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Genistein Improves Barrier Function and Proliferation of Human Umbilical Vein Endothelial Cells from Preeclampsia Patients

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Abstract: Preeclampsia (PE), which is characterized by hypertension in women who have normal blood pressure before pregnancy and is accompanied by proteinuria, edema, and major organ damage, is the major cause of the increased mortality in pregnant women and perinatal fetuses. So far, genistein is recognized as the only safe and effective natural phytoestrogen without estrogen-like adverse reactions. Recent studies show that genistein may play a significant role in controlling oxidative/nitrative stress during preeclampsia, indicating a possible beneficial role of genistein in the prevention of preeclampsia. However, the ability of genistein to prevent and treat pregnancy-induced hypertension, especially preeclampsia, is unknown. To study the effect of genistein on endothelial cell damage in preeclampsia, we isolated human umbilical vein cells (HUVEC) from 20 normal pregnant women and 40 preeclampsia patients (half treated with genistein and the other half untreated). We found that HUVEC barrier function, proliferation, nitric oxide synthesis, glutathione peroxidase activity, and Bcl-2 expression were significantly decreased in the PE group. Furthermore, 8-hydroxy-2'-deoxyguanosine levels and Bax expression were significantly increased comparing to control group. Genistein treatment reversed these changes in the PE group, suggesting that genistein reduces endothelial cell damage in preeclampsia HUVEC and providing a supporting the use of genistein for PE prevention and treatment.

Keywords: Genistein, Preeclampsia, Human Umbilical Vein Endothelial Cells, Oxidative Stress, Anti-apoptosis

1. Introduction

Preeclampsia (PE) generally occurs in pregnant women after 20 weeks of gestation. PE is characterized by hypertension in women who have normal blood pressure before pregnancy, accompanied by proteinuria, edema, and major organ damage [1]. With an incidence rate of about 3.9% of all pregnancies, PE is the main cause of the increased mortality in pregnant women and perinatal children [2]. With the liberation of the two-child policy in China at 2016, the number of maternal mothers has increased, and the PE incidence rate is on the rise [4]. As the etiology and pathogenesis of PE are still not clear, there is no ideal method for curing this disease completely. The most thorough treatment method is early delivery, which poses a high risk of complications for the mother and the fetus [3]. Thus, it is of great significance to study the pathogenesis of PE for the prevention and treatment of these high-risk pregnancies. So far, the two-stage model of PE is widely accepted [3], which suggests that maternal uterine spiral artery remodeling disorder leads to shallow placental implantation. Subsequently, placental ischemia, placental hypoxia, and oxidative stress cause a release of inflammatory cell factors that enter the maternal blood circulation, resulting in vascular endothelial damage and systemic inflammatory response. Thus, vascular endothelial cell injury has been considered as a key factor in the pathogenesis of preeclampsia.

Genistein is an isoflavone compound that was first proposed in 1899 by Perkin and Newbury [12]. Similar in structure with estrogen, genistein is a phytoestrogen that can

compete with 17 β-estradiol (E2) for binding to estrogen receptors, producing an estrogen-like effect [13]. E2 has the same affinity as estrogen to receptors α (ERα) and β (ERβ), but the ability of genistein to bind ERβ is 87% of E2, while the ability to bind ERα is only 4% of E2. Thus, genistein can selectively bind ERβ to produce a vasculature effect similar to E2, without stimulating adverse reproductive system-related effects [14]. Genistein has a variety of biological activities and pharmacological effects. Recently, a large number of related studies have shown that genistein has anti-inflammatory and antioxidative effects that can inhibit cardiac hypertrophy, protect vascular endothelial cells, and resist atherosclerosis [15, 16]. Genistein also has protective effects for cardiovascular disease [15, 16]. Genistein is an active ingredient found in soybeans, clovers, and other legumes. Genistein is known as a phytoestrogen, having a chemical structure similar to estrogen. Additionally, several studies have confirmed many physiological functions of genistein as an antioxidant, anti-inflammatory, cell cycle regulator, apoptosis inhibitor, and peripheral blood vessel relaxant [5]. Numerous studies have shown that vascular endothelial cells are critical regulatory targets of genistein [6, 27, 28]. Genistein regulates the proliferation, apoptosis, and permeability of endothelial cells, as well as the function of endothelial cells and exerts a protective effect on the vascular endothelium. Thus, an understanding of the protective effects of genistein on vascular endothelial cells, specifically human umbilical vein endothelial cells (HUVECs), from preeclampsia patients, and the possible mechanism by which genistein reduces anti-oxidative stress and anti-apoptosis is urgently needed. Our study found genistein reduces endothelial cell damage in preeclampsia HUVEC, which provides new insight into the use of genistein in the early prevention and treatment of vascular endothelial cell injury due to preeclampsia.

