Protective functions of *Lycium barbarum* polysaccharides in H_2O_2-injured vascular endothelial cells through anti-oxidation and anti-apoptosis effects

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Abstract. Cell injury in the cardiovascular endothelia caused by oxidative stress is among the major inducers of endothelium dysfunction and serves an important role in initiating cardiovascular diseases (CVDs). Therefore, protecting and improving the normal function of endothelial cells are considered key measures against CVDs. As a traditional Chinese medicinal component, *Lycium barbarum* is regarded to have high medicinal value. The present study aimed to investigate the potential anti-apoptosis and anti-oxidation effects of *Lycium barbarum* polysaccharides (LBPs) on injured rat artery endothelial cells, to demonstrate the experimental and medicinal values of LBPs. In the present study, the aortic endothelial cells of rats were cultivated and randomly divided into five groups: A control group, H_2O_2-injured group (H_2O_2 group), H_2O_2+LBPs (110 μg/ml) group (low-dose group, LT), H_2O_2+LBPs (220 μg/ml) group (medium-dose group, MT) and H_2O_2+LBPs (440 μg/ml) group (high-dose group, HT). Among these, the activity of superoxide dismutase (SOD), and the levels of malondialdehyde (MDA) and nitric oxide (NO) were detected by colorimetry. Additionally, the expression of B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) were detected by western blotting. It was observed that SOD activity and NO content decreased while MDA content increased significantly in the H_2O_2 group ($P<0.05$ vs. control); that SOD activity in the MT and HT group, and NO content in all three LBP groups were increased, while MDA content in the three LBP groups was decreased, compared with the H_2O_2 group (all $P<0.05$); that Bcl-2 expression decreased significantly in the H_2O_2 group while the expression of Bax increased significantly compared with the control group (both $P<0.05$); and that Bcl-2 expression in all three LBP groups increased, while Bax expression in the MT and HT groups decreased compared with the H_2O_2 group (all $P<0.05$), with these altered Bax levels being statistically similar to those in the control group ($P>0.05$). On light microscopy, the cells in the control group exhibited spindle-shaped morphology, consistent sizes, defined boundaries, and distinct nuclei of equivalent sizes with round or oval morphology. Additionally, the chromatin in the nuclei was evenly distributed, and all cells were adhered in a paving-stone arrangement. Notably, only few cells died. Conversely, the cells in the H_2O_2 group exhibited signs of damage and enlarged gaps, and focal cells died. In the HT group, the cells once again appeared adherent and exhibited similar morphological status to the normal cells. Overall, these results indicate that LBPs serve a protective role in oxidative-injured vascular endothelial cells through anti-apoptosis and anti-oxidation effects.

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Introduction

Cardiovascular diseases (CVDs), also termed circulatory system diseases, affect the heart and corresponding blood vessels and are established as a worldwide leading cause of mortality (1). In 2014, over 1.7 million fatalities occurred worldwide as a result of CVDs, with a total mortality rate of ~31%, and due to the availability of healthcare, populations in developing countries are more susceptible to CVDs (2). By 2030, it is estimated that the total number of mortalities from CVDs will exceed 2.3 million. To combat this, the World Health Organization plans to reduce the rates of non-communicable diseases (due to living, behavioral and environmental causes) to 25% by 2025, particularly with regard to CVDs (3).

Vascular endothelial cells (VECs) form a continuous cell mass layer over the endangium of vessels in the body. They not only complete the metabolic exchange of blood and interstitial fluid, but also serve as the largest endocrine gland in organisms (4). VECs produce and secrete over ten types of bioactive substances that participate in normal blood flow and blood coagulation, adjust vessel tensions, and control the proliferation and functions of smooth muscles (5). In addition,

VECs also serve an important role in adjusting the steady state of the cardiovascular system (6). The circulatory system, particularly the blood vessel component, is among the main targets of oxidative stress. Consequently, VECs serve a physiological defense function of 'first line of defense' against stress injuries to organisms (7,8). The function of endothelial cells is associated with the occurrence and development of CVDs (9). Among numerous causes for injury to endothelial cells, oxidative stress is an important inducer of vascular endothelial dysfunction (10). Therefore, the apoptosis of endothelial cells in the cardiovascular system is a major indicator of oxidative stress injury, and a prelude for the occurrence and evolution of various CVDs (11,12).

