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Research Article

Echinatin Inhibits Oxidative Stress and Inflammatory Processes in Trophoblast Cells by Inhibiting TLR4-MyD88-NF-κB Pathway in Preeclampsia

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Background. Preeclampsia (PE) is a common obstetric disorder hallmarked by impaired trophoblast invasion and a skew toward an inflammatory immune response. Echinatin, a flavonoid with established anti-inflammatory, antioxidant, and anticancer activities, may offer therapeutic benefits in PE. Our study aimed to investigate the effect of echinatin on preeclampsia *in vitro* and *in vivo* and to reveal the potential molecular mechanism of its action. *Methods*. Eighteen adult female Sprague Dawley rats were randomized into three experimental groups: a PE model group, a PE + echinatin treatment group, and a PE + echinatin treatment group with TLR4 overexpression. Placental tissue CK7 expression was assessed by immunohistochemistry. TUNEL immunofluorescence staining quantified placental cell apoptosis. Cell viability, proliferation, and migration were evaluated using cell counting kit-8, EdU incorporation, and Transwell assays, respectively. Oxidative stress parameters of malondialdehyde, superoxide dismutase, and glutathione peroxidase were measured. Flow cytometry determined cell apoptosis and intracellular reactive oxygen species (ROS) levels. Western blotting evaluated the expression of proteins related to the TLR4-MyD88-NF-κB signaling pathway, and the concentrations of TNF-α, IL-6, and IL-18 were measured with ELISA kits. *Results*. Echinatin mitigated placental damage, reduced apoptosis, and increased CK7 expression. It significantly enhanced HTR-8/SVneo cell viability and migration. Echinatin also counteracted H_2O_2-induced ROS production and cell death in HTR-8/SVneo cells. Moreover, it inhibited the expression of proteins within the TLR4-MyD88-NF-κB signaling cascade. Overexpression of TLR4 negated echinatin's protective effects. *Conclusion*. Echinatin exerts protective effects against oxidative stress and inflammation in PE by targeting the TLR4-MyD88-NF-κB pathway, suggesting its therapeutic potential for the management of preeclampsia.

1. Introduction

Globally, 3%–8% of pregnancies are complicated by preeclampsia, an unpredictable and progressive severe disease [1]. Preeclampsia is responsible for the annual mortality of over 46,000 pregnant women and 500,000 fetuses and infants. This condition disproportionately affects women residing in low- and middle-income nations or other vulnerable groups [2, 3]. Preeclampsia is a clinical syndrome involving multiple organs and systems, primarily characterized by cardiovascular manifestations, with its pathogenesis stemming from systemic inflammation, endothelial dysfunction, and widespread vasoconstriction, leading to hypertension and multi-organ hypoperfusion [4–7]. Preeclampsia is a medical condition characterized by the start of high blood pressure after the 20th week of pregnancy, accompanied by the dysfunction of many organ systems. It is important to note that this condition often resolves completely within 12 weeks following childbirth [8, 9]. A large body of epidemiological research suggests

that placental defects are thought to be caused by abnormal immune reactions of the mother to invading trophoblast cells, and preeclampsia is associated with primiparity, partner change, and oocyte donation for assisted reproduction [10, 11]. Although there is a wealth of literature supporting the immunological origins of preeclampsia, its specific underlying biological mechanisms remain unclear.

The use of natural products has great importance in the advancement of medical treatment strategies owing to their little toxicity, diverse structural composition, and substantial biological efficacy [12, 13]. It has been observed that a significant proportion, around 70%, of anti-infective medications are derived from natural materials that are sourced from the environment [14]. Hence, natural products serve as a significant reservoir of potential novel pharmaceuticals for addressing the therapeutic needs associated with preeclampsia. The presence of echinatin (Figure 1(a)), a bioactive flavonoid, has been detected in Glycyrrhiza plants, often referred to as licorice. This compound is acknowledged for its natural sweetening properties and is used as a food addition to enhance taste [15]. Additionally, echinatin has many biological effects, including antioxidant, anti-inflammatory, and anticancer properties, partly through regulating the Nrf2, NO, and NF-κB pathways [16–18].