2. Methods

2.1. Research Subjects

In accordance with the principle of informed consent, 60 pregnant patients were selected from the Department of Obstetrics and Gynecology at the First Affiliated 95 Hospital of Nanjing Military Region of the PLA and the Affiliated Hospital of Putian University between January 2017 and June 2018. The selected pregnant women had a single birth in hospital through cesarean section or natural delivery. 40 of these patients (average age: 30±4.20) were diagnosed with preeclampsia, and the other 20 patients (average age: 27±3.60) were uncomplicated pregnancies (control group). For the diagnostic and inclusion criteria used for pregnancy-induced hypertension disease and preeclampsia, please refer to the Diagnosis and Treatment of Hypertension and Pre-eclampsia in Pregnancy in New Zealand: A clinical practice guideline (2018). There was no statistically significant difference in patient age and body weight, as well as gestational age, between the groups (P>0.05). No premature rupture of membranes, premature birth, and/or signs of infection occurred in any of the pregnant women. All subjects did not smoke nor drink alcohol, and their diet and lifestyle were under normal conditions. Exclusion criteria included pregnancy complications, chronic hypertension, nephritis, diabetes, cardiovascular disease, and other internal surgical complications. None of the patients were related.

2.2. Solation and in Vitro Culturing of HUVECs

Newborn umbilical cords from all research subjects were obtained under aseptic conditions during delivery, placed in a sterile canister containing cold phosphate buffered saline (PBS), and sent to the laboratory for follow-up within 2 h. All umbilical cord samples were obtained with informed consent. At a sterile workbench, the primary HUVECs were treated with preheated (37°C) 0.1% type I collagenase for digestion and isolation. The cells were resuspended in 3-5 mL of M199 medium (Gene, USA). The suspension was transferred to a 25 cm^2 flask and placed in a 37°C, 95% humidity, and 5% CO_2 incubator (Memmert, Germany). HUVECs were cultured on polylysine (PLL) coated coverslips and cell slides, and immunohistochemical staining was used to detect the expression of human factor VIII (VIIIF), an endothelial cell-specific antigen. Cells were viewed on an inverted microscope (Olympus, Japan).

2.3. Cell Grouping

Primary HUVECs obtained from uncomplicated (normal) pregnant women served as the control group (n=20). Primary HUVECs obtained from the preeclampsia patients were divided into two groups: an untreated group (PE group; n=20) and a genistein intervention group (PE+Gen; n=20). HUVECs were cultured in M199 medium containing 10% fetal bovine serum (FBS; Gibco, USA) at 37°C, 20% O_2, and 5% CO_2. Newborn umbilical cords from all research subjects were obtained under aseptic conditions during labor, placed in a sterile canister containing cold phosphate buffered saline (PBS) before being sent to the lab.

2.4. Genistein Intervention

Genistein (purity ≥98%; Sigma, USA) was prepared using high-performance liquid chromatography. Cells in PE+Gen group were treated with 25 μmol/L genistein (Sigma, USA) for 4 h before experiments. 25 mg of genistein was dissolved in 250 μL of dimethyl sulfoxide (DMSO; Sigma, USA) to make a 0.1 mol/L genistein stock solution. From this stock solution, 8 μL of genistein was diluted in 3992 μL M199 medium to create the application solution. The genistein stock and application solutions were stored at -20°C.

