Journal of Medicinal Plants Research Vol. 5(14), pp. 3304-3311, 18 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Pretreatment with astragaloside IV protects H9c2 cells against hydrogen peroxide-induced apoptosis by scavenging of reactive oxygen species and regulation of Bcl-2 and Bax expression

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Accepted 22 April, 2011

**Astragaloside IV (ASI), a saponin isolated from *Radix Astragali*, has been found to have potent cardioprotective effects. This study was designed to investigate whether ASI prevents cardiomyocytes from hydrogen peroxide ($H_2O_2$)-induced apoptosis. H9c2 cells were pretreated with different concentrations of ASI (5, 10, 20 µg/ml) for 24 h and then exposed to 100 µM $H_2O_2$ for 24 h. The cell viability were examined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay, reactive oxygen species (ROS) generation was quantified by the 2',7'-dichlorofluorescin-diacetate (DCFH-DA) method. The activity of antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were determined photometrically. Apoptotic cells were detected by Hoechst 33258 staining, annexin-V binding and by assessment of caspase-3. Protein and mRNA expression of both Bax and Bcl-2 were determined by Western blotting and reverse transcription-polymerase chain reaction (RT-PCR). The cell apoptosis significantly increased after 24 h of $H_2O_2$ exposure. Pretreatment of H9c2 cells with ASI significantly increased the activities of antioxidant enzymes, scavenged ROS and reduced malondialdehyde (MDA) production. ASI increased the expression of Bcl-2, decreased the expression of Bax, and ultimately reduced $H_2O_2$-induced H9c2 cells apoptosis. In summary, these results suggest that ASI can block $H_2O_2$-induced apoptosis in H9c2 cells, and that the underlying mechanism involves in scavenging of ROS and modulating expression of Bcl-2 and Bax.**

**Key words:** Astragaloside IV, H9c2 cells, hydrogen peroxide, oxidative stress, apoptosis.

## INTRODUCTION

Oxidative stress is damage caused by an imbalance between reactive oxygen species (ROS) and antioxidants, and is thought to play an important role in cells injury. Lipid peroxidation refers to the oxidative degradation of lipid. Lipid peroxidation is used as an indicator of oxidative stress in cells. MDA is the end product of lipid peroxidation and is a reliable parameter of oxidative stress (Moore and Roberts, 1998; Del et al., 2005; Atmani et al., 2011). Antioxidant enzymes, such as including catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) are an important antioxidant defense in cells exposed to oxygen. They can cope with ROS generated and could be vital in protecting the cell against free radical damage. Hydrogen peroxide ($H_2O_2$), a major source of ROS, can cross the cell

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membrane (Halliwell and Gutteridge, 1984) and cause lipid peroxidation and DNA damage in cells (Janero et al., 1991; Droge, 2002). $H_2O_2$ can damage cells by inducing apoptosis and has been used in many studies on cardiovascular disease (Ren et al., 2009; Pechtelidou et al., 2008). Apoptosis, the process of programmed cell death, is recognized as a pivotal mechanism in the pathogenesis of cardiovascular diseases. The loss of cardiomyocytes by apoptosis is likely to contribute to the development and progression of heart failure in a variety of myocardial diseases (Olivetti et al., 1997). It is very important to inhibit cardiomyocyte apoptosis to prevent the development of heart failure. The Bcl-2 family proteins are involved in the regulation of apoptosis. Among the Bcl-2 family, Bcl-2 induces cell survival, whereas Bax promote cell death (Bruckheimer et al., 1998; Reed, 1998; Sun et al., 2007). ASI, a saponin isolated from *Radix Astragali*, has been found to have potent cardioprotective effects, including activities of anti-myocardial fibrosis (Zhang et al., 2007) and activities of improving cardiac function (Xu et al., 2007; Li et al., 2002). However, there have been no reports on protection of ASI against oxidative stress-induced cardiotoxicity. In the present study, we examined the protective effect of ASI for the first time and tried to find out the possible mechanisms.