Inflammatory response is a physiological reaction of the body to various pathological damages and stimuli. Inflammation can be triggered by various pathways in preeclampsia [19]. For example, bacterial antigens activate Toll-like receptors (TLRs) to trigger an inflammatory response, which may induce cell membrane oxidative stress and activate intracellular kinases [20]. Oxidative stress can lead to the activation of TLRs through the production of DAMPs. For example, high mobility group box 1 (HMGB1) is a nuclear protein that can be released from necrotic cells, where it acts as a DAMP and activates TLRs on immune cells. The TLRs are essential pattern recognition receptors that function prominently in the innate immune system. The responsibility of launching the inflammatory cascade lies with the detection of both pathogen-associated molecular patterns and damage-associated molecular patterns [21]. TLR4-MyD88-NF-κB signaling pathway is closely related to the inflammatory response and has been shown to be involved in the development of preeclampsia [22–24]. Blocking TLR4 and MyD88 may be a potential therapeutic method for preeclampsia. The inflammatory response can, in turn, lead to the production of more ROS, both through the respiratory burst in phagocytic cells and through mitochondrial damage, creating a feedback loop that exacerbates oxidative stress. Hydrogen peroxide (H_2O_2) is commonly employed to induce oxidative stress in various *in vitro* models, including investigations of preeclampsia using HTR-8/SVneo cells, an immortalized human trophoblast cell line. Hence, the primary objective of this study is to explore the effects of echinatin on the inflammatory response observed in both *in vivo* and *in vitro* models of preeclampsia, as well as its regulatory influence on the TLR4-MyD88-NF-κB signaling pathway.

2. Materials and Methods

2.1. Animals. Female Sprague Dawley rats, aged 8–10 weeks and weighing 210–250 g, were maintained under controlled conditions with 50–60% humidity, ambient temperatures of 22–24°C, and a 12 h light/dark cycle (06:00 am to 06:00 pm). They were granted ad libitum access to food and water. Pregnant rats were assigned randomly to three experimental groups: a model group receiving L-NAME treatment ($n = 6$), an L-NAME + echinatin group treated with both L-NAME and echinatin ($n = 6$), and an L-NAME + echinatin + TLR4 group, which underwent additional overexpression of TLR4 via intraperitoneal injection of a lentivirus ($n = 6$). Preeclampsia was induced with L-NAME (50 mg/kg/day) (N5751; Sigma, MO, USA) from gestational day (GD) 14 to GD19 through oral gavage. Concurrently, a lower dose of echinatin (1.5 mg/kg/day) was administered from GD0 to GD19. The echinatin was initially dissolved in DMSO to create a stock solution and then diluted in saline prior to its administration. Both serum and placental tissue samples were harvested on GD20. All animal procedures were authorized by the Ethics Committee of Shengli Oilfield Central Hospital.

2.2. Histological and Immunohistochemical Analysis. All sections from the placenta biopsies of the rat were stained with hematoxylin-eosin staining. Immunohistochemical (IHC) staining was performed with the automated Ventana BenchMark ULTRA staining system (Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA) according to the instructions of the manufacturers, using the prediluted primary antibodies: anti-cytokeratin 7 (1 : 500; Abgent, Cat#AJ1229a). Representative images were captured on a Nikon A1 confocal microscope (original magnification ×40) and prepared using Fiji software v.1.51h.

2.3. Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Staining. The induction of apoptosis of the transfected cells was assessed by the TUNEL assay (Beyotime Institute of Biotechnology). HTR-8/SVneo cells were cultured on glass coverslips overnight. Following fixation with 4% paraformaldehyde at room temperature for 1 h, the cells were blocked with 3% H_2O_2 (dissolved) for 20 min at room temperature. Following permeation with 0.1% Triton X-100 at 4°C for 2 min, the slides were incubated with 50 μL of the TUNEL reaction mixture for 1 h at 37°C, mounted with VECTASHIELD® mounting medium. Following the addition of DAPI solution at 37°C for 10 min away from light, the cell samples were analyzed using an inverted fluorescence microscope (Olympus Corporation) in five random fields.