Lycium barbarum polysaccharides (LBPs), water-soluble protein polysaccharides, are a constituent separated and extracted from the red medlar (also known as wolfberry) fruit of *L. barbarum,* a commonly used medicinal herb in Ningxia, China (13). LBPs have diverse functions, including eye-protective, anti-aging, anti-oxidant, immune adjustment, neuroprotective and anti-tumor effects (14-18). Notably, LBPs may protect the rat reproductive system through an anti-oxygenation mechanism; they may also prolong the apoptosis time of rat epithelial cells, inhibit ultraviolet light-induced peroxidase and free radical-induced cytochrome C expression, and reduce the oxidation of fractured DNA in rat testis cells. Furthermore, LBPs have been reported to markedly promote serum sex hormone levels and superoxide dismutase (SOD) activity, decrease malondialdehyde (MDA) level, decrease injury to spermatogenic cells caused by high temperature and H_2O_2, and promote normal development of germ cells of the testis (19). However, there is limited research on whether LBPs protect against oxidative stress in artery endothelial cells to prevent and cure CVDs. Therefore, the present study aimed to investigate the protective effects of LBPs, particularly regarding anti-apoptosis and anti-oxidation, in injured rat artery endothelial cells (RAECs), to thus provide experimental and theoretical foundations for the medicinal use of LBPs.

Materials and methods

Materials and equipment. The materials used in the present study included RAECs (Jiangsu Chi Scientific Co., Ltd.), complete Dulbecco's modified Eagle's medium (DMEM) containing FBS (both 3-7202; Jiangsu Chi Scientific Co., Ltd.), a Cell Counting kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.), LBPs (Ningxia Qiyuan Pharmaceutical, Co., Ltd.), anti-B-cell lymphoma 2 (Bcl-2) antibody (cat. no. ab59348; Abcam), anti-Bcl-2-associated X protein (Bax) antibody [E63] (cat. no. ab32503; Abcam), and β-actin monoclonal antibody (cat. no. TA811000), horseradish peroxidase (HRP)-goat anti-rat immunoglobulin G (IgG) (cat. no. TA130038) and HRP-goat anti-rabbit IgG (cat. no. TA130023) all from OriGene Technologies, Inc. A total protein extraction kit (KGP250) and bicinchoninic acid assay (BCA) kit (KGP902; Nanjing KeyGen Biotech. Co., Ltd.), a SOD assay kit-WST-1, a Microscale MDA assay kit (thiobarbituric acid method) and a nitric oxide (NO) assay kit (A012-1-2; Colorimetric; Nanjing Jiancheng Bio-Engineering Institute Co., Ltd.) were also used. The major instruments included electrophoresis apparatus, a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad Laboratories, Inc.), a light microscope (Olympus Corporation), an Amersham Imager 600 (GE Healthcare Life Sciences and a CO_2 incubator (Heraeus).

Cultivation and experimental grouping. RAECs cell line were cultivated in complete Dulbecco's modified Eagle's medium (DMEM) to a confluent state (80-90%) for passage at 37˚C in the CO_2 incubator at 5% CO_2. The cells were then divided into five groups: A control group, H_2O_2 injury group (H_2O_2 group), H_2O_2+LBPs (110 μg/ml) group (low-dose group, LT), H_2O_2+LBPs (220 μg/ml) group (medium-dose group, MT) and H_2O_2+LBPs (440 μg/ml) group (high-dose group, HT). The doses of LBPs and H_2O_2 were selected on the basis of preliminary experiments, as follows, in which they were safe and effective without causing toxicity to the RAECs.

Oxidative injury model induced by H_2O_2 analyzed through CCK-8. RAECs, cultured in DMEM supplemented with 10% FBS were seeded in 96-well plates at $2x10^4$ cells/well, treated with 0, 50, 100, 200, 300, 400 and 500 μmol/l H_2O_2 and incubated at 37˚C with 5% CO_2 for 2 h. Subsequently, cell viability was detected using CCK-8 as described previously (20) and cells were detected under a microplate reader at wavelength 450 nm. The optical densities (ODs) were recorded and used to calculate the cell survival rate at each H_2O_2 concentration. The H_2O_2 concentration that retained 50% survival of the cells was selected and used to establish an RAEC oxidative injury model for subsequent experiments.