## MATERIALS AND METHODS

### Materials

H9c2 cells were obtained from the American Type Culture Collection (ATCC, Shanghai, China). ASI was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO BRL Life Technologies (Grand Island, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Corporation (St. Louis, USA). Annexin V and propidium iodide (PI) were from Jingmei Biotechnology (Shenzhen, China). Primary antibodies and horseradish peroxidase conjugated secondary antibodies were from Boster Biological Technology (Wuhan, China). Trizol and RNA PCR kit were obtained from TaKaRa Biotechnology Co. (Dalian, China). RevertAid™ H Minus M-MuLV reverse transcriptase was from Biometra (Goettingen, Germany). Enhanced chemiluminescence kit was obtained from Amersham (Buckinghamshire, UK). Assay kits for antioxidant enzyme activities, dichlorofluorescein diacetate (DCFH-DA) detection kit and Hoechst 33258 were from Beyotime Institute of Biotechnology (Jiangshu, China). MDA test kit was from Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents were of analytical grade.

### Cell culture and experimental groups

H9c2 cells were cultured in DMEM, supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/ml of penicillin and of 100 µg/ml streptomycin) at 37°C in a humidified incubator with 5% $CO_2$. There were five groups in this study: (1) Control group, cells were untreated. (2) $H_2O_2$ group, cells were exposed to 100 µM $H_2O_2$ for 24 h. (3, 4, 5) ASI group, cells were treated with ASI (5, 10, 20 µg/ml) for 24 h and then exposed to 100 µM $H_2O_2$ for 24 h.

### MTT assay

Cell viability was assayed by using a MTT assay. Briefly, at the end of each treatment period, 20 µl of MTT was added to each well and the microplate was incubated at 37°C for 4 h. 150 µl DMSO was added to each well to dissolve the formazan after the medium was removed. The absorbance was measured at 490 nm by an ELISA reader (Sunrise RC, Tecan, Switzerland).

### Hoechst 33258 staining

Cells were collected and washed with PBS twice and fixed in 4% formaldehyde for 10 min at 4°C. After washing twice with PBS, the cells were incubated with Hoechst 33258 at room temperature in the dark for 5 min, nuclear morphology was observed under a fluorescent microscope (Nikon, Yokohama, Japan). The nuclei of apoptotic cells appeared irregularly shaped and hyper-condensed (brightly stained).

### Flow cytometric analysis

Cells were washed with PBS three times and suspended in 100 µl binding buffer (10 mM HEPES/NaOH, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). Cells were first stained with 5 µl of FITC-conjugated Annexin V and 10 µl of PI and 400 µl binding buffer was then added. They were analyzed by flow cytometry (FACScalibur, Becton-Dickinson, USA). Annexin V-FITC-positive, PI-negative cells were in early apoptosis. Double-stained cells were in late apoptosis (Sun et al., 2007).

### Determination of ROS and MDA

ROS were measured by using dichlorofluorescein diacetate (DCFH-DA) detection kit. Briefly, cells were washed three times with PBS. 40 µM of DCFH-DA was added to cultrues and incubated for 30 min at 37°C. The absorbance was read at 525 nm.

We quantify lipid peroxidation with the thiobarbituric acid method. The MDA was assessed by using MDA test kit according to the manufacturer's protocol. The absorbance of the colored product was read at 535 nm.

### Determination of antioxidant enzyme activities

The total SOD (T-SOD) activity was determined by the xanthine oxidase method using total superoxide dismutase assay kit according to the manufacturer's protocol. The absorbance was read at 550 nm. The SOD activity was expressed as units per milligrams of protein. One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

The GPx activity was determined by cellular glutathione peroxidase assay kit according to the manufacturer's protocol. The absorbance was read at 340 nm. The GPx activity was expressed as units per milligram of protein. One unit of GPx activity was defined as the amount required to oxidize 1 µmol NADPH to $NADP^+$ in 1 min at 25°C, pH 8.0.

