Effects of *Lycium barbarum* polysaccharides on the damage to human endometrial stromal cells induced by hydrogen peroxide

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Abstract. Previous studies have shown that *Lycium barbarum* polysaccharides (LBPs) serve an important role in antioxidant activity to protect the cells and tissues. However, the specific mechanism of LBPs in the prevention of endometrial damage remains to be elucidated. Using morphological observation, cell proliferation assay, the detection of superoxide dismutase (SOD) activity and the content of malondialdehyde (MDA) in cell culture supernatant fluid, the detection by western blot analysis and reverse transcription-quantitative polymerase chain reaction of the mRNA and protein expression levels of caspase-3 and Bcl-2 in endometrial stromal cells (ESCs), it was demonstrated that, *in vitro*, hydrogen peroxide (H_2O_2)-induced death of ESCs, increased the content of MDA and decreased the activity of SOD, and decreased the expression of Bcl-2 and increased the expression of caspase-3. LBPs can inhibit H_2O_2-induced cell death of ESCs, decrease the content of MDA in ESCs and increase the activity of SOD, as well as increasing the expression of Bcl-2 and decreasing the expression levels of caspase-3. These findings suggested that LBPs can inhibit H_2O_2-induced apoptosis of EECs and that LBPs are able to offer a significant protection against oxidative stress to ESCs.

Introduction

The uterus in mammals serves more than a reproductive role; it also serves other key physiological functions and so the maintenance of the normal structure and function of the endometrium is important. Pathological conditions, including ischemia, hypoxia, or inflammation, may cause excessive reactive oxygen species to accumulate in the body, upsetting the oxidation/antioxidant balance and causing oxidative stress to trigger endometrial cell damage (1,2). This is mainly manifested in infertility; endometrial damage is a difficult clinical problem.

With the development of assisted reproductive technology, numerous problems in fertility have been solved, but not endometrial damage. At the time of writing, the mechanism of endometrial damage remains to be elucidated and there remains a lack of effective diagnosis and prevention.

The fruits of *Lycium barbarum* are used in traditional Chinese medicine and are credited with numerous biological activities and pharmacological functions. They may serve a role in antiaging effects (3,4), in antitumor activity (5,6), immunomodulatory activity (7) and increased metabolism (8). Previous studies have indicated that of the variety of nutrients and trace elements in *Lycium barbarum*, the main effective components are *Lycium barbarum* polysaccharides (LBPs) and they have been reported (9-12) to exhibit a concentration-dependent antioxidant activity, including antilipid peroxidation, superoxide anion scavenging and anti-superoxide formation. However, any specific antioxidant properties of LBPs in the prevention of endometrium damage remain to be elucidated.

In the present study, selected human endometrial stromal cells were used to investigate the protective effect of LBPs on the hydrogen peroxide (H_2O_2)-induced injury of human endometrial stroma, and a scientific theoretical basis was investigated for the application of LBPs in reproductive health care.

Materials and methods

Endometrial specimen collection. Endometrial tissues were obtained from the hysterectomies of 20 patients with uterine fibroids in the Affiliate Hospital of Hebei Engineering University (Handan, China) between September 2013 and December 2014. The procedure was approved by the Ethics Committee of Hebei Engineering University, and informed consent was obtained from the patients with uterine fibroids prior to the hysterectomies. The patients were aged between 30 and 45 years old, with menstrual cycles from 24-35 days (mean, 28 days). No patient received any hormonal treatment for at least 3 months prior to the hysterectomies.

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Endometrial tissues were confirmed as in hyperplasia period and disease-free by postoperative pathology. Endometrial tissue was scraped immediately from the uterus under sterile conditions *in vitro*, and placed in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F12) (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), including 10% fetal bovine serum, penicillin and streptomycin (100 mg/ml; Gibco; Thermo Fisher Scientific, Inc.) in an ice bath, and transported to the laboratory within 2 h.

Preparation of LBPs. LBPs were purchased from Shanghai Organic Chemistry Institute (Shanghai, China). A stock solution was prepared of 5,000 mg/l in basal medium DMEM/F12 and maintained at -20˚C. For the following experiments, the final concentration of LBPs was 100 μg/ml produced by diluting the stock solution with DMEM/F12.

