Anti-oxidative Effect of Ligustrazine on Treatment and Prevention of Atherosclerosis

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Abstract

Purpose: To investigate the protective effects of ligustrazine on oxidative stress-induced atherosclerosis.

Methods: The indicators related to oxidative stress were determined using commercially available assay kits. MTT assay was used to assess the survival rate of human umbilical vein endothelial cells (HUVECs). HUVECs apoptosis was analyzed using fluorescence staining and flow cytometry. mRNA expression level and activity of caspases 3, 8, and 9 were determined via quantitative real-time polymerase chain reaction (PCR) and caspase 3, 8, and 9 assay kits.

Results: Ligustrazine concentration of < 80 µmol/L had negligible inhibitory effect on HUVECs viability and protected HUVECs against oxygen stress damage by regulating the indicators related to oxidative stress. Flow cytometry results show that ligustrazine ameliorated H2O2-induced apoptosis, while the proportion of cells that stepped into early apoptosis and late apoptosis or necrosis were 52.7 and 0.6 %, respectively, in the H2O2 group, and 38.2 and 1.3 %, respectively, in the ligustrazine group. In addition, ligustrazine attenuated the up-regulation of caspase 3, 8, and 9 mRNA expression levels and activity.

Conclusion: Ligustrazine can protect HUVECs against H2O2-induced injuries by regulating the indicators related to oxidative stress and suppressing the overexpression of caspases 3, 8, and 9. The protective mechanism of ligustrazine on H2O2-induced injury in HUVECs may be a caspase-dependent anti-apoptotic mechanism which provide important information for treating and preventing oxidative stress-induced atherosclerosis.

Keywords: Ligustrazine, Oxidative stress, Umbilical vein, Endothelial cells, Atherosclerosis

INTRODUCTION

Atherosclerosis (AS) is the primary cause of cardiovascular and cerebrovascular diseases [1]. Substantial evidence indicates that oxidative stress contributes to the progression of AS [2]. Oxidative stress can result in endothelial damage [3]. Injury to endothelial cells is the initiating factor of AS [4].
Recently, many studies have suggested that natural bioactive compounds from plants can protect endothelial cells against oxidative damage [6]. Ligustrazine (tetramethyl-pyrazine) is the major active ingredient extracted from *Ligusticum chuanxiong* and is widely applied in the treatment of vascular diseases in China [7]. Previous studies have reported that ligustrazine can effectively scavenge cytotoxic oxygen free radicals that can alleviate hepatic and kidney cell damage [8,9]. However, the potential mechanism of ligustrazine involved in AS was still obscure. Therefore, in the present study, the H$_2$O$_2$-induced oxidative stress model was established using human umbilical vein endothelial cells (HUVECs) to explore the anti-oxidative effects of ligustrazine on oxidative damaged endothelial cells and the underlying mechanism involved in the pathogenesis of AS.

**EXPERIMENTAL**

**Materials**

The HUVECs were provided by Nanchang University Medical School. The current study was approved by the Ethics Committee of Nanchang University (China). Trypsin and 3-(4, 5-dimethylthiazal-2-yl)-2, 5-diphenylterazolium (MTT) were purchased from Sigma (St. Louis, USA), and Dulbecco’s Modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Carlsbad, USA). Ligustrazine was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The LDH, MDA, GSH, NOS, nitric oxide (NO), and SOD assay kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

**Cell culture**

The HUVECs were cultured in 25 cm$^2$ plastic flasks at 37 °C in a humidified CO$_2$ incubator (95 % air and 5 % CO$_2$). The complete medium for cell maintenance was 4.5 g/L glucose DMEM containing 10% FBS, L-glutamine, 100 IU/mL penicillin, and 100 IU/mL streptomycin. When cells were 80% confluent, they were subcultured using 0.25 % trypsin and 0.02 % EDTA, and the medium was changed every two days. Cells between passages 3 and 10 were used in the present study.