**Table 1.** Preventive effects of ASI on cell viability against $H_2O_2$-induced injury determined by MTT assay.

| Group | Cell viability (%) |
|---|---|
| Control | 100 |
| $H_2O_2$ | 58.8 ± 4.5☆☆ |
| $H_2O_2$ + ASI (5 μg/ml) | 64.6 ± 4.0** |
| $H_2O_2$ + ASI (10 μg/ml) | 70.5 ± 5.5** |
| $H_2O_2$ + ASI (20 μg/ml) | 76.3 ± 4.7** |

The data from 5 independent experiments were expressed as means ± S.D. ☆☆P <0.01 compared to control group. **P <0.01 compared to $H_2O_2$ group.

The CAT activity was determined by using CAT assay kit according to the manufacturer's protocol. The absorbance was read at 520 nm. The CAT activity was expressed as units per milligram of protein. One unit of CAT activity was defined as the amount of CAT required to decompose 1 μmol $H_2O_2$ in 1 min at 25°C, pH 7.0.

#### Protein extraction and western blotting

H9c2 cells were collected after treatment, washed three times with PBS and incubated with 100 μl cell lysis buffer for 30 min on ice. After centrifugation, the extracted protein concentration was determined using the Bradford assay. The proteins were first subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes. The membranes were treated with TBST (pH 7.5, 10 mM Tris-HCl, 150 mM NaCl and 0.1%(v/v) Tween-20) containing with 5%(m/v) nonfat milk for 1 h at room temperature and incubated with the primary antibodies anti-Bax, anti-Bcl-2 or anti-cleaved caspase-3 at 37°C for 2 h. After being washed thrice with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature and washed with TBST, the protein bands were detected by enhanced chemiluminescence kit. β-actin was used as an internal control for each sample.

#### RNA extraction and semiquantitative real-time RT-PCR

Total RNA was extracted from cells using Trizol reagent following the manufacturer's instructions. RNA concentrations were quantified by spectrophotometry (UV300, Hampshire, England). The synthesis of copy DNA (cDNA) was carried out by using RevertAid™ H Minus M-MuLV reverse transcriptase. Real-time RT-PCR was run in an ABI 7700 Prism Sequence Detection System (Applied Biosystems, Foster City, USA). Total volume was 20 μl: 2 μl cDNA, 10 μl SYBR® Premix Ex Taq™, 0.4 μl of each primer (10 μM) and 7.2 μl ultrapure water. Cycler conditions were as follows: Taq activation at 95°C for 30 s, amplification at 40 cycles of 95°C for 5 s and final extension at 60°C for 30 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primer sequences were: Bax forward, 5′-GGC GAT GAA CTG GAC AAC-3′; Bax reverse, 5′-CCG AAG TAG GAA AGG AGG C-3′; Bcl-2 forward, 5′-CTT CCA GCC TGA GAG CAA CC-3′; Bcl-2 reverse, 5′-CAT CCC AGC CTC CGT TAT CC-3′; GAPDH forward, 5′-CAT GCC GCC TGG AGA AAC CTG CCA-3′; GAPDH reverse, 5′-TGG GCT GGG TGG TCC AGG GGT TTC-3′.

#### Statistical analysis

Statistical analysis was processed with SPSS 13.0 (SPSS Inc., Chicago, USA). All data are expressed as mean ± standard deviation (S.D.). Statistical comparison within groups was carried out with one way ANOVA, and the Student-Neuman-Keuls test was used to analyze between two groups. A $p<0.05$ was adopted as statistically significant.

## RESULTS

### ASI increases the viability of H9c2 cells exposed to $H_2O_2$ in a dose-dependent manner

Compared with control (Table 1), survival rate of cardiomyocytes was increased significantly after been exposed to 100 μM of $H_2O_2$ for 24 h. ASI increased the viability of H9c2 cells in a dose-dependent manner at concentration of 5 to 20 μg/ml.