Isolation, purification and culture of endometrial stromal cells (ESCs). Following several washes with phosphate buffered saline (PBS), the tissue was cut into 1-2 mm^3 pieces with sterile scissors and incubated with 5 ml DMEM/F12 containing 0.2% collagenase I (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) in an incubator with atmosphere of 5% CO_2 at 37˚C for 60 min. During the incubation, the tissue pieces were pipetted gently to disperse the cells. The whole cell suspension was centrifuged at 500 x g for 5 min at room temperature. The supernatant fluid, which contained ESCs, was centrifuged at 1,200 x g for 5 min at room temperature. The supernatant fluid was discarded, while the precipitate was resuspended in a culture bottle with 3 ml complete cell-culture medium [DMEM/F12+10% fetal bovine serum (FBS)+1% penicillin and streptomycin]. The ESCs attached to the culture bottle were washed several times with serum-free DMEM/F12 to remove red blood cells.

A trypan blue exclusion assay was performed to assess the proportion of living cells. Then, 1 ml cell suspension ($1x10^5$ cells/ml) was placed into a six-well plate containing coverslips and cultured in atmosphere of 5% CO_2 at 37˚C for 5 days for the identification and assessment of the purity of the cells.

Morphological observation and identification of ESCs. ESCs were cultured for 0 and 5 days and then stained with hematoxylin and eosin (H&E) following fixing in 4% paraformaldehyde for 1-2 h at 4˚C and dehydration with 70, 80, 90, 95 and 100% ethanol and 100% xylene. The morphology and structure of the ESCs were observed with an inverted microscope equipped with phase-contrast apparatus and with a light microscope.

Immunocytochemical staining was performed to identify the ESCs and assess their purity. PBS was used instead of a primary antibody as a negative control. Cells cultured on coverslips were fixed with 4% paraformaldehyde and treated with 0.25% Triton X-100. After blocking with 5% normal goat serum for 20 min at 37˚C, the rabbit anti-human primary antibody vimentin (1:100; cat. no. MA3-745) and cytokeratin (1:100; cat. no. PA5-14263; Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) were incubated with the cells at 4˚C overnight. The cells were subsequently incubated with goat anti-rabbit IgG (1:100; Boster Corporation, Wuhan, China) for 20 min at 37˚C and stained with 3,3'-diaminobenzidine (5 mg/ml; Sigma Aldrich; Merck Millipore) for 5 min at room temperature. The specimens were washed with PBS for 5 min three times and observed using light microscopy.

Experimental groups. ESCs were divided into four groups according to the different intervention factors. Control group: Serum-free DMEM/F12; H_2O_2 group: H_2O_2 and serum-free DMEM/F12; LBP group: LBPs and serum-free DMEM/F12; H_2O_2 + LBP group: H_2O_2, LBPs and serum-free DMEM/F12. The final concentration of H_2O_2 and LBPs was 10^{-4} M/l and 100 μg/ml respectively.

Morphological observations. ESCs ($4x10^4$/ml) were seeded in 6-well plates and were divided into the above four groups and cultured for 12 h. Morphology and structure of the ESCs were observed with an inverted microscope equipped with phase-contrast apparatus.

Cell proliferation assay. Cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. ESCs ($4x10^4$/ml) were seeded in 96-well plates and cultured with serum-free DMEM/F12 for 12 h for synchronization. They were divided into the four groups described previously, each group into 6-well plates and cultured for 24 h. Thereafter, MTT (5 mg/ml) was added to each well and the plates were incubated at 37˚C for 2 h. The medium was then replaced with 150 μl DMSO and agitated for 10 min at room temperature. The absorbance at 560 nm was measured using a microplate reader (Packard Instrument Company, Inc., Meriden, CT, USA).

Detection of superoxide dismutase (SOD) activity and malondialdehyde (MDA) content. The four groups of cells were cultured for 12 h, absorbing the culture supernatant fluid, according to the manufacturer's protocol of SOD assay kit and MDA assay kit (R&D Systems, Minneapolis, MN, USA), the activity of SOD was detected by autoxidation of pyrogallic acid, as previously described (13) and the content of MDA was detected by the thio-barbituric acid method, as previously described (14).