2.4. Cell Culture and Treatment. The HTR-8/SVneo cell line, which originates from human early pregnancy trophoblast cells, was obtained from the American Type Culture

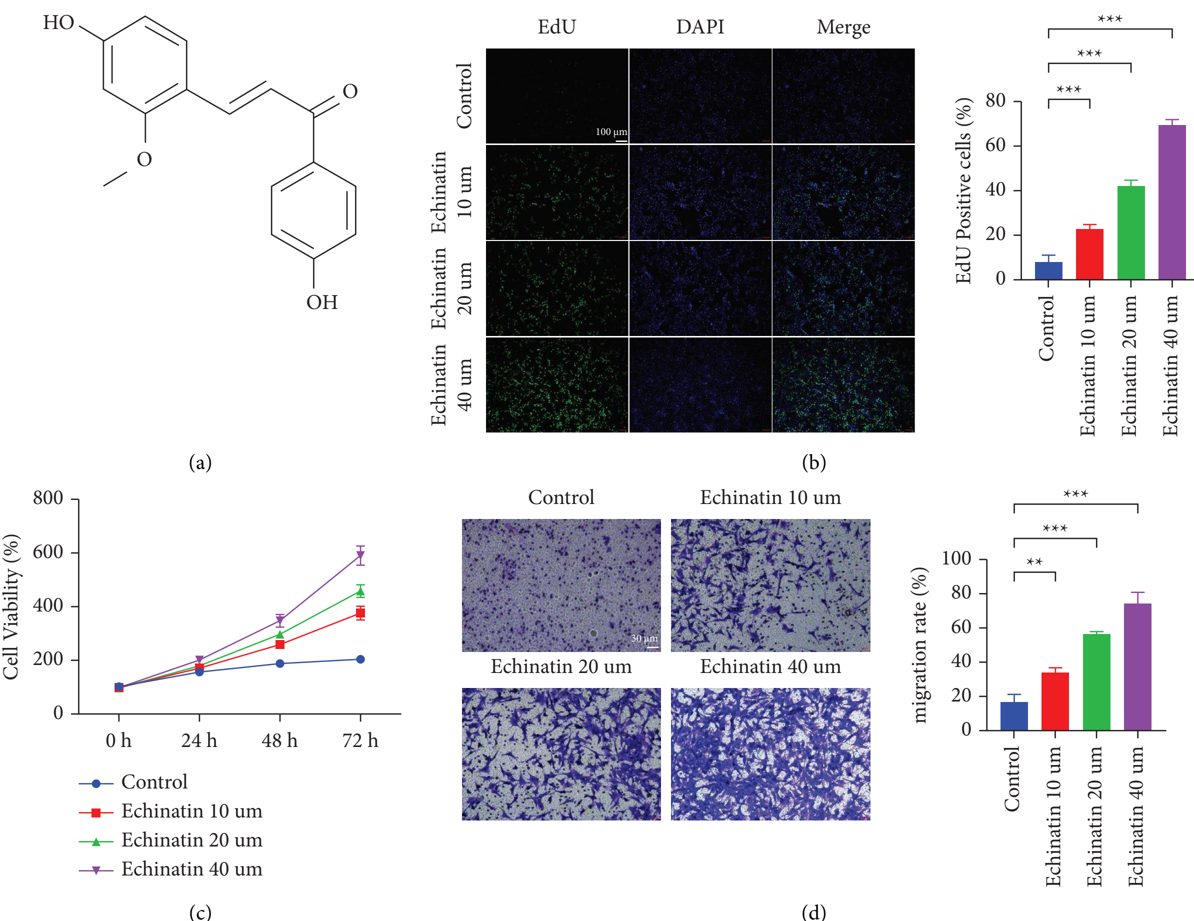


Figure 1: The application of echinatin leads to an increase in the vitality and migratory capacity of HTR-8/SVneo cells. (a) The molecular structure of echinatin is depicted. (b) The HTR-8/SVneo cell line was treated with three different concentrations of echinatin (10, 20, and 40 μM), and then, the vitality of the cells was assessed using the EdU test. (c) Various doses of echinatin were administered to the HTR-8/SVneo cell line for different time intervals (0, 24, 48, and 72 h). The vitality of the cells was evaluated using the CCK-8 test. (d) Transwell assays were conducted to analyze the migration of HTR-8/SVneo cells. The statistical values reported in this study include the mean ± standard deviation (SD). Significance levels are denoted as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Collection (ATCC) located in the United States. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with the addition of 5% carbon dioxide (CO_2) at a temperature of 37°C. In the experimental model of cell injury induced by H_2O_2, different concentrations of echinatin (10 μM, 20 μM, and 40 μM), TAK-242 (a TLR4 inhibitor), or the positive control drug magnesium sulfate (8 mmol/l) were administered to the cells prior to exposure to H_2O_2 (200 μM) for a duration of 12 h.

