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Original Research Article

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 H_2O_2-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 H_2O_2 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 H_2O_2-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 H_2O_2-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*

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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].

Cells usually have three model systems for oxidative stress: extracellular sources of superoxide anion (O^{2-}), hydroxyl radical (H_2O_2), and normobaric hyperoxia (elevated ambient oxygen) [5]. Among the three, H_2O_2 has been extensively used to induce endothelial cell-injury models *in vitro* because it can easily penetrate the plasma membrane and does not play a role in initiating lipid pre-oxidation and oxidizing DNA and amino acids [4].

Recently, many studies have suggested that natural bioactive compounds from plants can protect endothelial cells against oxidative damage [6]. Ligustrazine (tetramethy-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_2O_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-z-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_2O_2 concentration in oxidative stress model (MTT assay)

The concentration-dependent studies of HUVECs induced by H_2O_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_2O_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_2O_2-induced HUVECs

The cells were cultured as described above. The HUVECs were randomly divided into the control, H_2O_2, and five ligustrazine groups (5, 10, 20, 40, and 80 μmol/L + 100 μmol/L H_2O_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_2O_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_2O_2 was regarded as the H_2O_2 group. Subsequently, the effect of ligustrazine on the H_2O_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 ($\Delta\Psi_m$)

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, H_2O_2, 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 florescence 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 (∆Ct) method. ∆Ct 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, $n \geq 3$). 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 H_2O_2-induced HUVECs

Studies on the concentration-dependent survival rate of H_2O_2-induced HUVECs were performed in the present study. As shown in Figure 1, the survival rates gradually decreased with the increase in H_2O_2 concentration. When H_2O_2

Table 1: Primer sequences used for qRT-PCR

Gene	Forward primers (5'to 3')	Reverse primers (5'to 3')	Product size (bp)
β-actin	AGTTGCGTTACACCCTTTCTTG	CACCTTCACCGTTCCAGTTTT	152
Caspase-3	TGTGAGGCGGTTGTGGAAGAGT	AATGGGGGAAGAGGCAGGTGCA	182
Caspase-8	TGTCCTTCCTGAGGGAGCTGCT	TGAGCCCTGCCTGGTGTCTGAA	115
Caspase-9	TGGAGGATTTGGTGATGTCGAGCA	ATCTGGCTCGGGGTTACTGCCA	97

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 H_2O_2 can induce cell injury. Previous studies indicate H_2O_2 concentrations in the oxidative stress model system ranging from 10^{-5} mol/L to 10^{-3} mol/L, which coincide with our results [11].

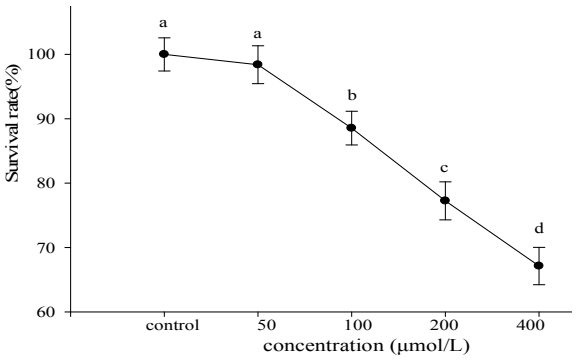
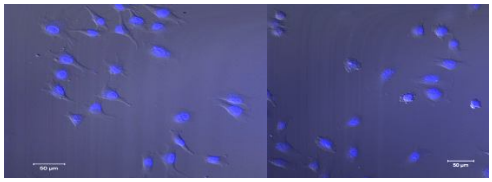


Figure 1: Survival rates of HUVECs induced by H_2O_2. Values are mean ± S.D. (n ≥ 4). Values with different letters are significantly difference in a concentration-dependent manner (p < 0.05).

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 H_2O_2 group, suggesting that H_2O_2 can result in HUVECs apoptosis.



A B

Figure 2: The micrograph of HUVECs in the control group (A) and H_2O_2 group (B) by CLSM. The results show that H_2O_2 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.

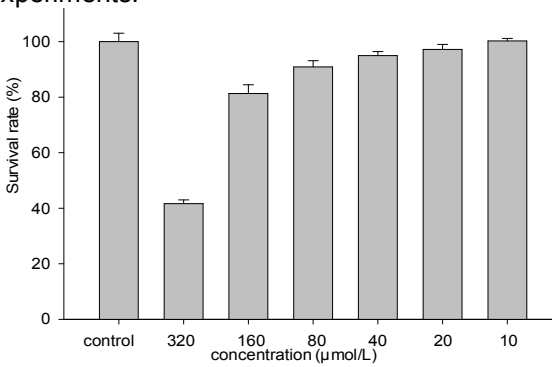


Figure 3: The effect of ligustrazine on the survival rates of HUVECs.

Protective effect of ligustrazine on the viability of H_2O_2-induced HUVECs

The protective effects of ligustrazine on the viability of H_2O_2-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 H_2O_2 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 H_2O_2-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, 82.00 ±1.20, and 86.91 ± 2.86 %, respectively. These rates suggest that ligustrazine can protect HUVECs against H_2O_2-induced injury.