2.5. Detection of HUVEC Barrier Function

HUVECs were inoculated on the fiber polycarbonate membrane upper chamber of a 12-well Transwell plate (0.4 μm pore size; Corning Costar, USA) at a density of 1×10^6 cells/well. After 72 h, the cells were fused into a monolayer, and fluorescently labeled bovine serum

albumin (BSA; Beijing Boaosen Co., Ltd., China) was added to the upper chamber of the Transwell plate. The clearer the barrier function damage, the higher the concentration of BSA entering, and the lower Transwell chamber. It takes a certain time for BSA to pass through the endothelial cells from the Transwell upper chamber into the lower chamber. Based on the literature, one hour was selected as the appropriate time for this experiment. After 1 h, the medium in the lower chamber was collected. The concentration of fluorescently labeled BSA that passed through the HUVEC monolayer into the collected medium was measured using a fluorescence microplate reader (Gene, USA) to assess barrier function. The excitation and absorption wavelengths were 485 nm and 520 nm, respectively. A greater BSA concentration indicated a more severely impaired cellular barrier. All operations were carried out in the dark.

2.6. Detection of HUVEC Proliferation

HUVECs with a density of 1×10^5 cells/well were seeded in 96-well culture plates with a total volume of 200 μl M199 medium. Each HUVEC group was treated in five replicates. The experiment was performed according to the instructions in the MTT assay kit (Sigma, USA). Absorbance was measured at 570 nm (A570) on an ELX800 multi-function microplate reader (BioTek, USA).

2.7. Detection of NO Concentration in HUVECs

HUVECs were seeded into a 24-well culture plate at a density of 1×10^6 cells/well with a total volume of 0.5 mL M199 medium per well and placed in a 37°C and 5% CO_2 incubator. After 24 h, the medium from each well was collected, and the concentration of NO was measured using the nitrate reductase method according to the instructions in the nitric oxide assay kit (Nanjing Institute of Bioengineering, China). An ELX800 multi-function microplate reader was used to measure the absorbance at 470 nm (A470). Five replicates were performed for each sample.

2.8. Enzyme-linked Immunosorbent Assay (ELISA) for the Detection of 8-hydroxydeoxyguanosine (8-OHdG) Levels in HUVECs

Culture medium from the HUVECs was collected in sterile test tubes and centrifuged at 2,500 rpm for 15 min at 4°C. The supernatant was collected and an 8-OHdG ELISA kit (HCB, Canada) was used to measure the 8-OHdG levels in the supernatants. Absorbance was measured using a microplate reader at a wavelength of 450 nm (A450).

2.9. Colorimetric Assay for the Detection of Glutathione Peroxidase (GSH-Px) Activity Levels in HUVECs

HUVECs were harvested and incubated with cell lysate buffer for 40 min in an ice bath. Cell lysates were centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was extracted and a GSH-Px detection kit (Biyuntian Biotechnology Institute, China) was used to measure GSH-Px enzyme activity. Absorbance was measured at a wavelength of 340 nm (A340) using a microplate reader.

2.10. Real-Time Polymerase Chain Reaction (RT-PCR) for the Detection of Bcl-2 and Bax Gene Expression in HUVECs

HUVECs were harvested and incubated with cell lysate buffer. Within 24 h after subculture, cells with a good growth state and an attachment rate of about 80% were selected for further experiments. Once cells were fully lysed, total ribonucleic acid (RNA) was extracted according to the instructions in the TRIzol total RNA extraction kit (Invitrogen, USA). The purity of the cell lysates was measured using an ultraviolet spectrophotometer. The A260/A280 ratio was quantified at 1.80 or higher. Complementary deoxyribonucleic acid (cDNA) was reverse transcribed using a reverse transcription kit (TaKaRa, Japan), and PCR amplification was performed according to the instructions in the PCR amplification kit (TaKaRa, Japan). Each sample was tested in triplicate using a Bio-Rad CFX96 Real-Time Quantitative PCR Detection System (Memmert, Germany). The primer sequences used were as follows: 1) Bcl-2 Forward: 5'-GTCCGTGCCTGCATTTAG-3' and Bcl-2 Reverse: 5'-ACCGCTTCAGACCTCCAG-3'; 2) Bax Forward: 5'-TCATCCAGGATCGAGCAG-3' and Bax Reverse: 5'-GCTCCATATTGCTGTCCA-3'; 3). The internal reference primer, β-actin, Forward: 5'-AGGCCAACCGTGAAAAGATG-3' and β-actin Reverse: 5'-TGGCGTGAGGGAGAGCATAG-3'. Bcl-2 and Bax primers were designed and synthesized by Shanghai Sangon Biological Co., Ltd., China. The PCR reaction conditions were set as follows: 94°C for 5 minutes, 94°C for 30 sec, 60°C for 30 sec, 74°C for 40 sec, for 40 consecutive cycles. The relative expression levels of Bcl-2 and Bax genes in the three groups of HUVECs were calculated using the 2^-ΔΔCt method.