Influence of LBPs on RAEC survival rate analyzed through CCK-8. Cells were seeded in 96-well plates at $2x10^4$ cells/well and treated with 0.0 (control), 27.5, 55.0, 110.0, 220.0, 440.0, 880.0, 4,400 and 8,800.0 μg/ml LBPs at 37˚C with 5% CO_2 for 2 h. The CCK-8 assay was performed as above. The cells were then detected under a microplate reader at wavelength 450 nm. The ODs were recorded and used to calculate the cell survival rate at each LBP concentration. The concentrations that exerted an inhibitory effect on RAECs were removed.

Determination of LBP effect on oxidative injury in RAECs. Cells were treated with H_2O_2 at the 50% inhibitory concentration and the LBP concentrations that did not result in inhibition of cell viability. Under these established interventions, cells were seeded in 96-well plates at $2x10^4$ cells/well and cultured at 37˚C with 5% CO_2 for 2 h. The CCK-8 assay was performed as above. Subsequently, the cells were detected under a microplate reader at wavelength 450 nm. The ODs were recorded and used to calculate the cell survival rates of all groups. The optimum protective clinical concentration of LBPs against oxidative injury in the RAECs was screened out and used in the following experiments.

Morphological observation of all groups. The cells in the established treatment groups (control, H_2O_2, LT, MT and HT groups) were placed under an inverted light microscope. Following removal of the medium, the cell morphologies and quantities were observed at x200 magnification.

Measurement of SOD, MDA and NO. Oxidative stress participates in numerous pathways associated with pathological

changes in endothelial cells (3). To determine whether LBP intervention could remove reactive oxygen species (ROS) and inhibit ROS-mediated oxidative stress, the cell culture was collected and centrifuged at room temperature for 5 min at 3,000 x g, and the commercial kits were used to detect the levels of SOD activity, MDA and NO by colorimetric methods, according to the manufacturer's instructions.

Western blot analysis of Bcl-2 and Bax expression. Oxidative stress ultimately causes cell apoptosis, and thus apoptosis factors were studied to determine whether LBPs could inhibit apoptosis caused by oxidative stress. The typical index of the Bcl class was selected, including the inhibitor of apoptosis protein Bcl-2 and the pro-apoptotic protein Bax, which were used to indirectly assess whether LBPs prevented apoptosis. Total protein was extracted from cells using the protein extraction kit, and quantified using the BCA kit, according to the manufacturer's instructions. Proteins (80 μg) were separated on 12% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. Membranes were then blocked (5% non-fat dry milk in PBS plus 0.1% Tween-20; room temperature; 2 h), incubated with primary antibody (4˚C; overnight) and second antibody (room temperature; 2 h) and then quantified. The primary antibodies used were anti-Bcl-2, diluted in 1% bovine serum albumin (BSA; Thermo Fisher Scientific, Inc.) to 1:200, anti-Bax, diluted in 1% BSA to 1:3,000, and anti-β-actin, diluted in 1% BSA to 1:500; the secondary antibodies were HRP-goat anti-rat IgG and HRP-goat anti-rabbit IgG, diluted in 1% BSA to 1:5,000. Bands were quantified following detection with the ECL kit using Image Lab 5.0 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analysis was conducted on SPSS 16.0 (SPSS, Inc.). Data were expressed as the mean ± standard deviation of at least three independent replicate experiments. The groups were compared by one-way analysis of variance followed by Fisher's least significant difference post hoc analysis, with a significance level of P<0.05.

Results

Optimal concentration of H_2O_2 for the oxidative injury model. H_2O_2 injured cells in a dose-dependent manner. The concentration of H_2O_2 that caused ~50% death rate in RAECs was 100 μmol/l (P<0.05 vs. control; Fig. 1). Therefore, 100 μmol/l was selected for the oxidative injury model to be tested in subsequent assays.