Western blot analysis. Cells from the above four groups were grown in 10 cm dishes and cultured for 12 h, followed by washing with PBS. The cells were subsequently lysed with lysis buffer (pH 7.4; 1 M Tris-HCl, 1% Triton X-100, sodium deoxycholate, 10% SDS). Solubilized proteins were centrifuged at 14,000 x g at 4˚C for 30 min. Extracted proteins were quantified by Coomassie Protein assay reagent (Sigma-Aldrich; Merck Millipore). Western blot analysis of Bcl-2 and caspase-3 were performed. In brief, 30 mg isolated protein was electrophoresed on 8% sodium dodecyl sulphate-polyacrylamide gel (100 V for 1.5 h), and transferred onto PVDF membranes. The membranes were treated with blocking solution [Tris-buffered saline (TBS) pH 7.2, 0.1% Tween, 5% milk] for 1 h at room temperature and incubated for 12 h at 4˚C with rabbit anti-human monoclonal Bcl-2 (cat. no. 13-8800) and caspase-3 (cat. no. 700182) antibodies (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), diluted 1:1,000 in TBS (pH 7.2) 0.1% Tween. After four washings for 15 min with

TBS (pH 7.2) 0.1% Tween, the membranes were incubated with the goat anti-rabbit IgG antibody horseradish peroxidase conjugate (cat. no. 35552) (Invitrogen; Thermo Fisher Scientific, Inc.), diluted 1:1,000 in TBS pH 7.2, 0.1% Tween for 1 h at room temperature. They were then washed four times for 15 min with TBS (pH 7.2), treated with an enhanced chemiluminescent method according to the protocols of an enhanced chemiluminesence detection kit (eECL western blot detection kit) (Invitrogen; Thermo Fisher Scientific, Inc.) and exposed to Kodak X-ray film for 0.5-20 min as necessary to detect the signals. The relative intensity of the immunoreactive bands on the films was quantified by a computer-assisted densitometry program (SmartView; Major Science, Saratoga, CA, USA). Proteins expression was quantified by comparison with internal control β-actin.

RNA extraction. Total RNA was extracted from the cells in the four groups by culturing for 12 h with the TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and the RNA was dissolved in RNase-free water. The integrity of the RNA was assessed by ethidium bromide agarose gel electrophoresis and the quantity of RNA was determined by the relative absorbance at 260 vs. 280 nm. cDNA (2 μg) was synthesized in a volume of 10 μl with a cDNA synthesis kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. The cDNA was stored at -20˚C.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR, primers (Invitrogen; Thermo Fisher Scientific, Inc.) were derived from the GeneBank database. β-actin was used as the housekeeping gene. The primers used were as follows: Caspase-3 forward, 5'-TATCCTGAGATGG GTTTA-3' and reverse, 5'-TGTTTCCCTGAGGTTTGC-3'; Bcl-2 forward, 5'-CTGGGAGAACAGGGTACGATAA-3' and reverse, 5'-CCCACCGAACTCAAAGAAGG-3'; β-actin forward, 5'-CGGGAAATCGTGCGTGAC-3' and reverse, 5'-CAGGAAGGAAGGCTGGAAG-3'.

The RT-qPCR reactions were performed using Brilliant SYBR-Green QRT-PCR Master Mixture according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.). RNA for caspase-3 and Bcl-2 was amplified using an ABI Prism 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). For all RT-qPCR studies, synthesizing by PCR procedure was performed with the following time courses: 94˚C for 10 min, 40 cycles at 94˚C for 15 sec, 59˚C for 30 sec, and 72˚C for 32 min for amplification. The amplified products were subjected to a stepwise increase in temperature from 59-94˚C and dissociation curves were constructed.

Target mRNA was quantified by measuring the threshold cycle and reading against a calibration curve. The relative amount of each mRNA was normalized to the housekeeping gene, β-actin mRNA. Results were analyzed using the relative standard curve method of analysis/$2^{-\Delta\Delta Cq}$ method of analysis (15).

Statistical analysis. All data are presented as bar graphs with the mean ± standard deviation of six independent experiments with samples from different subjects. Data were analyzed using SPSS for windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed by one-way analysis of variance, Student-Newman-Keuls was used to compare individual groups. $P<0.05$ was considered to indicate a statistically significant difference.