2.11. Western Blot Analysis of Bcl-2 and Bax Protein Expression in HUVECs

HUVECs were harvested and lysed. Total protein was extracted from the cell lysates and protein concentration was determined via the bicinchoninic acid (BCA) method. 0.1 mL of 10% sodium dodecyl sulfate (SDS) loading buffer was added to samples, which were then boiled for 5 min and stored at -20°C until ready for use. Samples were run on a 12% polyacrylamide gel electrophoresis (SDS-PAGE) gel at 60 volts for 2 h. Membranes were blocked with 5% skim milk in an airtight container for 1 h at room temperature (37°C) and then incubated with Bcl-2 primary antibody (1:100) overnight at 4°C. Membranes were washed three times with Tris Buffered Saline with Tween 20 (TBST) for 10 min and then incubated with alkaline phosphatase-labeled rabbit secondary antibody (1:5,000) for 1 h at room temperature (37°C). Membranes were rinsed three times with TBST for 10 min and color developed via the alkaline

phosphatase method. Experiments were repeated as above for the detection of internal reference protein, β-actin, and Bax, for which the primary antibodies used were β-actin antibody (1:1,000) and Bax antibody (1:500), respectively. A gel imaging analysis system was used to photograph and analyze the results. The relative expression of the target proteins, Bcl-2 and Bax, was calculated as the ratio of the optical density of the target band to the internal reference, β-actin band.

2.12. Statistical Analysis

Statistical analysis was performed using an SPSS 17.0 statistical package. The BSA concentration, MTT value, and NO concentration in the three groups of HUVECs were expressed as the mean ± standard deviation (SD). All data were analyzed using one-way ANOVA. For significance analysis, pairwise comparison between the groups was performed using Fisher's Least Significant Difference (LSD). $P < 0.05$ was considered statistically significant. The data correlation was tested using the Pearson rank correlation test.

3. Results

3.1. Differences in Monolayer Barrier Function, Proliferation, and NO Synthesis Between the Three Groups of HUVECs

The primary HUVECs were successfully cultured from pregnant women and almost completely adherent after 24 h. Early adherent cells were mostly small triangles and spheres. A few cells stretched into short spindle shapes, and single cells or clusters existed. Between 48-72 h, the cells grew the fastest and gradually appeared fusiform. Some cells arranged in a fish-like shape with a swirling arrangement. Nuclei were either clear and round or elliptical, and HUVECs were commonly found in the mitotic phase. The cells had one to two nucleoli rich in cytoplasm and containing small particles. Additionally, the cells formed a single layer and did not overlap, producing a paving stone appearance (Figure 1A). Immunohistochemical staining of VIIIF revealed brownish-yellow particles in the cytoplasm or nucleus, indicating a positive reaction (Figure 1B). Cells were harvested after 4 hours of treatment with or without genistein. So far, genistein is recognized as the only safe and effective natural phytoestrogen with no estrogen-like adverse reactions [17]. However, the ability of genistein to prevent and treat pregnancy-induced hypertension, especially preeclampsia, is unknown. To detect any possible change of preeclampsia HUVECs properties with genistein treatment, we tested BSA concentration, MTT, and NO concentration, respectively. The concentration of BSA that passed through the HUVEC monolayer was significantly higher in the PE group than in the control group, however, the BSA concentration was significantly lower in the PE+Gen group than in the PE group. The MTT results showed that the optical density (OD) value at A570 of the PE group of HUVECs was significantly lower than that of the control group, while the OD value of the PE+Gen group was significantly higher than that of the PE group. The NO concentration in the supernatants from the three groups of HUVECs showed that the NO content in the PE group was significantly lower than that in the control group, and that the NO content in the PE+Gen group was significantly higher than in the PE group. The above results from PE+Gen group were found to be statistically significant comparing with PE group ($P<0.05$; see Table 1), suggesting that HUVECs from PE patients treated with genistein can fully rescue the BSA, MTT, and NO level to normal levels from control patient HUVECs.