**Determination of H$_2$O$_2$ concentration in oxidative stress model (MTT assay)**

The concentration-dependent studies of HUVECs induced by H$_2$O$_2$ were conducted using MTT assay. HUVECs were counted and seeded into 96-well culture plates at a density of 5×10$^3$ cells/well. The cells were washed twice with PBS after incubation with various H$_2$O$_2$ concentrations (0, 50, 100, 200, and 400 μM) for 8 h. For each well, the cells were then incubated with 100 μL MTT (final concentration of 0.5 mg/mL) for 4 h. After MTT removal, the colored formazan was dissolved in 100 μL of DMSO. The absorption values were measured at 490 nm using a Thermo Scientific Multiskan MK3 Microplate Reader (Thermo Fisher, USA). The viability of HUVECs in each well was presented as percentage of control cells. Six independent replicates were performed for each group.

**Confocal laser scanning microscopy (CLSM)**

The HUVECs were cultured as described above and were stained with Hoechst 33258 for 30 min at 37 °C. Cell shape and nuclear morphology with apoptotic characteristics were observed immediately using Zeiss LSM 710 confocal laser scanning microscope (Carl Zeiss MicroImaging GmbH, Germany).

**Evaluation of ligustrazine cytotoxicity**

The effect of ligustrazine on HUVEC viability was evaluated via MTT assay in 96-well plates at a cell density of 5 × 10$^3$ cells per well. The cells were washed twice with PBS, after being pre-treated with ligustrazine (10, 20, 40, 80, 160, and 320 μmol/L) for 24 h. The MTT assay was done under the conditions described earlier.

**Evaluation of effect of ligustrazine on the viability of H$_2$O$_2$-induced HUVECs**

The cells were cultured as described above. The HUVECs were randomly divided into the control, H$_2$O$_2$, and five ligustrazine groups (5, 10, 20, 40, and 80 μmol/L + 100 μmol/L H$_2$O$_2$). Sub-confluent cells were pre-treated with the medium containing various concentrations (5, 10, 20, 40, and 80 μmol/L) of ligustrazine for 24 h. Thereafter, a final 100 μmol/L H$_2$O$_2$ concentration was added to the culture medium for 8 h, which was designated as the ligustrazine group. The control group was treated with the culture medium only, whereas the oxidative injury model of HUVECs established using H$_2$O$_2$ was regarded as the H$_2$O$_2$ group. Subsequently, the effect of ligustrazine on the H$_2$O$_2$-induced HUVECs was measured via MTT assay.

**Evaluation of oxidative stress parameters**

The percentage of LDH release, NO production, SOD, GSH-Px, and NOS activities, and MDA concentration were determined using
commercially available assay kits (Jiancheng Bioengineering Research Institute, Nanjing, China). All procedures complied with the manufacturer's instructions. In addition, the percentage of LDH release was defined as the release of LDH in the supernatant/(release of LDH in the supernatant + release of LDH from the cell lysate) × 100.

**Flow cytometry**

Here, HUVECs were harvested, washed, and double-stained with an Annexin V–fluorescein isothiocyanate apoptosis detection kit (BD Biosciences, USA). The cells were incubated in the dark at 4 °C for 10 min to 15 min and analyzed using a BD FACS Calibur™ flow cytometry system (Becton Dickinson, USA). All tests were done in triplicate.

**Determination of the mitochondrial membrane potential (ΔΨm)**

Rhodamine 123, a cationic fluorescent dye whose mitochondrial fluorescence intensity decreases quantitatively with the dissipation of the mitochondrial membrane potential, was used to evaluate perturbations in mitochondrial membrane potential [10]. The HUVECs were cultured as described above and divided into different groups (control, H2O2, and ligustrazine groups). After 24 h treatment, cells were harvested and washed twice with cold PBS and then incubated in the dark with rhodamine 123 (1 μmol/L) for 30 min at 37 °C. Fluorescence was measured using flow cytometry with an excitation wavelength of 485 nm.

**Detection of caspase activity**

The cells were cultured and treated as aforementioned. The fluorometric specific detection kits (KeyGEN, China) containing fluorescent substrates were used to analyze the activities of caspase 3, 8, and 9. The protocol for detecting caspase activity was conducted according to the manufacturer's directions. Experiments were performed in triplicate.

