cytochrome c. In brief, the cells were treated with AEP-w1 at designed concentration of 100, 200 and 400 μg/ml for 24 h and then treated with 200 μM H_2O_2 for 24 h. The cells were centrifuged at 1000 g for 5 min at 4 °C. The supernatants were saved, as these contained the cytosolic fraction of cytochrome c. And the resulting released cytochrome c in supernatant was determined by a commercial available cytochrome c assay kits according to the manufacturer's directions.

2.10. Measurement of cellular reduced glutathione (GSH)

Cell lysates were prepared as described in Chiu, Leung, Poon, Mak, & Ko (2006). GSH levels in cell lysates were measured using a GSH assay kit (Shanghai Qianchen Biotech Co., China) according to the manufacturer's instructions.

2.11. Statistical analysis

All datawere expressed as mean ± SD. Numerical data were compared using Student's *t*-test for paired observations between two groups. A value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Purification and physicochemical characters of the polysaccharide

In the present study, crude polysaccharides AEP were isolated from the root bark of *A. elata* and the yield was about 4.25%. Firstly, the AEP was separated through a DEAE-52 cellulose chromatography and eluted stepwise with 0, 0.3, 0.6 and 1.0 M NaCl solution, affording three independent elution peaks (AEP-1, AEP-2 and AEP-3) as detected by the phenol–sulfuric acid assay. Furthermore the neutral polysaccharide AEP-1, eluted by distilled water, was collected, concentrated and purified on a Sephadex G-200 column with 0.15 M NaCl at a flow rate of 0.5 ml/min, giving one purified polysaccharide (AEP-w1).

The total sugar, protein, and uronic acid contents, as well as molecular weight and monosaccharide compositions of the polysaccharide are summarized in Table 1. On HPGPC, AEP-w1 showed a single symmetrical peak, indicating it was a homogeneous polysaccharide. Correlation with the calibration curve of Dextran standards indicated that its molecular weight was about 4.5×10^4 Da. Lack of absorption at 280 and 260 nm by UV scanning indicated that AEP-w1 contained no protein and nucleic acid. The total carbohydrate and uronic acid content of AEP-w1 were 96.3% and 1.1%, determined by phenol–sulfuric acid method and m-hydroxydiphenyl colorimetric method, respectively. According to GC analysis, AEP-w1 appeared to be arabinogalactan, consisting of galactose, arabinose and trace glucose with molar ratios of 6.3:3.5:0.2.

3.2. Antioxidant activities of the polysaccharide

Superoxide anion, one of the precursors of the singlet oxygen and hydroxyl radicals, is known to indirectly initiate lipid peroxidation (Wickens, 2001). Therefore its scavenging is extremely important to antioxidation work. Superoxide radical was determined by the PMS–NADH superoxide generating system and the result was shown in Fig. 1 A. The superoxide anion-scavenging activity of AEP-w1 was 19.5–69.2% with increasing sample concentration raging from 25–400 μg/ml, whereas that of vitamin C was

Table 1
physicochemical characters of purified polysaccharide AEP-w1.

Sample	Molecular weight (Da)	Absorption at 280 and 260 nm	Total sugar (%)	Uronic acid (%)	Protein (%)	Monosaccharide content (molar ratio)		
						Arabinose	Galactose	Glucose
AEP-w1	4.5×10^4	–	96.3	1.1	–	6.3	3.5	0.2