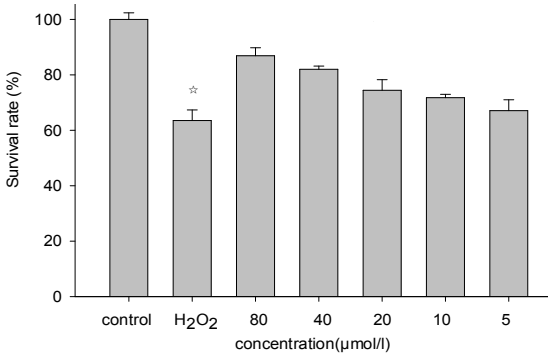


Figure 4: The protective effects of ligustrazine on HUVECs against oxidative injury.
Pentagram (☆) indicates statistically significant differences between the H_2O_2 group and control group.

Influence of ligustrazine on oxidative stress indicators of H_2O_2-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 H_2O_2-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 H_2O_2 for 8 h. However, pre-treatment with the different concentrations of ligustrazine (> 5 µmol/L) for 24 h attenuated the H_2O_2-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 H_2O_2 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 H_2O_2 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 H_2O_2 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 H_2O_2 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

Group	LDH release (%)	MDA (nmol/mgprot)	NOS (U/mgprot)	NO (µmol/L)	SOD (U/mgprot)	GSH-Px (U/mgprot)
Control	16.28±1.71	23.78±2.15	4.18±0.77	73.30±7.00	129.76±15.52	160.13±3.73
H_2O_2	34.89±0.89*^{a}	45.70±3.14*^{a}	1.95±0.20*^{a}	27.44±4.34*^{a}	45.16±3.25*^{a}	77.81±4.29*^{a}
5 µM of Ligustrazine	33.68±0.81^{a}	42.98±2.96^{ab}	2.15±0.50^{a}	32.20±4.13^{ab}	57.98±9.53^{a}	81.75±1.08^{ab}
10 µM of Ligustrazine	32.78±1.49^{b}	40.23±2.10^{abc}	2.23±0.29^{ab}	46.05±8.47^{ab}	60.64±8.85^{b}	83.37±2.15^{bc}
210 µM of Ligustrazine	29.86±0.44^{c}	38.66±4.91^{bcd}	2.60±0.35^{bc}	53.60±8.02^{ab}	74.00±3.63^{b}	90.44±4.06^{cd}
40 µM of Ligustrazine	27.42±0.83^{d}	36.52±2.68^{cd}	2.94±0.81^{cd}	68.07±3.30^{b}	82.51±1.68^{c}	96.96±2.43^{d}
80 µM of Ligustrazine	25.90±0.50^{d}	34.41±3.65^{d}	3.01±0.33^{d}	69.61±11.42^{b}	107.28±5.18^{c}	110.39±9.56^{e}

Note: Asterik (*) indicates statistically significant differences between the control and H_2O_2 group. a, b, c, d, e indicate statistically significant differences between the H_2O_2 group and ligustrazine group.

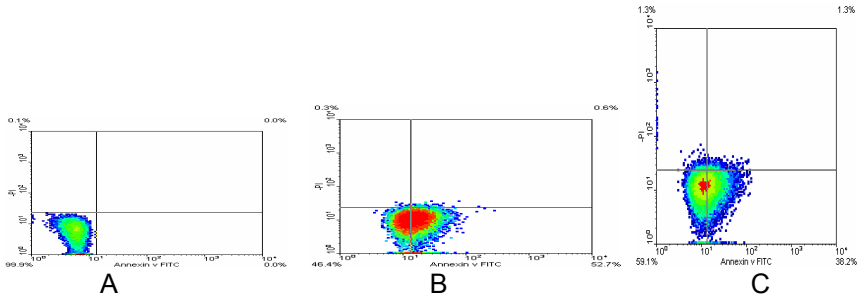


Figure 5: The flow cytometry of HUVECs apoptotic cells after treatment with the H_2O_2 and ligustrazine. Quadrant analysis of fluorescence intensity of gated cells in Annexin V-FITC and PI channels was from 16,000 events. A = control; B = H_2O_2 group; C = ligustrazine group (80 µmol/L).

Effect of ligustrazine on t 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_2O_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_2O_2 group (Figure 6). These data indicate that ligustrazine could protect HUVECs against oxidative stress via mitochondrial induction pathways.