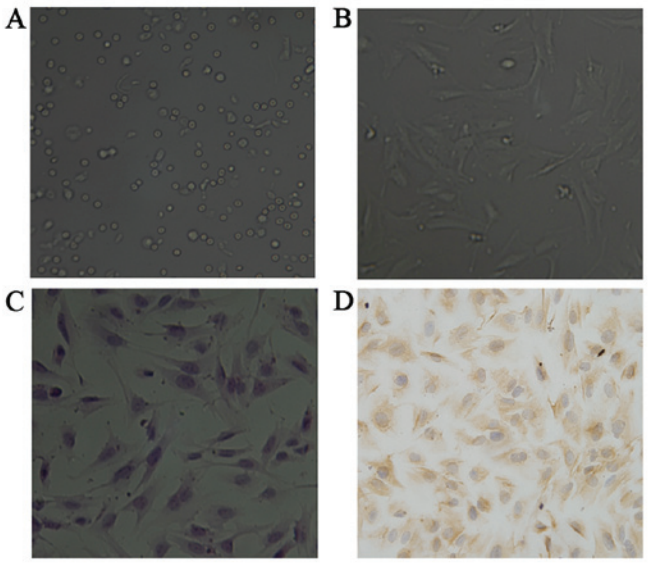

Figure 1. Observation and identification of ESCs under an inverted microscope equipped with phase-contrast apparatus and after staining with H&E (magnification, x400). (A) Round freshly isolated ESCs under phase-contrast. (B) ESCs 5-days-old growing as spindle and polygonal-shaped cells with long cytoplasmic processes under phase-contrast. (C) ESCs 5-days-old stained by H&E under the light microscope. (D) ESCs 5-days-old positively stained by vimentin under the light microscope. ESCs, endometrial stromal cells; H&E, hematoxylin and eosin.

Results

Morphological observation of ESCs. The ESCs were observed using an inverted microscope equipped with phase-contrast apparatus. Differential centrifugation identified the purity of the isolated ESCs as 92-95%. Trypan blue staining demonstrated that the viability of the EECs was 96-98%. Recently isolated ESCs exhibited mostly round morphology (Fig. 1A), later growing as spindle and polygon-shaped cells with long cytoplasmic processes, usually reaching confluence after 5 days culture, exhibiting a single-cell monolayer growth pattern (Fig. 1B). The ESCs were stained by H&E staining after being cultured for 5 days (Fig. 1C) to indicate the cell morphology.

Identification of ESCs. The ESCs were detected by immunocytochemical staining. The cytoplasm of positive ESCs was stained as brownish/yellow granules. The ESCs were positive for vimentin (Fig. 1D).

LBP inhibits H_2O_2-induced cell death. After the four groups of ESCs had been cultured for 12 h, the changes in the ESCs morphology and structure were observed using an inverted microscope equipped with phase-contrast apparatus. No significant changes were observed in the morphology and structure of ESCs in the LBPs group compared with the control group, while those in the H_2O_2 group became irregular