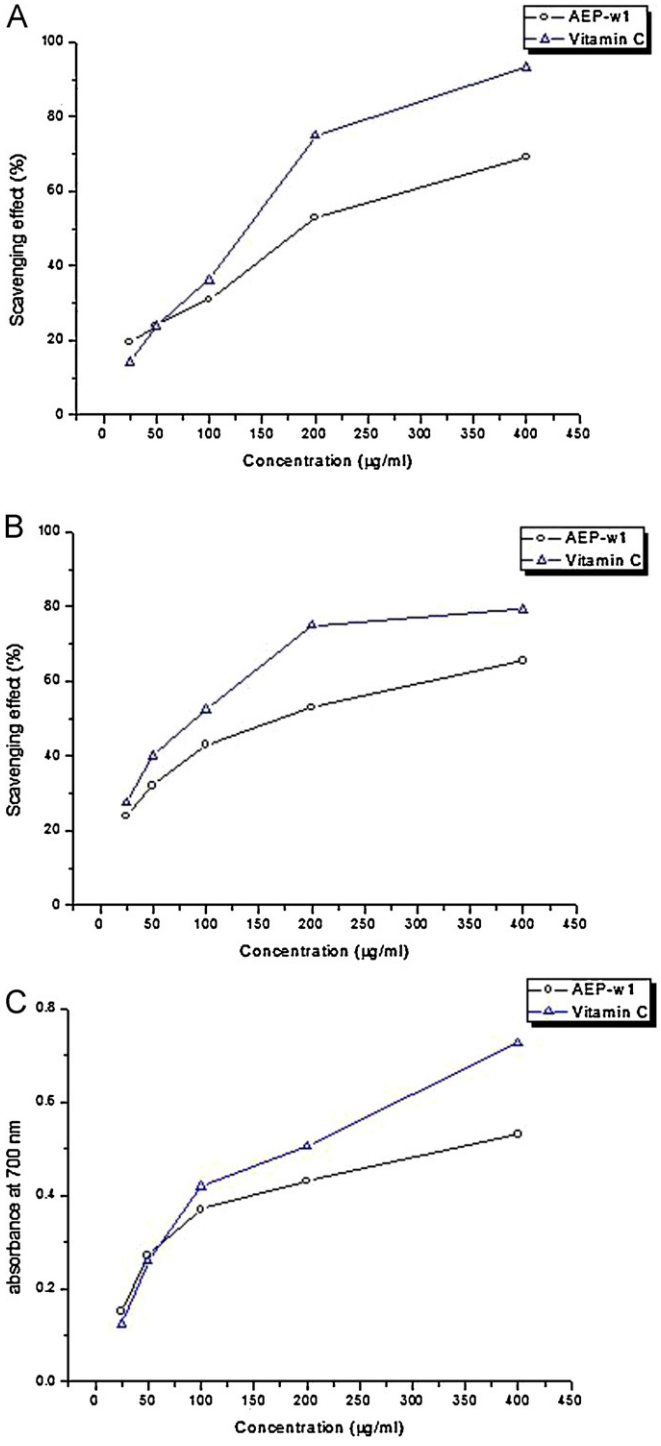

Fig. 1. Scavenging effects of AEP-w1 on (A) superoxide radicals; (B) hydroxyl radicals; (C) reducing power with ascorbic acid as a positive control. All data were shown as mean ± SD of three separate experiments.

16.4–79.5% at the same dosages. AEP-w1 exhibited weaker scavenging effect than vitamin C at all concentrations, but beyond the concentration of 100 μg/ml, the scavenging activity was closed to vitamin C. Therefore, AEP-w1 had an appreciable scavenging power on superoxide anion in the higher doses and it was possible that the antioxidant activity of AEP-w1 was related to its scavenging ability on superoxide radical.

Hydroxyl radical is the most reactive free radical among the oxygen radicals and can cause severe oxidative injury by superoxide anion and hydrogen peroxide, which is associated with the indirect production of hydroxyl radicals (Erel, 2004; Macdonald, Galley, & Webster, 2003). Thus, the hydroxyl radical scavenging ability was measured in the present study. Fig. 1B depicted the scavenging effects of AEP-w1 on the hydroxyl radical. The scavenging power correlated well with increasing concentrations. Moreover, AEP-w1 showed pronounced high radical scavenging activity (65.4%), which was closed to that of vitamin C at the concentration 400 μg/ml. The results of the scavenging activity of AEP-w1on hydroxyl radical suggested that it was likely to contribute toward the observed antioxidant effect.

In the reducing power assay, the presence of antioxidant substance in the tested samples would cause a reduction of Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}), and the color change reflects the reducing power of the antioxidants (Qi et al., 2006). Higher absorbance value means stronger reducing power. Fig. 1C depicted the reducing power of the samples and vitamin C. AEP-w1 exhibited significant reducing power at all concentration points. With the concentration increased from 25 to 400 μg/ml, the absorption of AEP-w1 was increased from 0.19 to 0.53. However, the reducing power of AEP-w1 was lower than vitamin C. Our data showed that AEP-w1 probably play a role in the antioxidation observed, because of its reducing power.