Selection of LBP dosage. The RAECs was not affected by different concentrations of LBPs except at the concentration of 8,800 μg/ml, which significantly reduced cell viability compared with the control (P<0.05; Fig. 2). Thus, 8,800 μg/ml was removed from further experiments.

Protective effect of LBPs. Following selection of the safe LBP dosages, the oxidative injury model was treated with different concentrations of LBPs to test the effect of the LBPs on cell injury (Fig. 3). LBPs at ≤55 or 4,400 μg/ml did not exert a protective effect in the oxidative-injured RAECs. By contrast, LBP concentrations between 110 and 880 μg/ml significantly protected the RAECs (P<0.05 vs. control), in an apparent dose-dependent manner between 110 and 440 μg/ml. Thus, the concentration of 440 μg/ml LBPs exerted the most marked protective effect, and 110, 220 and 440 μg/ml were used as low, medium and high intervention dosages of LBPs, respectively, to characterize their protective effects against oxidative cell injury.

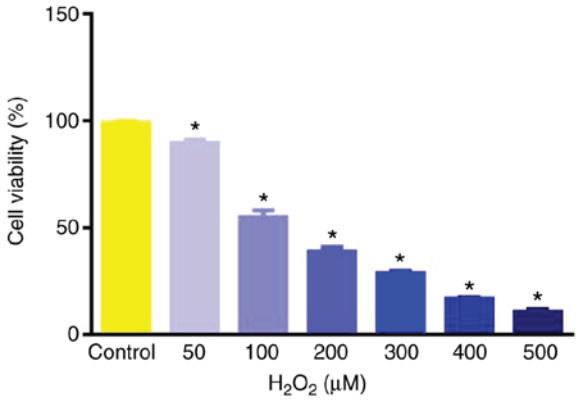

Figure 1. Effect of H_2O_2 on rat artery endothelial cell viability. *P<0.05 vs. control.

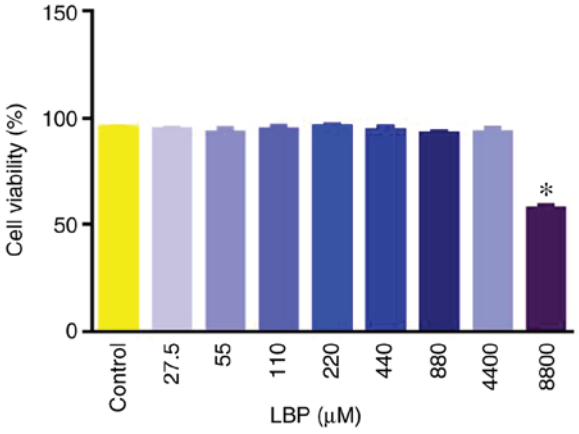

Figure 2. Effect of LBPs on rat artery endothelial cell viability. *P<0.05 vs. control. LBPs, *Lycium barbarum* polysaccharides.

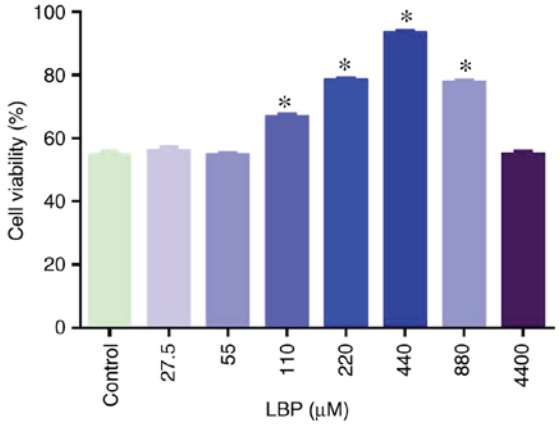

Figure 3. Effect of LBPs on cell viability of rat artery endothelial following H_2O_2-associated oxidative stress injury. *P<0.05 vs. control. LBPs, *Lycium barbarum* polysaccharides.