### ASI prevents H9c2 cells from $H_2O_2$-induced apoptosis

After Hoechst 33258 staining, most nuclei had regular contours and were round in the control group (Figure 1A). After exposure to 100 μM $H_2O_2$ for 24 h, the nuclei appeared irregularly shaped and hyper-condensed (brightly stained) (Figure 1B). Pre-treatment with 20 μg/ml ASI reduced the $H_2O_2$-induced increase in the number of apoptotic nuclei containing condensed chromatin (Figure 1C).

Flow cytometry revealed that 7.8 ± 1.3% of cells were in apoptosis (Figures 2A and F) in the control group and 25.6 ± 2.3% (Figures 2B and F) in the $H_2O_2$ group. After pretreatment with ASI (5, 10, 20 μg/ml), the percentage of apoptotic cells was 20.8 ± 1.3, 16.2 ± 2.3 and 12.0 ± 1.7%, respectively (Figures 2C to F).

Caspase-3 activity was determined by Western blotting. As shown in Figure 3C, the cleaved caspase-3 protein levels were increased in $H_2O_2$ group (cleaved caspase-3/β-actin = 0.43 ± 0.07) compared to control group (cleaved caspase-3/β-actin = 0.07 ± 0.03) and were significantly decreased (cleaved caspase-3/β-actin = 0.35 ± 0.05, 0.24 ± 0.04 and 0.16 ± 0.04, respectively) in ASI groups (5, 10, 20 μg/ml) compared to the $H_2O_2$ group.

### Inhibition of ROS generation and lipid peroxidation by ASI

The level of intracellular ROS was measured with fluorescence probe DCFH-DA. Compared with control (Table 2), the intracellular ROS manifested as fluorescence intensity increased remarkably in H9c2 cells

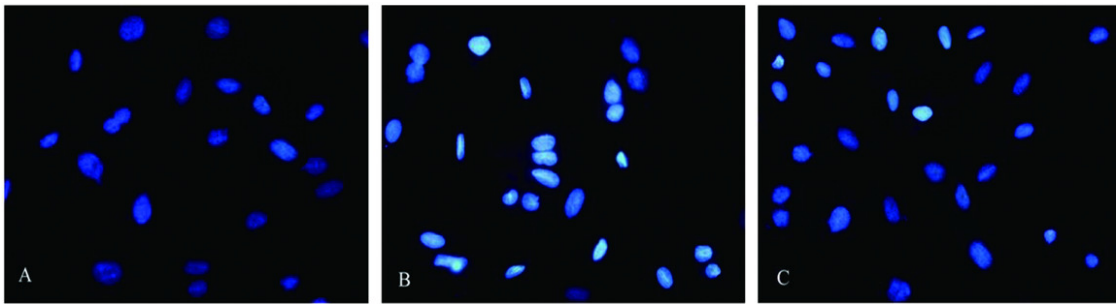


**Figure 1.** Images (original manification, × 400) of Hoechst 33258-stained H9c2 cells after a 24-h exposure to 100 μM $H_2O_2$ with or without ASI. The cells with irregularly shaped and hyper-condensed nuclei were counted as apoptotic cells. (A) Control group (B) $H_2O_2$ group (C) ASI (20 μg/ml) + $H_2O_2$ group