3.3. AEP-w1 attenuated H_2O_2-induced damage in H9c2 cells

For the purpose of evaluating whether AEP-w1 protects H9c2 cells from oxidative stress induced by H_2O_2, an MTT assay was first carried out to measure the cell viability of H9c2 cells 12 and 24 h after exposure to 50–400 μM H_2O_2. A significant decrease in viability of H9c2 cells was achieved in a dose- and time-dependent manner, which allowed the cells to be maintained with 200 μM H_2O_2 for 24 h in the next experiments (data not shown). As shown in Fig. 2, the addition of 200 μM H_2O_2 to the H9c2 cells for 24 h resulted in a drastic increase of cytotoxicity, as revealed by a 72.1% fall in cell viability compared with the normal control cells, whereas pretreatment with AEP-w1 had a significant protective effect against H_2O_2-mediated cytotoxicity in H9c2 cells at the designed concentration range of 100–400 μg/ml in a dose-dependent manner. Especially when the concentration of AEP-w1 was up to 200 μg/ml, over 60% viability was observed during the test interval.

3.4. AEP-w1 inhibited H_2O_2-induced apoptosis in H9c2 cells

Significant cell apoptosis was induced in H9c2 cells after challenge with 200 μM H_2O_2 by the annexin V/propidium iodide staining in the present study, consistent with the observation demonstrated previously by Chen, Tu, Wu, & Bahl (2000).

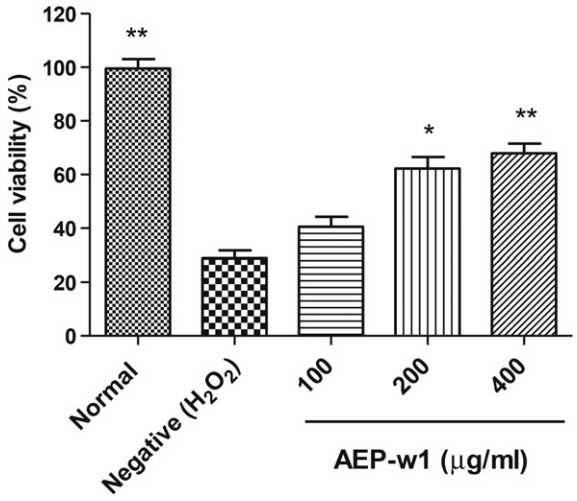

Fig. 2. Protective effect of AEP-w1 on H_2O_2-induced cytotoxicity in H9c2 cells. All data were shown as mean ± SD of three separate experiments. $^*P < 0.05$, $^{**}P < 0.01$ vs. negative control.

However, pretreatment with AEP-w1 at the concentrations of 200 and 400 μg/ml significantly protect H9c2 cells from H_2O_2-induced oxidative stress as compared with negative control ($P < 0.05$ or $P < 0.01$) (Fig. 3).

3.5. AEP-w1 suppressed mitochondrial dysfunction and cytochrome c release caused by H_2O_2 in H9c2 cells

Mitochondria play a key role in apoptosis related to oxidative stress and produce a considerable quantity of reactive oxygen species (ROS), including superoxide and hydrogen peroxide, which can cause damage to the macromolecules of cells (Van Houten, Woshner, & Santos, 2006). Therefore, an intact mitochondria organelle with normal functions would be in favor of inhibiting the apoptosis of myocardial cell. To verify the possibility, the influence of AEP-w1 on the function of mitochondria was analyzed by employing a fluorescent dye Rh123 on a spectrophotofluorimeter. As shown in Fig. 4A, exposure of H9c2 cells to H_2O_2 caused a dramatic change in mitochondrial transmembrane depolarization, characterized as a fall in mitochondrial membrane potential. Pretreatment with AEP-w1 could restore the mitochondrial membrane