**Quantitative real-time (qRT)-PCR analysis of mRNA expression**

The total RNA was extracted from cultured cells using Trizol reagent (Invitrogen, USA). The RNA concentrations were determined at 260 nm, and the samples were then stored in a freezer. First-strand cDNA was synthesized from 1 μg of total RNA using PrimerScript™ RT-PCR kit (TaKaRa Code: DRR041A) according to the manufacturer’s instructions. The expression levels of genes (β-actin, caspase-3, caspase-8, and caspase-9) in each sample were determined via qRT-PCR in the ABI 7900HT Real-Time PCR system (Applied Biosystems, USA). The fluorescence signals were detected with the ABI 7900HT Version 2.3 sequence detection system (Applied Biosystems, USA). The PCR conditions were as follows: 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, and 60 °C for 1 min. The gene expression data were normalized to the endogenous control β-actin, and the relative mRNA expression was calculated using the comparative cycle threshold (ΔCt) method. ΔCt is the difference between the Ct values of the target gene and β-actin. The primers used for qRT-PCR are listed in Table 1.

**Statistical analysis**

Statistical analysis was performed using the SPSS 17.0 package. The values are presented as mean ± standard deviation (SD, n ≥ 3). One-way ANOVA and Student’s t-test were conducted to determine statistical significance. Differences between groups were considered significant at p < 0.05.

**RESULTS**

**Concentration-dependent survival rate of H2O2-induced HUVECs**

Studies on the concentration-dependent survival rate of H2O2-induced HUVECs were performed in the present study. As shown in Figure 1, the survival rates gradually decreased with the increase in H2O2 concentration. When H2O2

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**Table 1: Primer sequences used for qRT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primers (5’ to 3’)</th>
<th>Reverse primers (5’ to 3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>AGTTGCGTTACACCCCTTTCTTG</td>
<td>CACCTTACCCGTTCCAGTTTT</td>
<td>152</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>TGTGGAGCCGGTGTGGGAAGAGT</td>
<td>AATGGGGAAAGGCCAGGTTGCA</td>
<td>182</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>TGCCCTCTCCTGAGGGAGCTGCT</td>
<td>TGAGGCCCTGCTGGTGCTGAA</td>
<td>115</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>TGGAGGATTTGTTGAGTGTGAGCA</td>
<td>ATCTGGCTCGGGGTATTACGGCA</td>
<td>97</td>
</tr>
</tbody>
</table>
concentration was higher than 100 µmol/L, the survival rate decreased by 11.45 ± 2.62 % (p < 0.05, compared with control), suggesting that 100 µmol/L of H2O2 can induce cell injury. Previous studies indicate H2O2 concentrations in the oxidative stress model system ranging from 10^{-5} mol/L to 10^{-3} mol/L, which coincide with our results [11].

**Apoptosis of HUVECs detected via Hoechst 33258 fluorescence staining**

The CLSM results indicate that normal cells display weak fluorescence, whereas apoptotic cells show increasing bright fluorescence and typical apoptotic bodies. As shown in Figure 2, the chromatin in nucleus seemed to be condensed and marginalized in the H2O2 group, suggesting that H2O2 can result in HUVECs apoptosis.

**Cytotoxicity of ligustrazine on HUVECs**

The effect of ligustrazine on the viability of normal HUVECs was concentration dependent (Figure 3). When the concentration was more than 160 µmol/L, the survival rate was 81.29 ±3.18 %, which is significantly less than that of the control group (p < 0.05). However, ligustrazine at concentrations ≤ 80 µmol/L had negligible inhibitory effect on HUVECs survival. Therefore, ligustrazine concentrations less than 80 µmol/L were used for the subsequent experiments.

**Protective effect of ligustrazine on the viability of H2O2-induced HUVECs**

The protective effects of ligustrazine on the viability of H2O2-induced HUVECs were evaluated via MTT assay. As shown in Figure 4, the survival rate of HUVECs was about 64%±3.8% after exposure to 100 µmol/L of H2O2 for 8 h. However, pre-incubation of HUVECs with ligustrazine (5, 10, 20, 40, and 80 µmol/L) for 24 h can increase the viability of H2O2-induced HUVECs in a dose-dependent manner (p < 0.05), and the survival rates were 67.06 ± 3.95, 71.75 ±1.24, 74.44 ± 3.80, 80.00 ±1.20, and 86.91 ± 2.86 %, respectively. These rates suggest that ligustrazine can protect HUVECs against H2O2-induced injury.