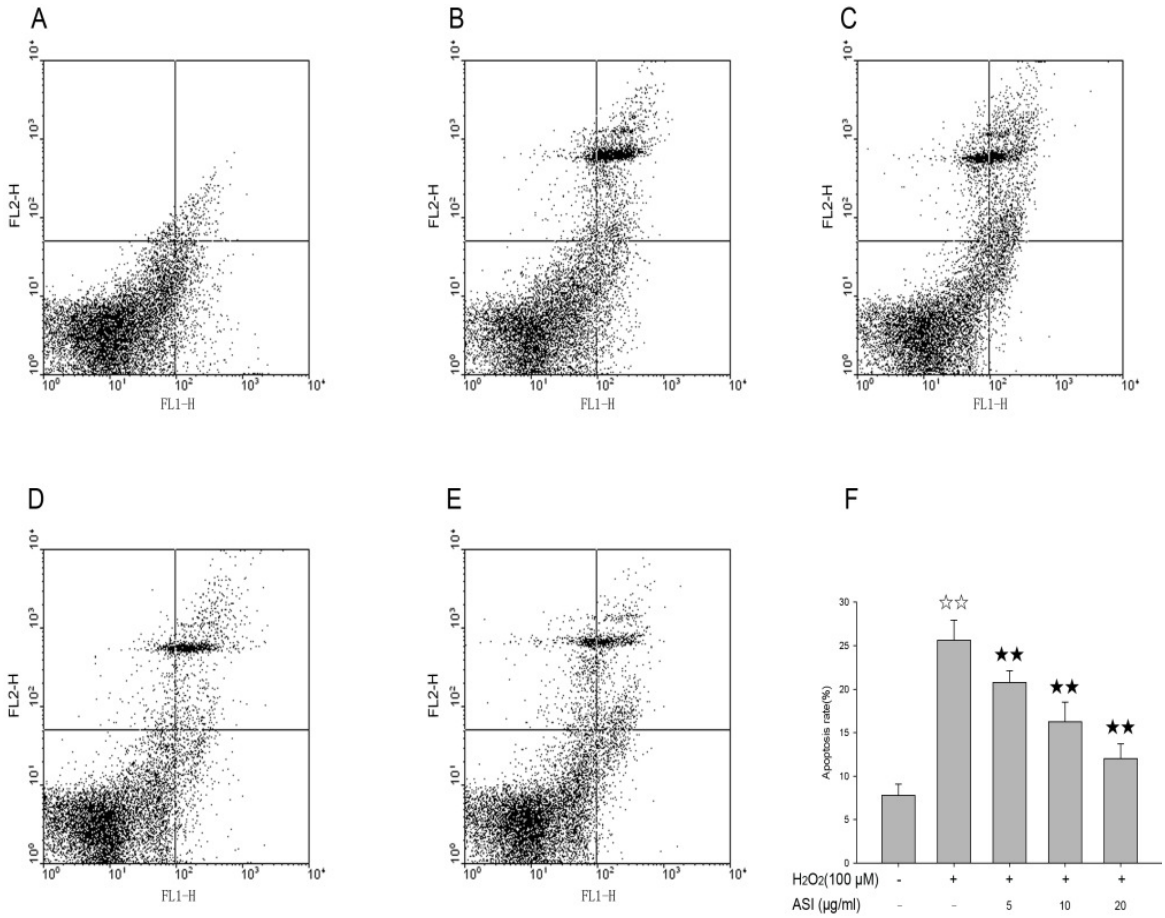


**Figure 2.** Annexin V-FITC/PI assay for apoptosis rate. H9c2 cells were pretreated with different concentrations of ASI (5, 10, 20 μg/ml) for 24 h and exposed to 100 μM $H_2O_2$ for 24 h. Cells were collected and the apoptosis rates were determined by flow cytometry using Annexin V-FITC/PI double labeling. (A) Control group; (B) $H_2O_2$ group; (C) 5 μg/ml ASI group; (D) 10 μg/ml ASI group; (E) 20 μg/ml ASI group; (F) shows a column bar graph analysis. The data are repeated for three independent examinations and plotted as the means ± S.D.☆☆ P <0.01 compared to control group. **P <0.01 compared to $H_2O_2$ group.

exposed to $H_2O_2$. The fluorescence intensity decreased remarkably by pretreatment with ASI (5, 10, 20 μg/ml) in a dose dependent manner. In comparison with the control group (Table 2), the content of MDA increased