2.5. EdU Assays. The HTR-8/SVneo cells, which had a positive transfection status, were subjected to enzymatic digestion and subsequent quantification. Subsequently, the cells were amalgamated with a complete culture medium and evenly dispensed onto 96-well plates, with a cell density varying between 10,000 and 20,000 cells per well. Following this, the plates were carefully positioned inside a regulated setting within a cell culture incubator, where they remained for a period ranging from 48 to 72 h. A 4% paraformaldehyde cell fixation solution was applied to each well, with a volume of 100 μL. The wells were then incubated at room temperature for 30 min. Following that, the cells were subjected to a sequence of 2–3 rinses with a 0.5% solution of Triton X-100, a compound known for its ability to permeate cell membranes. The cell nuclei were subjected to staining using a DAPI solution at ambient temperature for 10 min. After completing the staining procedure, fluorescence microscopy was used to observe the cells, and the subsequent acquisition of photographs was swiftly carried out. The quantification of cells expressing the EdU marker was performed using the ImageJ software.

2.6. Cell Counting Kit-8 (CCK-8) Assays. Cells in the logarithmic growth phase were obtained, and the cell solution was afterwards adjusted to a concentration of about 10,000 cells per well. The resulting cell solution was then evenly dispersed over 96-well plates. Each well was supplemented with a 100 μL amount of sample, which was thereafter incubated in a 37°C, 5% CO_2 incubator for 48 h. Following this, a 10 μL amount of CCK-8 (Abcam, USA) was

added to each well, and the samples were then incubated for a duration of 1–2 h in an environment with controlled light exposure. After the completion of the cultural activity, the measurement of optical density (OD) at a specific wavelength of 450 nm was performed using an enzyme-linked immunosorbent assay (ELISA) reader. The equation for determining cell viability as a percentage is given by the following expression: cell viability (%) = [A (treatment) – A (blank)]/[A (negative control) – A (blank)] × 100%. In this equation, the variable A denotes the absorbance value associated with the well that contains the culture medium and CCK-8 solution, excluding any cellular presence. In contrast, the negative control, denoted as A, corresponds to the absorbance measurement of the well containing cells in the absence of any treatment.

2.7. Transwell Assays. To aid the experiment on cell invasion, the top part of the Transwell chamber was coated with Matrigel. The cells were inserted into the top chamber of the Transwell instrument after being placed in a serum-free medium. Subsequently, a fully saturated medium was introduced into the lower chamber. After a 24 h period of cellular proliferation, the cells underwent fixation using a 4% paraformaldehyde solution, followed by staining with violet crystals. Afterwards, the number of invading cells was assessed using microscopic examination.

2.8. Cell Apoptosis Assays. HTR-8/SVneo cells in the phase of logarithmic development were seeded into six-well plates and subjected to a 48 h incubation period. The cells were collected and subjected to two rounds of washing. Subsequently, they were resuspended in 400 μL of labeling buffer, following the instructions provided by the apoptosis kit (Sigma-Aldrich, St. Louis, MO, USA). The cells were treated with 5 μL of Annexin V-FITC and 10 μL of propidium iodide (PI; BD Pharmingen, CA, USA). The analysis of cell apoptosis was conducted using a FACScan flow cytometer (BD Biosciences, CA, USA).

2.9. Detection of Oxidative Stress Products. The production of the fluorescent probe known as 2′-7′-dichlorofluorescin diacetate (DCFH-DA) was carried out by Beijing Qingke. The resulting product was acquired from Sigma-Aldrich, a reputable supplier based in St. Louis, MO, USA (catalog number D6883). The measurement of reactive oxygen species (ROS) in HTR-8/SVneo cells was determined using the fluorescent probe DCFH-DA, which is generated as a result of ROS activity. The cells that received treatment were subjected to washing using phosphate-buffered saline (PBS) and thereafter exposed to a solution containing a concentration of 10 μmol/L of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). After being incubated at a temperature of 37°C for 30 min, the cells were analyzed using a fluorescence microscope that was equipped with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. The HTR-8/SVneo cells from each experimental group were collected and underwent centrifugation at a force of 1,000 g for 10 min to remove the supernatant above the cell pellet. Following this, the cellular samples underwent sonication and centrifugation at a force of 8,000 g at a temperature of 4°C for 10 min, resulting in the collection of the supernatant. The levels of malondialdehyde (MDA) concentration, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities were evaluated using a kit supplied by the Nanjing Jiancheng Bioengineering Institute in China.