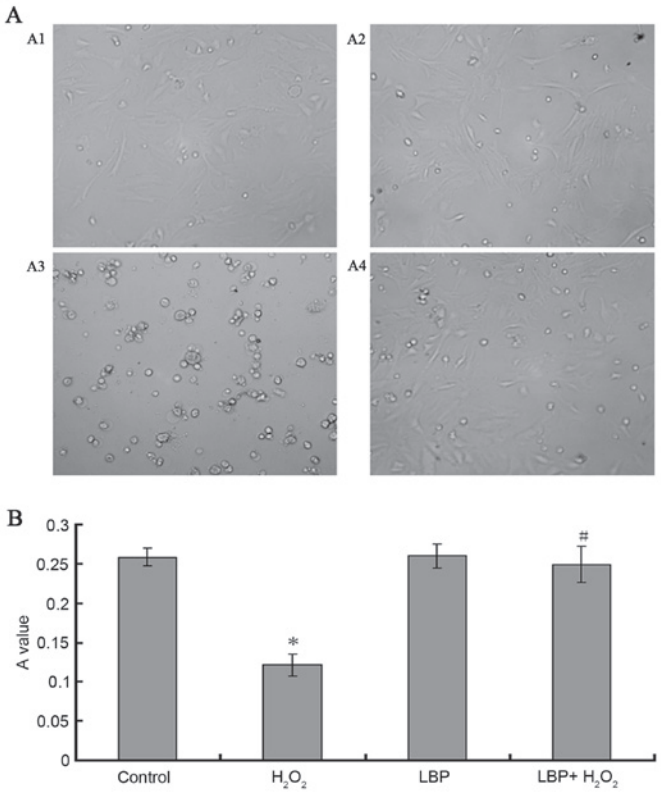

Figure 2. Morphological observations and proliferation assays of ESCs, ESCs were incubated with different intervention factors for 12 h. (A) Morphological observation of ESCs under an inverted microscope equipped with phase-contrast apparatus. A1, Control; A2 H_2O_2; A3, LBPs; A4, H_2O_2 + LBPs. (B) The proliferation assay of ESCs was evaluated by MTT. Control, serum-free DMEM/F12; H_2O_2, H_2O_2 and serum-free DMEM/F12; LBP, LBPs and serum-free DMEM/F12; H_2O_2 + LBP, H_2O_2, LBPs and serum-free DMEM/F12, the final concentration of H_2O_2 and LBPs was 10^{-4} M/l and 100 μg/ml respectively. The data are presented as the mean ± standard deviation (*P<0.05 vs. control group, $^\#$P<0.05 vs. the H_2O_2 group). ESCs, endometrial stromal cells; H_2O_2, hydrogen peroxide; LBPs, *Lycium barbarum* polysaccharides; MTT, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide; DMEM/F12, Dulbecco's modified Eagle's medium/nutrient mixture F-12.

in shape and declined in number. The H_2O_2 + LBP group demonstrated significantly less deterioration compared with the H_2O_2 group (Fig. 2A).

MTT assay. After the cells had been cultured for 12 h, the change in the number of the ESCs was observed by detecting the purple crystals in each culture well at an absorbance value of 550 nm. The result demonstrated that compared with the control group, the number of ESCs in the LBP group had no significant change (P=0.7976), while H_2O_2 significantly decreased the proliferation of ESCs in a time dependent manner (P<0.0001), the H_2O_2 + LBP group demonstrated significantly less deterioration compared with the H_2O_2 group (Fig. 2B).

LBP inhibits H_2O_2-induced content of MDA and activity of SOD in ESCs. The result of colorimetric analysis demonstrated that compared with the control group, no significant change was observed in the content of MDA and the activity of SOD in the LBP group, while the content of MDA increased and the activity of SOD decreased significantly in H_2O_2 group (P<0.0001 and P=0.0002, respectively). Compared with the

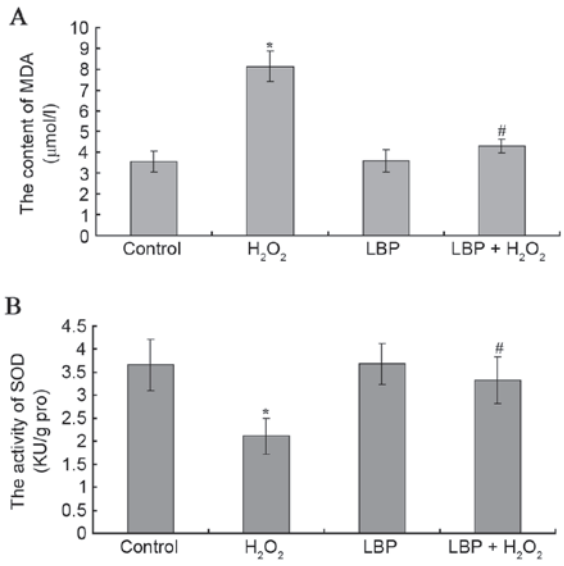

Figure 3. MDA content and SOD activity. The content of (A) MDA and activity of (B) SOD were assessed. Control, serum-free DMEM/F12; H_2O_2, H_2O_2 and serum-free DMEM/F12; LBP, LBP and serum-free DMEM/F12; H_2O_2 + LBP, H_2O_2, LBP and serum-free DMEM/F12, the final concentration of H_2O_2 and LBPs was 10^{-4} M/l and 100 μg/ml respectively. The data are presented as the mean ± standard deviation (*P<0.05 vs. control group, $^\#$P<0.05 vs. the H_2O_2 group). MDA, malondialdehyde; SOD, superoxide dismutase; DMEM/F12, Dulbecco's modified Eagle's medium/nutrient mixture F-12; H_2O_2, hydrogen peroxide; LBPs, *Lycium barbarum* polysaccharides.

H_2O_2 group, the content of MDA decreased and the activity of SOD increased significantly in the H_2O_2 + LBP group (P<0.0001 and P<0.0001; Fig. 3).