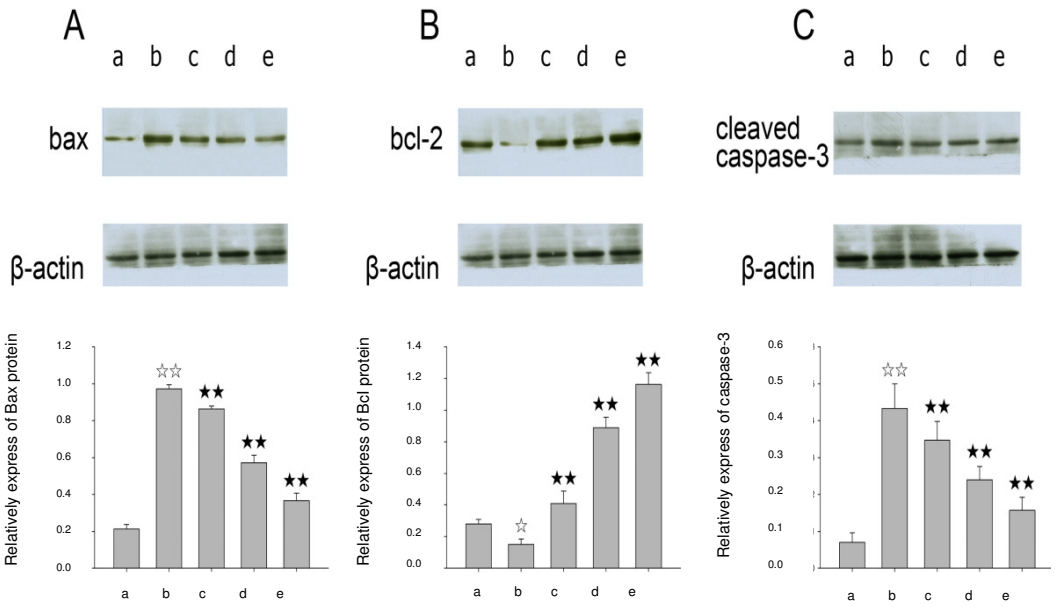


**Figure 3.** Western blot analysis was performed with antibodies anti-Bax, anti-Bcl-2 or anti-cleaved caspase-3. The protein bands were detected by enhanced chemiluminescence. Laser densitometry was used for the analysis of the data. β-Actin was used as the internal control. (A) Bax (B) Bcl-2 (C) cleaved caspase-3 (a) Control group. (b) H2O2 group. (c, d, e) ASI group (5, 10, 20 μg/ml). Values are means ± S.D. from three independent experiments. ☆P<0.05 and ☆☆P <0.01 compared to control group. ★★P <0.01 compared to $H_2O_2$ group.

**Table 2.** The effects of ASI on the level of MDA and the intracellular ROS under $H_2O_2$ treatment in H9c2 cells. Cells were pretreated with different concentrations of ASI (5, 10, 20 μg/ml) for 24 h and then exposed to 100 μM $H_2O_2$ for 24 h.

| Group | ROS | MDA (nmol/ mg protein) |
|---|---|---|
| Control | 29.2 ± 4.8 | 0.98 ± 0.12 |
| $H_2O_2$ | 97.6 ± 6.3☆☆ | 2.98 ± 0.17☆☆ |
| $H_2O_2$ + ASI (5 μg/ml) | 81.9 ± 8.2★★ | 1.64 ± 0.14★★ |
| $H_2O_2$ + ASI (10 μg/ml) | 70.3 ± 6.6★★ | 1.37 ± 0.13★★ |
| $H_2O_2$ + ASI (20 μg/ml) | 39.9 ± 6.1★★ | 1.19 ± 0.13★★ |

The data represent means ± S.D. from 5 independent experiments. ☆☆P <0.01 compared to control group. ★★P <0.01 compared to $H_2O_2$ group.

remarkably in the $H_2O_2$ group. ASI (5, 10, 20 μg/ml) significantly reduced lipid peroxidation in a dose dependent manner.

## Effects of ASI on CAT, GPx and SOD

Compared with control (Table 3), notable reductions in CAT, GPx and T-SOD activities were found after exposure to $H_2O_2$ for 24 h. Pretreatment with ASI (5, 10, 20 μg/ml) significantly elevated the activities of CAT, GPx and T-SOD in a dose dependent manner.