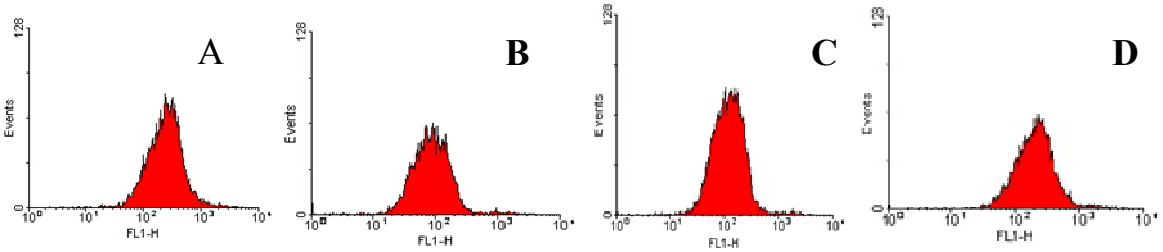


Figure 6: Effect of ligustrazine on mitochondrial membrane potential in HUVECs.
The $\Delta\psi_m$ depolarization was detected using flow cytometry with Rh123 staining. A = control group; B = H_2O_2 group; C = low ligustrazine concentration (20 µmol/L); D = high ligustrazine concentration (80 µmol/L).

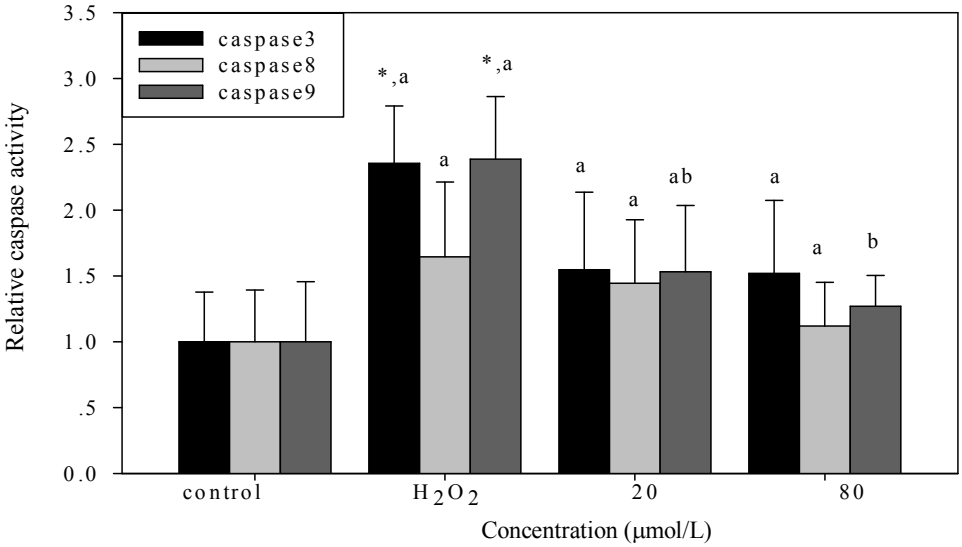


Figure 7: Effect of ligustrazine on the activities of caspase 3, 8 and 9 in HUVECs.
Asterisks (*) indicate statistically significant differences between the control, H_2O_2 group, and ligustrazine group ($p < 0.05$). Values with different letters indicate statistically significant differences between the H_2O_2 group and ligustrazine group ($p < 0.05$).

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_2O_2 group, suggesting that ligustrazine can protect HUVECs against oxidative stress and, hence, apoptosis.

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_2O_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.

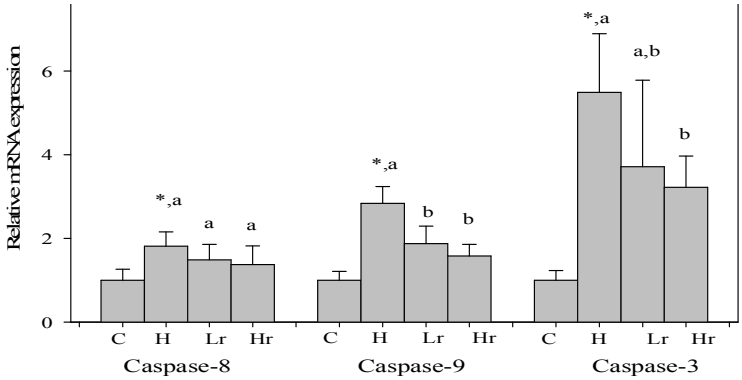


Figure 8: Effect of ligustrazine on the mRNA expression of caspase 3, 8, 9 in HUVECs. C = control group; H = H_2O_2 group; Lr = Low ligustrazine concentration (20 μmol/L); Hr = High ligustrazine concentration (80 μmol/L). Asterisk (*) indicates statistically significant differences between control, H_2O_2 group, and ligustrazine group ($p < 0.05$). Values with different letters indicate statistically significant differences between H_2O_2 and ligustrazine groups ($p < 0.05$).

DISCUSSION

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_2O_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_2O_2 as inducer of HUVEC apoptosis. As reported previously, the viability and proliferation of HUVECs significantly decreased after H_2O_2 exposure. Morphological analysis showed similar results, with the chromatin in nucleus appearing to be condensed and marginalized in the H_2O_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_2O_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_2O_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_2O_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 to further confirm the protection against H_2O_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 ($\Delta\Psi_m$), 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 apoptogenic 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 $\Delta\Psi_m$ 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_2O_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_2O_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.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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