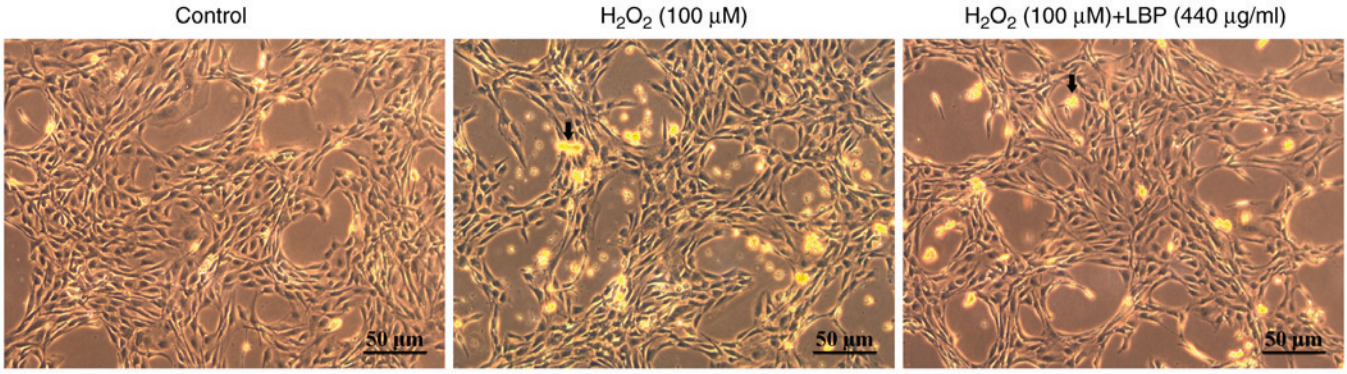

Figure 4. Morphology of rat artery endothelial cells in the different groups. Arrows indicate cells with typical morphological changes (magnification, x200).

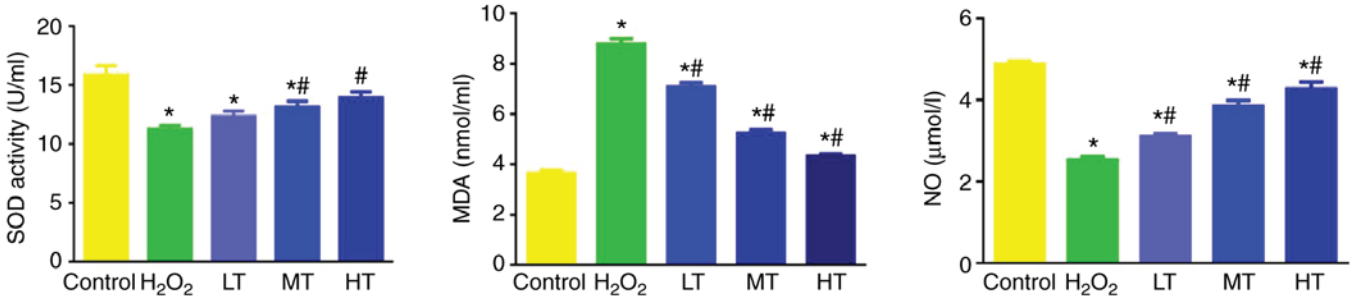

Figure 5. SOD activity and MDA and NO contents. *P<0.05 vs. control; #P<0.05 vs. H_2O_2. SOD, superoxide dismutase; MDA, malondialdehyde; NO, nitric oxide; LT/MT/HT, low/moderate/high-dose *Lycium barbarum* polysaccharide groups.