## Adjustment of the expression of Bcl-2, Bax by ASI

The Bcl-2 protein levels were reduced in $H_2O_2$ group (Bcl-2/β-actin = 0.15 ± 0.04, Figure 3B) compared to control group (Bcl-2/β-actin = 0.28 ± 0.03), and were significantly increased (Bcl-2/β-actin = 0.41 ± 0.08, 0.89 ± 0.07 and 1.16 ± 0.08) in ASI groups (5, 10, 20 μg/ml) compared to the $H_2O_2$ group. Bcl-2 mRNA levels were significantly

**Table 3.** The effects of ASI on the activities of antioxidant enzymes in H9c2 cells.

| Group | CAT (U/mg protein) | Gpx (U/mg protein) | T-SOD (U/mg protein) |
|---|---|---|---|
| Control | 11.5 ± 1.5 | 25.9 ± 2.7 | 21.3 ± 2.6 |
| $H_2O_2$ | 5.6 ± 1.2☆☆ | 10.2 ± 1.8☆☆ | 12.1 ± 2.6☆☆ |
| $H_2O_2$+ ASI (5 μg/ml) | 12.9 ± 1.8★★ | 20.5 ± 1.8★★ | 21.9 ± 2.7★★ |
| $H_2O_2$+ASI (10 μg/ml) | 17.3 ± 1.1★★ | 30.9 ± 3.3★★ | 26.5 ± 3.2★★ |
| $H_2O$+ASI (20 μg/ml) | 19.8 ± 1.0★★ | 41.6 ± 1.7★★ | 35.3 ± 3.1★★ |

All values are means ± S.D. of three replicates. ☆☆P <0.01 compared to control group. ★★ P <0.01 compared to $H_2O_2$ group.

**Table 4.** The mRNA expression of Bax and Bcl-2 by real-time RT-PCR analysis in H9c2 cells (GAPDH was used as the internal control).

| Group | Bax | Bcl-2 |
|---|---|---|
| Control | 1 | 1 |
| $H_2O_2$ | 4.63 ± 0.61☆☆ | 0.45 ± 0.13 |
| $H_2O_2$ + ASI (5 μg/ml) | 3.44 ± 0.51★★ | 3.92 ± 0.70★★ |
| $H_2O_2$ +ASI (10 μg/ml) | 2.18 ± 0.25★★ | 6.82 ± 0.63★★ |
| $H_2O_2$ +ASI (20 μg/ml) | 1.40 ± 0.31★★ | 10.58 ± 1.23★★ |

Results are the means ± S.D. from three independent experiments. ☆☆P <0.01 compared to control group. ★★P <0.01 compared to $H_2O_2$ group

decreased in cells exposed to $H_2O_2$ compared to levels in control cells (Table 4). In contrast, Bcl-2 levels were significantly increased compared to the $H_2O_2$ group in the 5, 10 and 20 μg/ml ASI groups.

The Bax protein levels were increased in $H_2O_2$ group (Bax/β-actin = 0.97 ± 0.02, Figure 3A) compared to control group (Bax/β-actin = 0.21 ± 0.03) and were significantly decreased (Bax/β-actin = 0.86 ± 0.02, 0.57 ± 0.04 and 0.37 ± 0.04, respectively) in ASI groups (5, 10, 20 μg/ml) compared to the $H_2O_2$ group. Bax mRNA levels were significantly increased in the $H_2O_2$ group. In contrast, Bax levels were significantly decreased compared to the $H_2O_2$ group in the 5, 10 and 20 μg/ml ASI groups (Table 4).