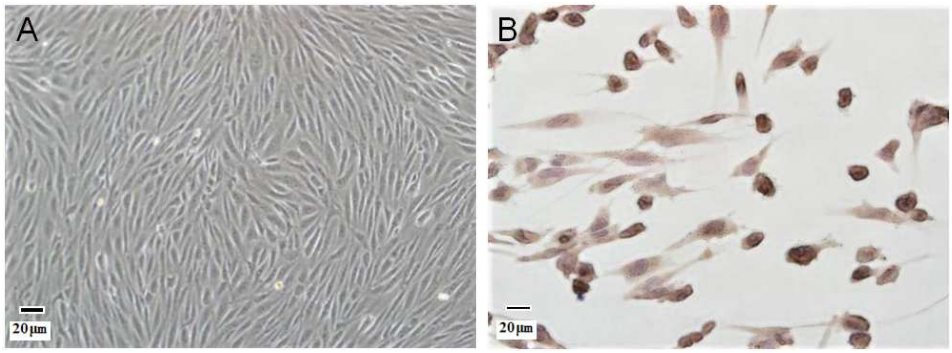

Figure 1. *Identification of primary human umbilical vein endothelial cells (HUVECs). A. Growth and morphology of the primary HUVECs under a microscope (100×); B. Immunohistochemical staining of VIIIF in primary HUVECs viewed under a high power microscope (400×).*

Table 1. *Changes in monolayer barrier function (via bovine serum albumin, BSA, Transwell assay), proliferation (via MTT assay), and nitric oxide (NO) synthesis (via nitric oxide assay) between the control, preeclampsia (PE), and genistein intervention (PE+Gen) groups of human umbilical vein endothelial cells.*

	BSA Concentration (μmol/L)	MTT Assay Absorbance (A570 nm)	NO Concentration (μmol/L)
Control group	12.56±0.39	1.09±0.07	265.42±8.56
PE group	20.37±0.61*	0.52±0.03*	184.73±5.69*
PE+Gen group	15.09±0.84Δ	0.94±0.05Δ	278.15±7.34Δ

Data is presented as the mean ± standard deviation. Note: *P<0.05 versus control group; ΔP<0.05 versus PE group; no statistic difference between control group and PE+Gen group (P>0.05).

3.2. Differences in 8-OHdG Concentration and GSH-Px Activity Between the Three Groups of HUVECs

The structure of genistein contains a number of phenolic hydroxyl groups, which have a strong antioxidant effect. 8-OHdG is a sensitive marker for evaluating DNA oxidative damage induced by reactive oxygen species (ROS), and GSH-Px is an important peroxide-decomposing enzyme in vivo that catalyzes the transformation of the reduced compound, glutathione (GSH), to its oxidized form, glutathione (GSSG). GSSG then scavenges intermediates of peroxide-free radicals and exerts antioxidant effects [20]. The above results suggest that genistein can antagonize the oxidative damage of vascular endothelial cells of preeclampsia patients, and its mechanism may be related to enhancing antioxidant enzyme activity and reducing DNA oxidative stress. To test this, we found that the concentration of 8-OHdG in the HUVECs from the PE group was significantly higher than that in the control group, but was dramatically reduced after the genistein intervention (Figure 2). However, there is no significant difference between the PE+Gen and control groups, indicating that genistein can fully rescue the 8-OHdG phenotype in PE HUVECs. In addition, the GSH-Px activity in the PE group HUVECs was significantly lower than that in the control group. After the genistein intervention, the activity of GSH-Px was significantly higher than in the PE group ($P<0.05$, Figure 3). However, the difference between the PE+Gen and control groups was not significant, indicating that genistein can also rescue the GSH-Px activity in PE HUVECs.