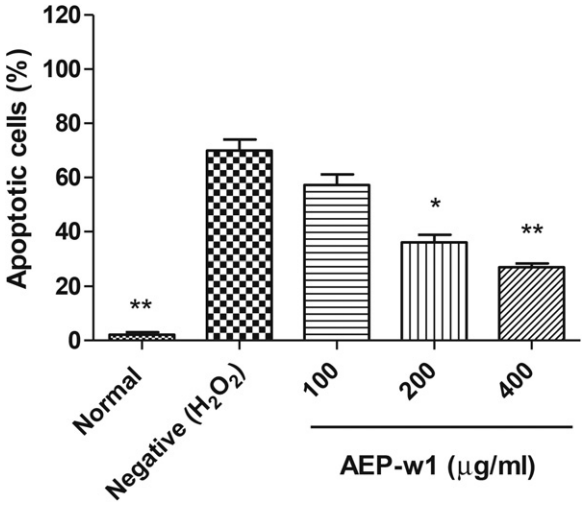

Fig. 3. The inhibition effect of AEP-w1 on H_2O_2-induced apoptosis in H9c2 cells. All data were shown as mean ± SD of three separate experiments. $^*P < 0.05$, $^{**}P < 0.01$ vs. negative control.

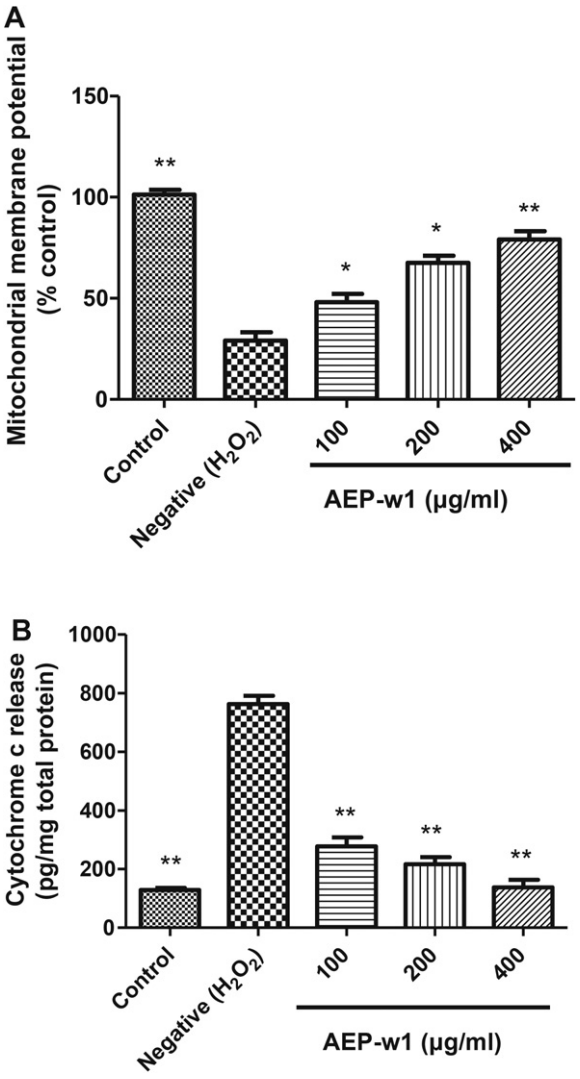

Fig. 4. The inhibition effect of AEP-w1 on H_2O_2-induced mitochondrial dysfunction and cytochrome c release in H9c2 cells. All data were shown as mean ± SD of three separate experiments. $^*P < 0.05$, $^{**}P < 0.01$ vs. negative control.

potential to the normal level. Normally mitochondrial membrane potential change can eventually leads to cytochrome c release from the mitochondria. To provide in-depth insight, the cytochrome c release was examined by ELISA kits in cytosolic parts. AEP-w1 pretreatment at the concentrations of 100, 200 and 400 μg/ml suppressed the H_2O_2-induced release of cytochrome c by 63%, 72% and 82%, respectively, relative to that of challenged control (Fig. 4B).