Effect of LBP on H_2O_2-induced protein expression levels of caspase-3 and Bcl-2 in ESCs. After cells were cultured for 12 h, the results of western blot analysis demonstrated that the control group cells had positive expression levels of caspase-3 and Bcl-2, and that the LBP group had similar expression levels of caspase-3 and Bcl-2 to the control group (P>0.05). The expression levels of caspase-3 in the H_2O_2 group increased significantly compared with that of the control group (P<0.0001), and decreased significantly in the LBP + H_2O_2 group compared with that of the H_2O_2 group (P<0.05); while the expression levels of Bcl-2 increased significantly in the H_2O_2 group compared with that of the control group (P<0.0001), and decreased in the LBP + H_2O_2 group compared with that of the H_2O_2 group (P<0.0001; Fig. 4A and B).

LBP inhibits H_2O_2-induced mRNA expression levels of caspase-3 and Bcl-2 in ESCs. After cells were cultured for 12 h, the result of RT-qPCR demonstrated that the control group cells had positive expression of caspase-3 and Bcl-2, as did the LBP group. Compared with the control group, the expression level of caspase-3 of the H_2O_2 group was increased significantly (P<0.0001) and the expression level of Bcl-2 was decreased significantly (P<0.05). Compared with the H_2O_2 group, the expression levels of caspase-3 in the LBP + H_2O_2 group was decreased significantly (P<0.0001), although the expression of Bcl-2 increased significantly (P<0.0001; Fig. 4C).

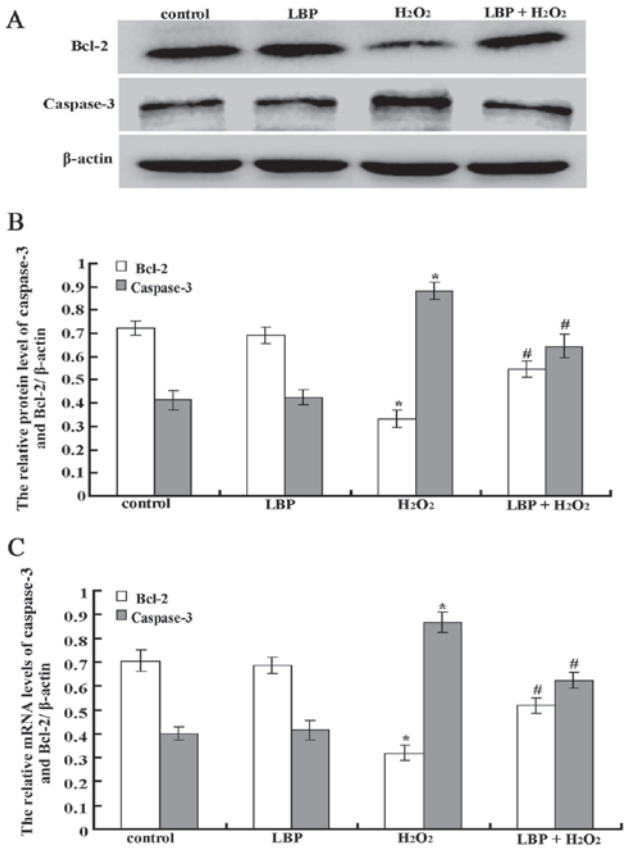

Figure 4. LBPs inhibit H_2O_2-induced expression of caspase-3 and Bcl-2 in ESCs. (A) The protein expression of caspase-3 and Bcl-2 was analyzed by western blotting. (B) The protein expression levels of caspase-3 and Bcl-2 were quantified by densitometry. (C) The mRNA expression levels of caspase-3 and Bcl-2 was analyzed by reverse transcription-quantitative polymerase chain reaction. Control, serum-free DMEM/F12; H_2O_2, H_2O_2 and serum-free DMEM/F12; LBP, LBPs and serum-free DMEM/F12; H_2O_2 + LBP, H_2O_2, LBPs and serum-free DMEM/F12, the final concentration of H_2O_2 and LBPs was 10^{-4} M/l and 100 μg/ml. The data are presented as the mean ± standard deviation (*P<0.05 vs. control group, $^\#$P<0.05 vs. the H_2O_2 group). LBPs, *Lycium barbarum* polysaccharides; H_2O_2, hydrogen peroxide; ESCs, endometrial stromal cells; DMEM/F12, Dulbecco's modified Eagle's medium/nutrient mixture F-12; H_2O_2, hydrogen peroxide.