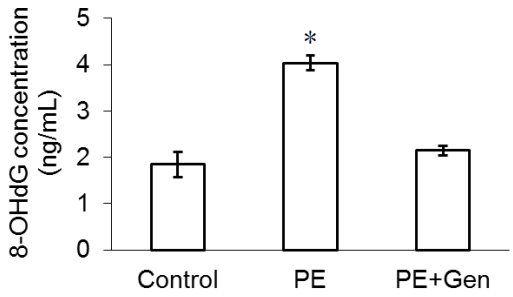

Figure 2. *The concentration of 8-hydroxy-2'deoxyguanosine (8-OHdG) in culture medium from control, preeclampsia (PE), and genistein treated (PE+Gen) human umbilical vein endothelial cells. 8-OHdG levels were measured using an enzyme-linked immunosorbent assay and are presented as the mean ± standard deviation.*

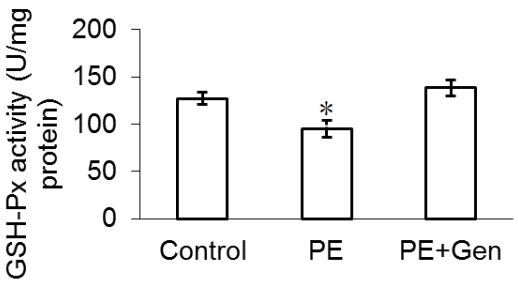

Figure 3. *Glutathione peroxidase (GSH-Px) activity in the control, preeclampsia (PE), and genistein treated (PE+Gen) groups of human umbilical vein endothelial cells. Data was obtained through colorimetric assay and is presented as the mean ± standard deviation.*

3.3. Differences in Bcl-2 and Bax Gene and Protein Levels Between the Three Groups of HUVECs

Given that the five criteria of defining PE patients above were all reversed by genistein treatment, we asked what has been changed at the genetic level with genistein treatment. Bcl-2 family proteins are divided into inhibitory apoptosis proteins (such as Bcl-2, Bcl-xl, and Bcl-w) and apoptosis-promoting proteins (such as Bax, Bad, and Bak), which play a vital role in the controlled process of apoptosis. Since Bcl-2 and Bax gene expression have a great impact in PE patients, we tested Bcl-2 and Bax mRNA level using RT-qPCR. The level of Bcl-2 mRNA in the PE group of HUVECs was significantly lower than that of the control group, and the level of Bax mRNA in the PE group was up-regulated in comparison to the control group (Figure 4). After genistein intervention, Bcl-2 mRNA expression increased and Bax mRNA expression significantly decreased in comparison to untreated preeclampsia patients (Figure 4). To further confirm the protein level of Bcl-2 and Bax, we used western blot to detect their expression. We found that Bcl-2 protein expression in HUVECs from the PE group was significantly lower than in the control group, and Bax protein expression was up-regulated (Figure 5). After genistein intervention, the level of Bcl-2 protein expression in the HUVECs significantly increased and Bax protein expression significantly decreased, in comparison to the PE group (Figure 5). Overall, there was no difference in Bcl-2 and Bax mRNA level or their protein level between the PE+Gen and control groups. Our data suggest that PE HUVECs with genistein treatment not only rescues BSA, MTT, NO, 8-OHdG levels, and GSH-Px activity, but also rescues the genetic level of Bcl-2 and Bax. This evidence supports the use of genistein to prevent and treat PE patients.