2.10. Western Blot. The RIPA buffer was used for the purpose of extracting the whole protein content from the HTR-8/SVneo cells inside each experimental group. After quantifying the protein, an appropriate amount of protein was mixed with an equal volume of loading buffer and then exposed to boiling for a 3 min to facilitate denaturation. The proteins were subjected to separation by the use of polyacrylamide gel electrophoresis. Subsequently, they were transferred onto a polyvinylidene fluoride (PVDF) membrane using a wet transfer method. After blocking the membrane, primary antibodies against TLR4 (ab22048; Abcam, USA), MyD88 (ab133739; Abcam, USA), p-p65 (ab28856; Abcam, USA), p65 (ab32536; Abcam, USA), p-IkB (ab133462; Abcam, USA), and IkB (ab32518; Abcam, USA) were incubated overnight at 4°C. Following that, the membrane underwent incubation with secondary antibodies for a 2 h, while maintaining a consistent temperature comparable to the ambient conditions. Chemiluminescence was used as a method for detection and quantification, while the ImageJ program was used for the analysis of grayscale values associated with each band. The quantification of the target protein's expression level was determined by calculating the ratio of grayscale values between the specific protein of interest and the internal reference protein GAPDH (ab9485; Abcam, USA).

2.11. Enzyme-Linked Immunosorbent Assay (ELISA). The concentrations of tumor necrosis factor-alpha (TNF-α), IL-6, and IL-18 in the supernatant of cell cultures were determined using ELISA kits (Eusebio Biotechnology Co., Ltd., Shanghai, China) following the manufacturer's instructions. The antigens were diluted with a carbonate-coated upwelling buffer. Except for the blank well, all wells received a 50 μL addition of an enzyme-labeled reagent. Subsequently, the wells were incubated at 37°C for one hour. Then, each well was treated with a total volume of 100 μL tetramethylbenzidine and 50 μL termination buffer. The OD value was measured at a wavelength of 450 nm using a microplate reader (DALB; Shanghai, China).

2.12. Statistical Analysis. The statistical analyses of the data were performed using SPSS software (version 18.0; USA). The data shown in all bar graphs are supplied in the form of the mean value accompanied by the standard deviation. For comparing two groups with normally distributed data, Student's *t*-test was employed, whereas a two-tailed Mann–Whitney test was used for non-normally distributed data.

Additionally, q-tests were applied to perform multiple comparisons among the groups. A P value below the predetermined threshold of 0.05 indicates statistical significance.