**Influence of ligustrazine on oxidative stress indicators of H2O2-induced injury**

LDH release, MDA and NO concentrations, and SOD, GSH-Px, and NOS activities were investigated to further confirm the protective effects of ligustrazine on H2O2-induced injury in HUVECs. As shown in Table 2, LDH release was...
16.28 ± 1.71 % in the control group, and a dramatic increase (34.89 ± 0.98 %) was observed after exposure to 100 µmol/L of H₂O₂ for 8 h. However, pre-treatment with the different concentrations of ligustrazine (> 5 µmol/L) for 24 h attenuated the H₂O₂-induced increase in LDH release (p < 0.05, Table 2).

The MDA concentration in the control group was 23.78 ± 2.15 nmol/mg of protein, whereas that in HUVECs treated with 100 µmol/L of H₂O₂ for 8 h significantly increased (45.70 ± 3.14 nmol/mg of protein) (p < 0.05, Table 2). Meanwhile, the MDA concentrations in cells pre-treated with various concentrations of ligustrazine (5, 10, 20, 40, and 80 µmol/L) for 24 h decreased.

As shown in Table 2, the NO level and NOS activity in HUVECs exposed to 100 µmol/L of H₂O₂ for 8 h (p < 0.05, with 27.44 ± 4.34 µmol/L and 1.95 ± 0.20 U/mg of protein, respectively) significantly decreased compared with those of the control group (73.30 ± 7.00 µmol/L and 4.18 ± 0.77 U/mg of protein, respectively). However, 24 h pre-treatment with ligustrazine (5, 10, 20, 40, and 80 µmol/L) could trigger a distinct, dose-dependent increase in these indicators.

Treating HUVECs with 100 µmol/L of H₂O₂ caused a significant decrease in SOD and GSH-Px activities (45.16 ± 3.25 and 77.81 ± 4.29 U/mg of protein, respectively) compared with that in the control group (129.76 ± 15.52 and 160.13 ± 3.73 U/mg of protein, respectively) (p < 0.05, Table 2). However, pre-incubation with ligustrazine (5, 10, 20, 40, and 80 µmol/L) for 24 h attenuated the changes in SOD and GSH-Px activities.

All of these results suggest that ligustrazine can be an anti-oxidative agent protecting HUVECs against oxidative stress.

**Effect of ligustrazine on apoptosis**

The percentages of cells that stepped into early apoptosis and late apoptosis or necrosis were 52.7% and 0.6%, respectively, in the H₂O₂ group, and 38.2% and 1.3%, respectively, in the ligustrazine group (80 µmol/L) (Figure 5). These results suggest that ligustrazine has a protective effect on HUVECs against oxidative damage, which is particularly important in maintaining normal physiological function and preventing the formation of atherosclerotic plaques.

**Table 2: Effect of ligustrazine on oxidative stress in HUVECs**

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH release (%)</th>
<th>MDA (nmol/mgprot)</th>
<th>NOS (U/mgprot)</th>
<th>NO (µmol/L)</th>
<th>SOD (U/mgprot)</th>
<th>GSH-Px (U/mgprot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.28±1.71</td>
<td>23.78±2.15</td>
<td>4.18±0.77</td>
<td>73.30±7.00</td>
<td>129.76±15.52</td>
<td>160.13±3.73</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>34.89±0.89*</td>
<td>45.70±3.14*</td>
<td>1.95±0.20*</td>
<td>27.44±4.34*</td>
<td>45.16±3.25*</td>
<td>77.81±4.29*</td>
</tr>
<tr>
<td>5 µM of Ligustrazine</td>
<td>33.68±0.81a</td>
<td>42.98±2.96ab</td>
<td>2.15±0.50a</td>
<td>32.20±4.13ab</td>
<td>57.98±9.53a</td>
<td>81.75±1.08ab</td>
</tr>
<tr>
<td>10 µM of Ligustrazine</td>
<td>32.78±1.49b</td>
<td>40.23±2.10abc</td>
<td>2.23±0.29abc</td>
<td>46.05±8.47ab</td>
<td>60.64±8.85b</td>
<td>83.37±2.15bc</td>
</tr>
<tr>
<td>210 µM of Ligustrazine</td>
<td>29.86±0.44c</td>
<td>38.66±4.91bcd</td>
<td>2.60±0.35cd</td>
<td>53.60±8.02ab</td>
<td>74.00±3.63b</td>
<td>90.44±4.06cd</td>
</tr>
<tr>
<td>40 µM of Ligustrazine</td>
<td>27.42±0.83d</td>
<td>36.52±2.68bcd</td>
<td>2.94±0.81cd</td>
<td>68.07±3.30b</td>
<td>82.51±1.68c</td>
<td>96.96±2.43d</td>
</tr>
<tr>
<td>80 µM of Ligustrazine</td>
<td>25.90±0.50d</td>
<td>34.41±3.65d</td>
<td>3.01±0.33d</td>
<td>69.61±11.42c</td>
<td>107.28±5.18e</td>
<td>110.39±9.56e</td>
</tr>
</tbody>
</table>