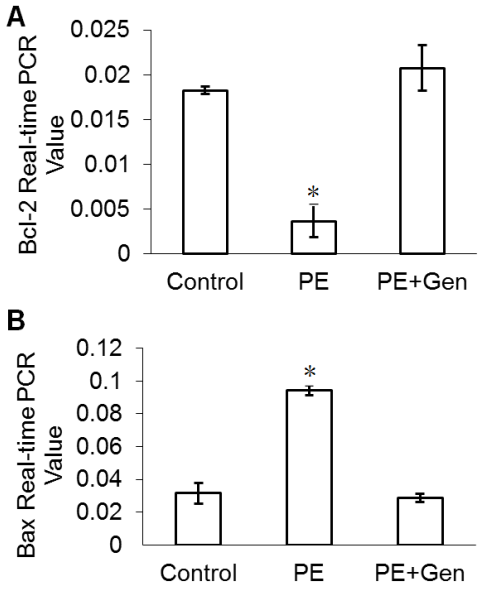

Figure 4. *Expression levels of Bcl-2 (A) and Bax (B) genes in the control, preeclampsia (PE), and genistein treated (PE+Gen) groups of human umbilical vein endothelial cells, as measured by real-time PCR (RT-PCR). Bars represent the mean ± standard deviation. Note: *P<0.05 versus PE group; **P<0.01 versus PE group.*

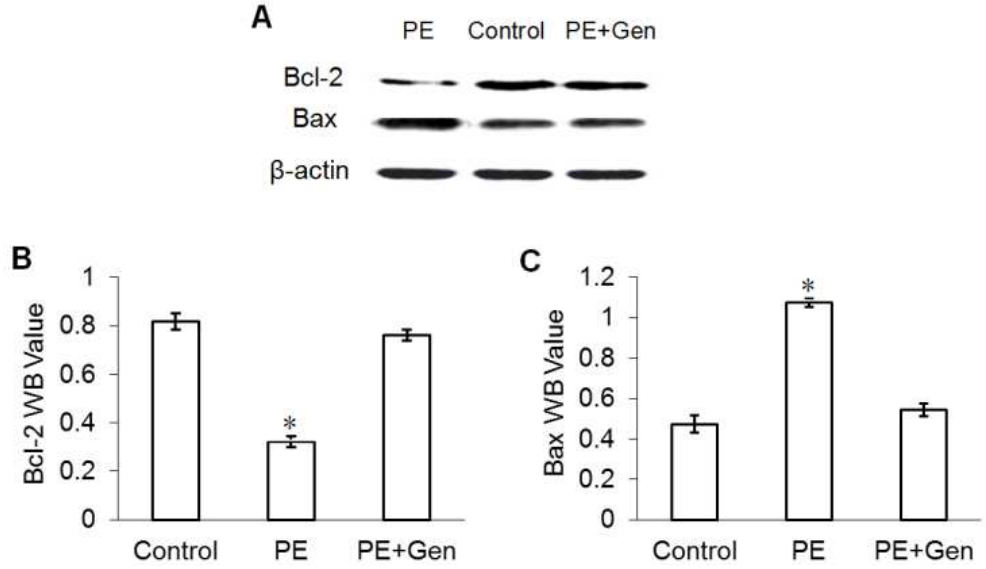

Figure 5. *Expression levels of Bcl-2 and Bax proteins in the control, preeclampsia (PE), and genistein treated (PE+Gen) groups of human umbilical vein endothelial cells (HUVECs). A. Western blot analysis of Bcl-2 and Bax proteins in the control, PE, and PE+Gen groups of HUVECs. β-actin was used as the internal reference protein. B. Relative Bcl-2 protein expression levels between the three groups of HUVECs measured by western blot image analysis. C. Relative Bax protein expression levels between the three groups of HUVECs measured by western blot image analysis. Bar graphs represent the mean ± standard deviation. Note: *P<0.05 versus PE group; **P<0.01 versus PE group.*