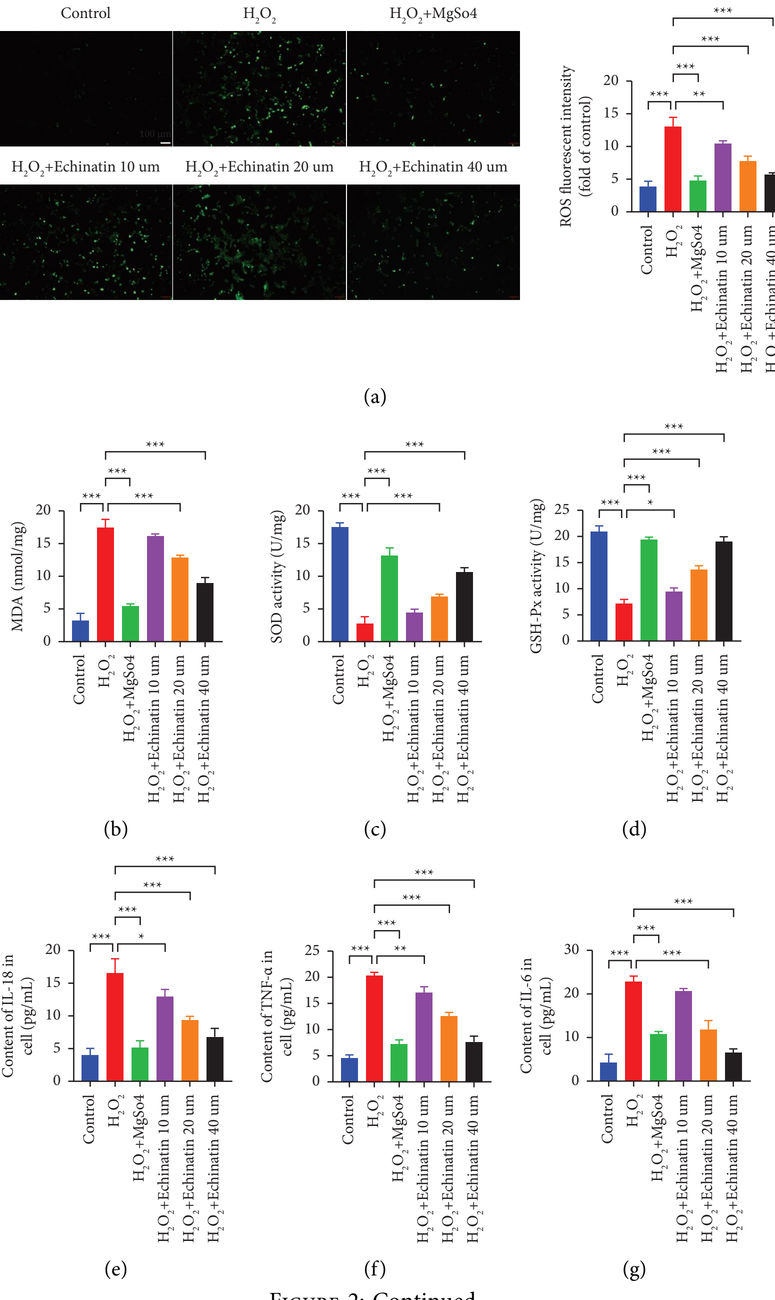
3. Results

3.1. Echinatin Enhances the Viability and Migration Ability of HTR-8/SVneo Cells. To examine the impact of echinatin on HTR-8/SVneo cells, the cells were subjected to treatment with several concentrations of echinatin, namely, 10, 20, and 40 μM. The findings shown in Figure 1(b) demonstrate that echinatin had a dose-dependent effect on the increase in EdU-positive cells in HTR-8/SVneo cells. Furthermore, the CCK-8 test demonstrated that the vitality of HTR-8/SVneo cells was greatly enhanced by echinatin (Figure 1(c)). The findings from the Transwell experiment indicate that echinatin had a dose-dependent effect on the migration of HTR-8/SVneo cells, as shown in Figure 1(d).

3.2. Echinatin Alleviates H_2O_2-Induced Inflammation and Oxidative Stress in HTR-8/SVneo Cells. To investigate the possible impact of echinatin on H_2O_2-induced cytotoxicity in HTR-8/SVneo cells, a treatment was administered to the cells including both H_2O_2 and echinatin. The experimental results indicate that the application of H_2O_2 resulted in an increase in ROS levels in HTR-8/SVneo cells. Furthermore, the impact of H_2O_2 was shown to be diminished in a way that correlated with the dosage administered, as shown in Figure 2(a). Furthermore, the application of H_2O_2 resulted in elevated concentrations of MDA (Figure 2(b)), SOD activity (Figure 2(c)), GSH-Px activity (Figure 2(d)), interleukin-18 (IL-18) (Figure 2(e)), TNF-α (Figure 2(f)), and interleukin-6 (IL-6) (Figure 2(g)) in HTR-8/SVneo cells. However, the administration of echinatin reversed these effects (Figures 2(b)–2(g)). The application of H_2O_2 resulted in the initiation of programmed cell death, known as apoptosis, in HTR-8/SVneo cells. Conversely, the administration of echinatin had a mitigating effect on H_2O_2-induced apoptosis in these cells, as shown in Figure 2(h). To enhance the credibility of the observed relieving impact of echinatin on oxidative stress in HTR-8/SVneo cells, we included magnesium sulfate in the cellular model of oxidative stress. The therapeutic use of magnesium sulfate in the treatment of preeclampsia has been extensive. The findings indicated that the group treated with echinatin at a concentration of 40 μM had a reduction in the inflammatory response, oxidative stress, and cell death in HTR-8/SVneo cells, which was similar to the group treated with magnesium sulfate (Figures 2(a)–2(h)).