*Note: Asterisk (*) indicates statistically significant differences between the control and H₂O₂ group. a, b, c, d, e indicate statistically significant differences between the H₂O₂ group and ligustrazine group.*

**Figure 5:** The flow cytometry of HUVECs apoptotic cells after treatment with the H₂O₂ and ligustrazine. Quadrant analysis of fluorescence intensity of gated cells in Annexin V-FITC and PI channels was from 16,000 events. A = control; B = H₂O₂ group; C = ligustrazine group (80 µmol/L).
Effect of ligustrazine on mitochondrial membrane potential ($\Delta \Psi_m$)

The changes in mitochondrial membrane potential reflect the initial cell apoptotic phenomenon. Here, $\Delta \Psi_m$ was measured using flow cytometry. A substantial decrease in $\Delta \Psi_m$ was observed in HUVECs upon exposure to H$_2$O$_2$. However, after pre-treatment with different concentrations of ligustrazine (20 and 80 µmol/L), $\Delta \Psi_m$ increased to a value higher than that in the H$_2$O$_2$ group (Figure 6). These data indicate that ligustrazine could protect HUVECs against oxidative stress via mitochondrial induction pathways.

![Figure 6: Effect of ligustrazine on mitochondrial membrane potential in HUVECs.](image)

The $\Delta \psi_m$ depolarization was detected using flow cytometry with Rh123 staining. A = control group; B = H$_2$O$_2$ group; C = low ligustrazine concentration (20 µmol/L); D = high ligustrazine concentration (80 µmol/L).

Effect of ligustrazine on caspase 3, 8, and 9 activities

Caspase 3, 8, and 9 activities are associated with specific intracellular polypeptide degradation during apoptosis. Caspase 8 and 9 are initiator caspases, and caspase 3 is considered as the main executor of apoptosis. The results in Figure 7 indicate that caspase 3, 8, and 9 activities are lower in the ligustrazine group than those in the H$_2$O$_2$ group, suggesting that ligustrazine can protect HUVECs against oxidative stress and, hence, apoptosis.

![Figure 7: Effect of ligustrazine on the activities of caspase 3, 8 and 9 in HUVECs.](image)

Asterisks (*) indicate statistically significant differences between the control, H$_2$O$_2$ group, and ligustrazine group ($p < 0.05$). Values with different letters indicate statistically significant differences between the H$_2$O$_2$ group and ligustrazine group ($p < 0.05$).

Effect of ligustrazine on mRNA expression of caspase 3, 8, and 9

The mRNA expression levels of caspase 3, 8, and 9 were measured via qRT-PCR. The results reveal that the mRNA levels of caspase 3, 8, and 9 are significantly up-regulated in the H$_2$O$_2$ group compared with those in the control group ($p < 0.05$). Pre-treatment with ligustrazine in endothelial cells led to a decrease in the mRNA expression levels of caspase 3, 8, and 9 that coincide with the enzyme changes (Figure 8), suggesting that the anti-apoptotic mechanism of ligustrazine may be caspase dependent.
A variety of factors, such as inflammatory cytokines, reactive oxygen species (ROS), and lipid oxidation enzymes, could result in vascular endothelial cell damage, of which the damage caused by ROS is the most crucial [12]. Oxygen free radicals, the main oxidizing substances, participate in the body's normal physiological activity. Oxyradicals are closely related to the body's metabolism and signal transduction and play an important role in cell physiological function. Normally, a small amount of oxyradicals are by-products of cellular metabolism. However, under certain conditions, large amounts of oxyradicals are generated from the intracellular system, which may cause irreversible oxidative damages. H$_2$O$_2$ has been suggested as inducer of apoptosis in several types of cells [13]. In the current study, the oxidative injury model was established with H$_2$O$_2$ as inducer of HUVEC apoptosis. As reported previously, the viability and proliferation of HUVECs significantly decreased after H$_2$O$_2$ exposure. Morphological analysis showed similar results, with the chromatin in nucleus appearing to be condensed and marginalized in the H$_2$O$_2$ group upon observation via CLSM.