3.6. AEP-w1 inhibited the cellular GSH reduction caused by H_2O_2 in H9c2 cells

GSH is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Hughes, 1964; Scholz, Graham, Gumpricht, & Reddy, 1989). As shown in Fig. 5, H_2O_2 challenge prominently decrease intracellular GSH levels in H9c2 cardiomyocytes. While AEP-w1 treatments caused 1.1–1.5 fold increase in cellular GSH levels, as compared with that of the negative control. From the data we can conclude that the cytoprotection against H_2O_2-induced apoptosis afforded by AEP-w1 pretreatment was associated with increases in

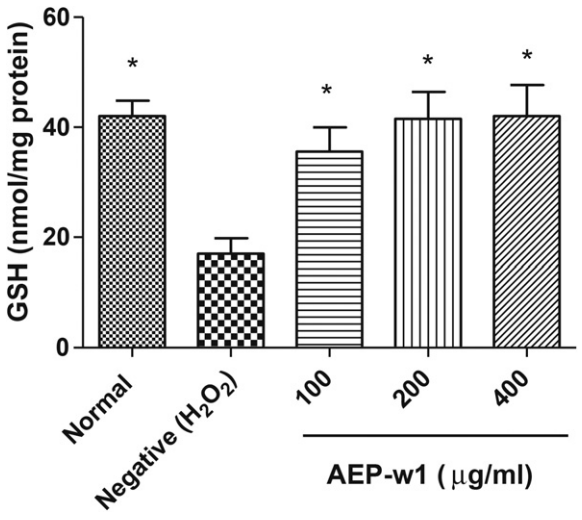

Fig. 5. The effect of AEP-w1 on H_2O_2-induced cellular GSH levels change in H9c2 cells. All data were shown as mean ± SD of three separate experiments. $^*P < 0.05$ vs. negative control.

cellular GSH levels. Thus depletion of cellular GSH was at least in part contributed to the cell death caused by oxidative stress.

4. Conclusions

Oxidative stress contributed to many abnormalities associated with diverse cardiovascular diseases, including hypertension, hypercholesterolemia, atherosclerosis, heart failure and type II diabetes (Hamilton et al., 2004). Accumulating evidences indicated that a variety of cardiovascular disease were associated with an accumulation of oxidative damage to lipids, proteins and DNA due to imbalance between the oxidant and antioxidant systems of the body, including superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) within the myocardial cells (Antonsson, Montessuit, Lauper, Eskes, & Martinou, 2000; Kim et al., 2005). Of special note, oxidative stress-induced apoptosis in cardiomyocytes aggravated the progression of heart failure (Tien et al., 2010). To protect the heart from the damage by oxidative stress, medicinal herbs have given rise to much attention as a very valuable source for natural antioxidant products. The antioxidant activity of many extracts and constituents from medicinal herbs has been widely documented in vivo and in vitro (Park et al., 2007; Piccinelli et al., 2004; Shao et al., 2001). Accordingly, antioxidants may decrease cellular injury and apoptosis through a radical-scavenging mechanism (Angeloni, Spencer, Leoncini, Biagi, & Hrelia, 2007; Bognar et al., 2006).

In the present study, we successfully extracted and isolated one homogeneous polysaccharide from the root bark of *A. elata* and pretreatment with AEP-w1 exhibited significant protective effects against H_2O_2-induced oxidative stress in myocardial H9c2 cells. In vitro antioxidant assay, AEP-w1 at the concentration ranging from 25 to 400 μg/ml showed strong superoxide radicals and hydroxyl radical scavenging activity and reducing power. Furthermore AEP-w1 pretreatment could significantly reduced apoptotic cell death caused by H_2O_2 in H9c2 cells, indicating AEP-w1 had significant protective effects against oxidative stress-induced injury on H9c2 cells. More importantly, AEP-w1 suppressed the features of mitochondrial dysfunction, including changes in the mitochondrial membrane potential and cytochrome c release from cytoplasm in H_2O_2-treated cells. In addition, H_2O_2-induced cellular GSH content fall in H9c2 cells was dramatically attenuated by AEP-w1. Taken together, the strong protective effects of AEP-w1 in H9c2 cells suggest that it may be useful for preventing cardiovascular diseases caused by oxidative stress.

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