## DISCUSSION

The rhizome of *R. Astragali* has been used in traditional Chinese medicine as a tonic and anti-aging agent for a very long time. ASI, a natural saponin of *R. Astragali*, has been shown to exhibit various biological and pharmacological activity, including anti-cancer (Hu et al., 2009; Qi et al., 2010), anti-HBV virus (Wang et al., 2009), anti-fibrotic effects (Liu et al., 2009), and prevention of clinical diabetic complications (Motomura et al., 2009). Some studies have shown ASI has potential cardioprotective effects (Zhang et al., 2007; Xu et al., 2007; Li et al., 2002). However, its protection mechanisms are still elusive. In this study, we examined the effect of ASI on the viability of H9c2 cells exposed to $H_2O_2$ by MTT. Enzymatic antioxidant status was studied by estimating the activities of CAT, GPx and SOD**.** Lipid peroxidation was assessed by examining MDA. Apoptotic cells were detected by Hoechst 33258 staining, annexin-V binding and by assessment of caspase-3. The major finding of the present study is that pretreatment with ASI (5, 10, 20 μg/ml) for 24 h markedly reduces the decrease in viability of H9c2 cells exposed to $H_2O_2$. ASI decreased levels of ROS and inhibited lipid peroxidation in a dose-dependent manner. ASI dose-dependently inhibited apoptosis in H9c2 cells exposed to $H_2O_2$. The mechanisms were associated with activation of antioxidant enzymes and scavenging ROS not only, but also up-regulation of Bcl-2 expression and down-regulation of Bax expression.

Apoptosis plays an important role in pathogenesis in cardiovascular diseases. The loss of cardiomyocytes by

apoptosis contributes to the development of heart failure; inhibition of cardiomyocyte apoptosis may have therapeutic implications (Razavi et al., 2005). ROS, such as superoxide anion, $H_2O_2$ and hydroxyl radical, can damage cardiomyocytes by inducing apoptosis (Abbate et al., 2006; von et al., 1999), and are a target for therapeutic intervention (Guaiquil et al., 2004). Oxidative stress induced by $H_2O_2$ may contribute to the pathogenesis of ischemic-reperfusion injury in the heart (Janero et al., 1991). Lipid peroxidation refers to the oxidative degradation of lipid. Lipid peroxidation is used as an indicator of oxidative stress in cells and tissues. MDA is the end product of lipid peroxidation and is used as a marker to measure the level of oxidative stress in an organism (Moore and Roberts, 1998; Del et al., 2005; Atmani et al., 2011). Antioxidant enzymes such as CAT, GPx and SOD are major intracellular antioxidant defenses. They can scavenge ROS and plays an important role in protecting the cell against oxidative stress induced injury. It has shown that CAT, GPx and SOD overexpression can provide cardioprotective effects against the toxicity of ROS (Fiers et al., 1999). In present results, incubation with $H_2O_2$ inactivated the activities of CAT, SOD and GPx, and led to enhanced concentration of ROS and MDA in H9c2 cells. ASI elevated the activities of antioxidant enzymes, scavenged ROS and reduced MDA activity in dose-dependent manner. Our findings suggest that ASI could protect H9c2 cells from $H_2O_2$-induced apoptosis and the underlying mechanisms were due to its antioxidant properties.

Many genes are associated with the regulation of apoptosis. The Bcl-2 gene family is important regulator of apoptosis. Studies have shown that Bcl-2 protect cells from apoptosis, whereas Bax induce cells apoptosis (Reed, 1998; Adams and Cory, 1998) in the Bcl-2 family members. When Bcl-2 is overexpressed, cells are protected (Wang et al., 1998). When Bax is hyperexpressed, cells die (Lu et al., 1998). In this study, when H9c2 cells were exposed to $H_2O_2$ for 24 h, we found that the Bcl-2 expression was down-regulated and the Bax expression was up-regulated. These alterations were ameliorated by pretreatment with ASI. Taken together, Bcl-2 and Bax are involved in mediating the anti-apoptotic effects of ASI in $H_2O_2$-exposed H9c2 cells.

In conclusion, our data demonstrates that ASI is protective against $H_2O_2$-induced apoptosis in cultured H9c2 cells. Our data also demonstrates that the anti-apoptotic effects are due to its antioxidant properties and its ability to regulate of Bcl-2 and Bax expression. The anti-apoptotic effect might contribute to prevention and treatment of oxidative stress-mediated cardiovascular disorders.

## ACKNOWLEDGEMENTS

This work was supported by Research Foundation for Institutions of Higher Education of Liaoning Province (No. 2008823). We wish to thank Lin Song and Zhao Jiawei for assistance with the Western blot analysis. Thanks to Felix Zhang for his kind advice on the manuscript.

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