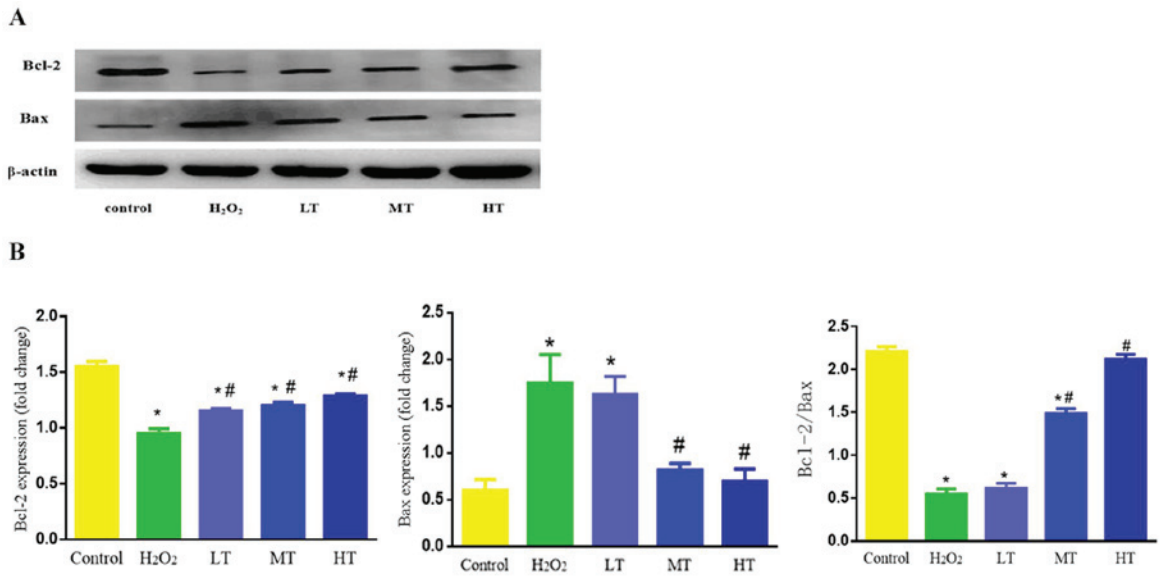

Figure 6. Western blot analysis of Bcl-2, Bax and Bcl-2/Bax protein expression. (A) Gel bands; (B) graph of quantified expression levels. *P<0.05 vs. control; #P<0.05 vs. H_2O_2. Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; LT/MT/HT, low/moderate/high-dose *Lycium barbarum* polysaccharide groups.

Morphology of the cells. Normal RAECs under the microscope (magnification, x200) exhibited shuttle-like morphology with round or oval, clear nuclei and an ordered, paving stone arrangement (Fig. 4). Furthermore, the cell nuclei were of similar sizes, with evenly distributed chromatin, and were located centrally in the cells. These cells formed a relatively confluent monolayer. Conversely, under the stimulation of 100 μmol/l H_2O_2, the gaps in the RAEC monolayer were enlarged and the adherence of cells was lost. Regions of the cell nuclei were strongly stained, indicating nuclei shrinkage. When the nuclei shrank, the chromatin became darker, which was matched by cell enlargement in certain cells. Granular, condensed chromatin was rarely identified. In the presence of 440 μg/ml LBPs, the majority of cells exhibited the same morphological characteristics as the normal control group (Fig. 4).

SOD activity, MDA and NO levels in cell culture supernatant. As depicted in Fig. 5, SOD activity, NO and MDA contents were altered between the groups. Notably, SOD activity and NO content significantly decreased while MDA content significantly increased in the H_2O_2 group compared with control group ($P<0.05$). In turn, SOD activity in the MT and HT group, and NO content in all three LBP groups were increased, while MDA content in the three LBP groups was decreased, compared with the H_2O_2 group (all $P<0.05$); however, the levels remained significantly altered compared with the control ($P<0.05$; Fig. 5).

Bcl-2 and Bax protein expression. Under the simulation of H_2O_2, the protein expression of apoptosis factors was also altered (Fig. 6). Notably, Bcl-2 expression decreased and Bax expression increased in the H_2O_2 group compared with control group (both $P<0.05$). In turn, Bcl-2 expression in all three LBP groups increased, while Bax expression in the MT and HT groups decreased compared with the H_2O_2 group (all $P<0.05$), with these altered Bax levels being statistically similar to those in the control group ($P>0.05$; Fig. 6B). The changes observed among the LBP groups appeared dose-dependent (Fig. 6A and B).

Furthermore, the expression ratio of Bcl-2/Bax protein in the H_2O_2 group decreased compared with the control group ($P<0.05$). The Bcl-2/Bax ratios in the MT and HT groups increased compared with the H_2O_2 group (both $P<0.05$), with the HT group deemed statistically similar to the control group ($P>0.05$; Fig. 6B). These changes again appeared dose-dependent (Fig. 6A and B).