Discussion

An increasing number of studies suggest that LBPs may have powerful antioxidant activity *in vitro* and *in vivo*, be able to remove free radicals and prevent lipoprotein lipid peroxidation, and to protect the biological macromolecules, including DNA, membrane lipids and cytoplasmic proteins, from oxidative damage (16,17). Wu *et al* (18) demonstrated that LBPs (10 mg/kg) significantly reduced blood glucose, nitric oxide and MDA in streptozotocin-induced diabetic rats. Li (19) also demonstrated that LBPs (20-50 mg/kg) protected liver and kidney tissue from oxidative damage in streptozotocin-induced diabetic rats. Luo *et al* (20) demonstrated that LBPs alleviated heat-induced damage of rat testes, as well as H_2O_2-induced DNA damage in mouse testicular cells, by increasing their resistance to oxidative stress-induced injury.

Oxidative stress causes membrane lipid peroxidation (21), directly damaging proteins and nucleic acids. H_2O_2 is an important intermediate in the body's redox reaction (22) and using exogenous H_2O_2 to treat a cell is one of the most common methods of establishing an oxidative stress damage model, which can effectively induce intracellular reactive oxygen species generation and cell damage (23). MDA is the product of the lipid peroxidation of free radicals with biomembrane polyunsaturated fatty acids and its content reflects the level of oxygen free radicals and the degree of lipid peroxidation. In addition, MDA crosslinks with nucleic acids and proteins and damages them. MDA is thus not only the one of the metabolites of cellular damage but also one of the substances leading to cell damage. SOD is the main antioxidant enzyme that can degrade and remove H_2O_2 and other free radicals. The present study identified that H_2O_2 can promote ESCs apoptosis, increase the content of MDA and decrease the activity of SOD, while LBPs suppress H_2O_2-induced ESCs apoptosis, reduce the content of MDA and increase the activity of SOD. This suggests that H_2O_2 has a damaging effect on ESCs, while LBPs serve a protective role in ESCs threatened by H_2O_2.

Apoptosis in mammalian cells has two pathways, intracellular and extracellular, which can activate caspase family proteins and cause cell lysis (24). The intracellular pathway is also called the mitochondrial pathway and is mainly regulated by the Bcl-2 family proteins (25), including apoptosis promoting proteins (Bax, Bak, Bid and Bik) and antiapoptotic proteins (Bcl-2 and Bcl-xl). In normal physiological conditions, apoptosis-promoting proteins and anti-apoptotic proteins form different dimers to counteract the activity of apoptosis promoting proteins and to achieve a balance between promoting and suppressing apoptosis (26). The Bcl-2 protein, located on the outer membrane of the mitochondria, has an antiapoptosis function and high expression levels can protect the integrity of the mitochondrial membrane, avoiding mitochondrial apoptosis factors, including the release of cytochrome *c* to prevent cell apoptosis (cytochrome *c* is a key factor in the formation of apoptotic bodies). The caspase family is divided into two subsets, the apoptosis initiator proteins (caspase-2, 8, 9, 10 and 15) and apoptosis effector proteins [caspase-3, 6 and 7, and poly (ADP-ribose) polymerase] (27). Mitochondria are activated and releases apoptosis factors, including cytochrome *c*, the latter activating apoptosis initiating caspase proteins, thus activating the apoptosis effect and finally destroying the cells (28,29). The results from the present study suggested that H_2O_2 reduces the protein and mRNA expression levels of Bcl-2 in ESCs, and increases the protein and mRNA expression level of caspase-3. LBPs suppress H_2O_2-induced ESCs damage, decreases the protein and mRNA expression levels of Bcl-2, and increases the protein and mRNA expression level of caspase-3. It is therefore speculated that ESCs oxidative stress induced apoptosis is mainly performed through the mitochondrial pathway, as this process is chiefly regulated by the Bcl-2 protein family. This suggests that the LBPs can improve the antiapoptosis protein expression levels of Bcl-2, protect the integrity of the mitochondrial membrane, prevent the release of cytochrome *c* from mitochondria into the cytoplasm and induce the activation of caspase-3 to trigger cell apoptosis.

In conclusion, LBPs are capable of inhibiting H_2O_2-induced ESCs apoptosis, reducing the content of MDA and increasing the activity of SOD. LBPs are also capable of inhibiting H_2O_2-induced ESCs damage, decreasing the protein and mRNA expression levels of Bcl-2, and increasing the protein and mRNA expression levels of caspase-3. This has

significance for the clinical prevention and treatment of endometrial damage.

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