4. Discussion

It is now widely accepted that the characteristics of the pathophysiological manifestations of preeclampsia can be divided into two stages: (1) the reduction of placental perfusion in early pregnancy, and (2) the extensive activation and/or damage of systemic vascular endothelial cells in the second trimester or beyond [7-9]. Multiple organ systems have varying degrees of involvement [7-9]. The two-stage theory suggests that preeclampsia patients have defects in the infiltration of early gestational cells in uterine spiral arteries. This leads to shallow implantation of the placenta, reducing uterine-placental perfusion and causing placental ischemia, hypoxia, placental oxidation disorders, and atherosclerosis. Consequently, the body releases many virulence factors into the circulation. Thus, the pathological changes of preeclampsia develop from the location of the placenta to the whole body. These virulence factors directly or indirectly injure endothelial cell precursors, causing a variety of pathophysiological changes and clinical manifestations [10]. In 1988, Rodger et al. found that cytotoxic factors, which can lead to vascular endothelial cell activation, dysfunction, and structural damage, exist in the serum of patients with pregnancy-induced hypertension, including preeclampsia [11]. The cytotoxic factor is one of the central links in the pathogenesis of preeclampsia [11]. The results of this study also show that apoptosis, proliferation, and barrier function of HUVECs from preeclampsia patients are impaired. As a result, the secretion of the endothelium-derived relaxing factor, NO, is reduced. This leads to vasoconstriction, diastolic dysfunction, and pathological changes, such as vasospasm and a rise in blood pressure [11]. Our study demonstrated that, the proliferation, barrier function, and NO excretive ability of HUVECs significantly improved after genistein intervention.

Bcl-2 family proteins play a vital role in the controlled process of apoptosis. Some researchers contend that Bcl-2 may act as an endogenous antioxidant to antagonize oxidative damage while regulating apoptosis [22]. Giving newly-weaned Wistar mice multivitamins can significantly reduce oxidative damage, apoptosis rate, and caspase-3 activity while up-regulating Bcl-2 protein expression in intestinal epithelial cells [22]. The results from the Wistar mice study suggest that there is a link between Bcl-2-mediated apoptosis and oxidative damage, and that oxidative stress occurs with apoptosis. In the process of apoptosis, it is generally believed that oxidative stress may affect the structure and function of mitochondria and trigger the release of apoptosis-related factors [22]. A variety of antioxidant substances have a regulatory effect on the apoptotic process. A previous study has found that, in PC12 cells, genistein can downregulate the expression of proapoptotic genes, caspase-3, and caspase-9, and up-regulate the expression of anti-apoptotic genes, Bcl-xl and LRP5, as well as antagonize the apoptosis of nerve cells [23]. In this study, primary HUVECs were successfully isolated and cultured, and the apoptosis-related factors were detected. The results show that gene and protein expression levels of Bcl-2 in HUVECs from preeclampsia patients were significantly lower than those from normal pregnant women. Bax gene and protein expression levels were significantly up-regulated in preeclampsia patients. After genistein intervention, Bcl-2 gene and protein expression levels were significantly higher than that of the patient group, and the Bax gene and protein expression levels were significantly decreased.

5. Conclusion

In summary, the results of this study show that oxidative stress and vascular endothelial cell injury play an important role in the pathogenesis of preeclampsia, and are consistent with previous studies [24, 25]. The balance between oxidation and anti-oxidation in patients with preeclampsia is broken, oxidative stress exists, and the production of intracellular ROS increases, causing damage and apoptosis of vascular endothelial cells. Furthermore, endothelial cells in preeclampsia patients have a weakened proliferative capacity, as well as reduced angiogenesis and repairability. An imbalance of vasoactive factors and systemic arteriolar spasm leads to a series of preeclampsia pathological changes and clinical symptoms [24, 25]. Previous studies show that up-regulating endogenous anti-oxidants may alleviate placental oxidative stress and provide therapy for these complications of pregnancy [26]. The experimental results further indicate that the phytoestrogen, genistein, may inhibit the damage of vascular endothelial cells caused by oxidative stress in patients with preeclampsia by up-regulating the levels of antioxidation factors and regulating apoptosis-related factors. This study confirms that genistein has protective effects on HUVECs, and its possible mechanisms include antioxidative stress and anti-apoptosis. These findings suggest that genistein may be effective for the prevention and treatment of preeclampsia. Although the clinical effects of genistein are very promising and it has already entered the market of health products, a large amount of basic and clinical research is still needed to confirm its effect on various diseases and its physiological mechanisms.

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

Acknowledgements

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