Discussion

Due to the environmental, diet and lifestyle factors in modern society, CVDs have become increasingly frequent and tend to occur at younger ages (21-23). Thus, there has been increasing focus on the prevention of these diseases. Red medlar is a tradition medical and food product used in China (24). In traditional Chinese medicine, medlar is considered to nourish the liver, kidney and heart, and balance 'yin' and 'yang' in the body (25). Therefore, the biological effects of medlar should be a focus of clinical research. Despite its history of use, the mechanisms underlying the effects of medlar in body remain unclear. LBPs, as the effective constituents of medlar, may directly block the action of toxins in cells and activate microglial cells in the central nervous system (26). Compared with the potent stimulatory effects of glossy ganoderma and ginseng reported in the immune system, LBPs may moderately stimulate the immunoreactivity of the central nervous system (27). A number of studies have indicated that LBPs can exert marked anti-oxidant and anti-aging effects against oxidation-induced cell injury (28,29). Notably, LBPs could protect lens epithelial cells from H_2O_2 stress by promoting the expression of anti-apoptotic protein Bcl-2 and upregulating Bcl-2/Bax expression (30). Furthermore, LBPs protected seminiferous epithelial cells from thermal-induced injuries, protected DNA from oxidation injury in rat testis cells and markedly decreased the blood glucose of diabetic rats (31).

Oxidative stress occurs under normal conditions in the body, and there are a series of adaptive mechanisms that protect cells from associated injuries. Normally, the production and elimination of ROS are under dynamic equilibrium, and thus they exert no lasting damage to cells. However, strong harmful stimulation may disrupt this balance and generate abundant levels of ROS that exceed the scavenging activity of the antioxidant system. As a consequence, the body generates an oxidative stress response to promote cell apoptosis, though pathological injury may still result (32). It is established that the ROS-mediated oxidative stress is a key inducer of cell apoptosis (33). ROS may also serve as a messenger in cells that is able to promote the activation of apoptosis to indirectly cause cell injury (34). The cell injury also induces the production of more ROS in cells. Taken together, oxidative stress is an important mechanism of organ injury and participates in the occurrence and development of various diseases (35).

Previous animal experiments by our group demonstrated that under exhaustive exercise and heat stress conditions, LBPs markedly inhibited oxidative stress and liquid peroxidation, improved compliance of the thoracic aorta, vessel diastolic function and exercise tolerance, and adjusted oxidase activities (36); however, the detailed mechanisms were undetermined. Therefore, the aim of the present study was to investigate the effect of LBP on H_2O_2-induced oxidative stress injury in RAECs, to thus determine the mechanism of the LBP protective effects. Initially, various H_2O_2 concentration concentrations were tested to determine the optimum dose for an oxidative stress model, and the H_2O_2 concentration of 100 μmol/l was selected based on its induction of ~50% cell survival.

Subsequently the effect of LBPs on the viability of RAECs was assessed, where it was observed that the cells were not inhibited by concentrations of LBPs <8,800 μg/ml. However, following the application of different concentrations of LBPs in the model cells, it was observed that the LBP concentrations below 55 or higher than 4,400 μg/ml did not exert a protective effect in the oxidative-injured RAECs; the effective concentration of LBPs was between 110-880 μg/ml, with the protective effect was most marked at 440 μg/ml. Therefore, 110-440 μg/ml doses were applied in subsequent assays to evaluate the mechanism of the LBP protective effect.

It is established that SOD is a primary scavenger of free radicals in the body and may also eliminate hazardous metabolic substances (37,38). Ageing has been associated with the production and accumulation of oxygen radicals in the body (39). It has been clinically demonstrated that SOD is associated with physiological pathology and the production/development of various diseases in humans (40,41). SOD is able to adjust blood lipids and, it may prevent atherosclerosis and CVDs caused by hyperlipidemia and reduce the content of lipid peroxide (42,43). The activity level of SOD, as a free-radical scavenger, reflects the ability of cells clear free radicals *in vivo* (44). MDA, as a major product of membrane lipid peroxidation, is also an injury marker that reflects the extent of cell injury. Additionally, MDA content is an important index that reflects membrane lipid peroxidation (45). Thus, the present study detected the change in both SOD activity and MDA content prior to and following LBP treatment in H_2O_2-injured cells.