ROS affects lipids and leads to lipid peroxidation, which thus produces MDA. MDA might combine with proteins, amino acids, and other cellular components and hence change the structure of phospholipids. Moreover, LDH is released into the culture medium when cell trauma occurred [7]. In the current study, ligustrazine significantly increased SOD and GSH-Px activities but decreased LDH release and MDA content. Thus, after pre-treatment with ligustrazine, HUVECs have the ability to resist damage from oxygen free radicals, reduce lipid peroxidation, and maintain biomembrane integrity. Moreover, according to the flow cytometry analysis of apoptosis, ligustrazine ameliorates apoptosis induced by oxidative stress. This observation is consistent with a previous study, which showed that ligustrazine can ameliorate apoptosis [14]. The current study demonstrates that ligustrazine is effective in protecting cells against oxidative damage induced by H$_2$O$_2$, which is particularly important in preventing the formation of atherosclerotic plaques.

NOS, the most important active enzyme in maintaining the physiological function of endothelial cells, has the ability to remove free radicals in vivo. Moreover, NOS is an enzyme responsible for the formation of NO [15], which is responsible for vasodilation, blood pressure regulation, cardiac contractility, and the mediation of immunity during bacterial infections and inflammation [16]. Compared with those of the control group, the NO and NOS expression levels of the H$_2$O$_2$ group decreased. However, the NO and NOS expression levels increased after pre-treatment with ligustrazine. The results suggest that the effect of ligustrazine on a meliorating H$_2$O$_2$-induced apoptosis is partly through the regulation of the NO pathway.

Massive vascular endothelial cell apoptosis occurs in atherosclerotic plaque. Moreover, excessive HUVEC apoptosis is involved in the initial stage of AS [17]. Hence, the prevention and control of AS by inhibiting the excessive apoptosis of vascular endothelial cells is greatly important.

Apoptosis may be mediated by two main signal transduction pathways, the death receptor pathway and mitochondrial induction pathways [18]. The triggering mechanism of the caspase cascade in apoptosis was explored in the current study.
study to further confirm the protection against H$_2$O$_2$-induced apoptosis by ligustrazine. Recent evidence suggests that the opening of the mitochondrial permeability transition (MPT) pore is a critical event in the process, which leads to apoptosis under oxidative stress. The opening of the MPT pore can cause the dissipation of the inner mitochondrial transmembrane potential ($\Delta$Ψ$_{mito}$), culminating in the disruption of outer membrane integrity, which then leads to the release of intermembrane proteins from the mitochondrion [19]. Once the pro-apoptotic factors move from the mitochondria into the cytosol, these apoptotic proteins activate caspase proteases, amplifying apoptosis. Caspases are family members of cysteine proteases that mediate cell death and are critical regulators of apoptosis[20]. Studies have indicated that at least 11 kinds of caspase exist. Caspases 8 and 9 participate in the beginning of apoptosis, whereas caspase 3 is involved in the implementation of apoptosis. Our results show that the addition of ligustrazine attenuated the decrease in $\Delta$Ψ$_{mito}$ and the up-regulation of caspase 3, 8, and 9 mRNA and activity, suggesting that the anti-apoptotic mechanism of ligustrazine against oxidative stress is a caspase-dependent pathway involved in mitochondrial induction pathways.

CONCLUSION

In summary, ligustrazine can protect HUVECs against H$_2$O$_2$-induced injury by regulating the indicators related to oxidative stress, enhancing anti-oxidant enzyme activity, and suppressing the overexpression of caspases 3, 8, and 9. The protective mechanism of ligustrazine on H$_2$O$_2$-induced injury in HUVECs may be a caspase-dependent, anti-apoptotic mechanism involved in mitochondrial induction pathways. The results of the present study provide important information for the treatment and prevention of oxidative stress-induced AS.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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