It was observed that the treatment with LBPs at moderate and high doses (220 and 440 μg/ml) significantly enhanced

SOD activity in the H_2O_2-induced oxidative stress group; notably, SOD activity was improved to a statistically similar level to that of the control group with 440 μg/ml LBPs. This may be associated with lycium polysaccharide reducing the fluidity of the plasma membrane and thus reducing damage by free radicals. The MDS levels in the oxidative injury group were also inhibited in a dose-dependent manner by LBPs, probably due to the LBPs enhancing the activity of anti-oxidase enzymes in free radical clearance.

The common pathological cause of many CVDs is endothelial dysfunction. NO is an important vasodilatation factor produced by VECs. The reduced rate of clearance and excessive production of ROS are important causes of endothelial dysfunction. The present data indicate that LBPs may clear high levels of ROS to thus exert an antioxidant effect, and thereby enhance NO usage rate and adjust vasodilatation function, which would ultimately improve the resistance of cells to oxidative stress. Collectively these results indicate that LBPs may prevent H_2O_2-induced cell apoptosis and modulate, at least in part, the ROS-NO axis.

Cell apoptosis is a structured form of cell death controlled by gene under certain physiological or pathological conditions. This programmed cell death involves the activation, regulation and expression of diverse genes (46). Cell apoptosis is essential in maintaining the body at steady state and the normal physiological functions of organs (47). Cell apoptosis results from stimulation by external factors and pathological events in cells. It is controlled by specific genes, and is induced and inhibited by stimulating factors (including H_2O_2) in the cell microenvironment (30) Exogenous factors may also exert stimulatory effects through certain signaling pathways (48). The Bcl-2 gene family serves a meditative and regulatory role in cell apoptosis (49). Key anti-apoptosis and pro-apoptosis factors in this family are Bcl-2 and Bax (50). As a proto-oncogene, Bcl-2 is a mitochondrial membrane protein and is considered the most important anti-apoptosis gene (51). Conversely, Bax expression may enhance antagonism of Bcl-2 and thereby promote cell apoptosis; H_2O_2 may activate this Bax expression (52). The apoptosis precursor proteins, including Bax, may alter translocation through mitochondrial membranes, but their activation may be resisted by the anti-apoptotic proteins of mitochondrial membranes, which form heterodimers and maintain membrane integrity (53). For instance, the anti-apoptotic protein Bcl-2 protects mitochondria by inhibiting Bax activation and subsequent translocation of cytochrome C through the mitochondrial membrane (54); in this regard, a lack of Bcl-2 would allow the breakdown of mitochondrial outer membrane integrity and the release of cytochrome C (55). Thus, the Bcl-2 family adjusts cell apoptosis mediated by the mitochondrial pathway through anti-apoptosis and pro-apoptosis factors, as well as participating in the crosstalk of the mitochondria and death receptor pathways (56). In cases of apoptosis mediated by oxidative stress, the expression of Bcl-2-related proteins may be altered, affecting cell apoptosis. Notably, the present data indicated that oxidative stress reduced the ratio of Bcl-2/Bax. This was improved following LBP intervention. Thus, LBPs may moderately protect cells from H_2O_2-induced injury and produce an apoptosis-inhibitory effect, ultimately attenuating the oxidative stress condition. Notably, the higher-dose LBP intervention almost recovered cell condition to a normal state. Overall these results may aid to develop methods for attenuating or preventing oxidative stress in endothelial cells for the clinical treatment of CVDs.

In conclusion, H_2O_2-induced oxidative stress in RAECs, while LBPs upregulated SOD and NO and downregulated MDA through an apparent antioxidant defense mechanism. Furthermore, LBPs increased Bcl-2/Bax expression potentially through an antiapoptosis system. Therefore, LBPs may protect VECs from H_2O_2-induced injury through anti-oxidation and anti-apoptosis effects.

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Availability of data and materials

All data sets generated or analyzed during the study are included in the published article.

Authors' contributions

GL conceived and designed the experiments. SX performed the experiments and prepared the manuscript. XH performed experiments. LZ and LN analyzed the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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