3.3. Echinatin Alleviates Inflammation, Oxidative Stress, and Cell Apoptosis via the TLR4-MyD88-NF-κB Pathway. Oxidative stress and inflammation are closely related, as oxidative stress can induce inflammation and inflammation can affect oxidative stress [25]. Following the activation of TLR4, a series of multimolecular complexes are initiated, resulting in the activation of signal cascades. This ultimately leads to the early activation of NF-κB and the subsequent generation of pro-inflammatory cytokines, including TNF-α and IL-6 [26]. NF-κB serves as a prevalent transcription factor in the context of inflammation and oxidative stress, wherein its activation also stimulates the transcription of genes associated with pro-inflammatory and pro-oxidative responses [27]. To examine the impact of echinatin on HTR-8/SVneo cells *via* the TLR4-MyD88-NF-κB pathway, the Western blot analysis revealed a dose-dependent reduction in the protein expression levels of TLR4, MyD88, p-NF-κB, and p-IkB upon treatment with echinatin (Figure 3(a)). These findings indicate that echinatin effectively inhibits the TLR4-MyD88-NF-κB pathway in HTR-8/SVneo cells. Subsequently, an assessment was conducted to determine the potential of echinatin in modulating the viability, migration, inflammation, oxidative stress, and cell death of HTR-8/SVneo cells *via* the inhibition of the TLR4-MyD88-NF-κB pathway. The results of the experiment demonstrated that the inhibitory impact of echinatin on the protein expression levels of TLR4, MyD88, p-NF-κB, and p-IkB in HTR-8/SVneo cells was reversed when co-treated with echinatin and TLR4 overexpression plasmids, as shown in Figure 3(b). The administration of echinatin resulted in an increase in the number of cells positive for EdU, but the overexpression of TLR4 hindered this increase, as shown in Figure 3(c). In a similar vein, the upregulation of TLR4 hindered the proliferation of HTR-8/SVneo cells that were stimulated by echinatin, as shown in Figure 3(d). In addition, the upregulation of TLR4 resulted in the reversal of the migratory effects caused by echinatin in HTR-8/SVneo cells, as shown in Figure 3(e). Subsequently, an examination was conducted to explore the association between echinatin and the TLR4-MyD88-NF-κB pathway in the regulation of inflammation, oxidative stress, and cell death generated by H_2O_2. It was observed that the presence of echinatin resulted in the inhibition of ROS buildup in H_2O_2-treated HTR-8/SVneo cells. However, this effect was reversed when TLR4 was overexpressed, as shown in Figure 4(a). Furthermore, the overexpression of TLR4 counteracted the impact of echinatin on the concentrations of MDA (as shown in Figure 4(b)), SOD activity (as depicted in Figure 4(c)), GSH-Px activity (as shown in Figure 4(d)), IL-18 (as presented in Figure 4(e)), TNF-α (as demonstrated in Figure 4(f)), and IL-6 (as indicated in Figure 4(g)) in H_2O_2-treated HTR-8/SVneo cells. The overexpression of TLR4 resulted in the initiation of apoptosis in H_2O_2-treated HTR-8/SVneo cells, a process that was effectively suppressed by the administration of echinatin, as shown in Figure 4(h). In summary, the findings of our study indicate that echinatin has the potential to mitigate inflammation, oxidative stress, and cell death generated by H_2O_2 *via* the TLR4-MyD88-NF-κB pathway.

To substantiate the therapeutic efficacy of echinatin, we established a PE rat model. Our findings indicate that echinatin mitigates placental damage (Figure 5(a)), enhances placental proliferation (Figure 5(b)), and suppresses cell apoptosis (Figure 5(c)). Notably, these beneficial effects were negated by the overexpression of TLR4, implying that echinatin's ameliorative impact on the placental tissues of PE rats is, at least in part, mediated by TLR4.



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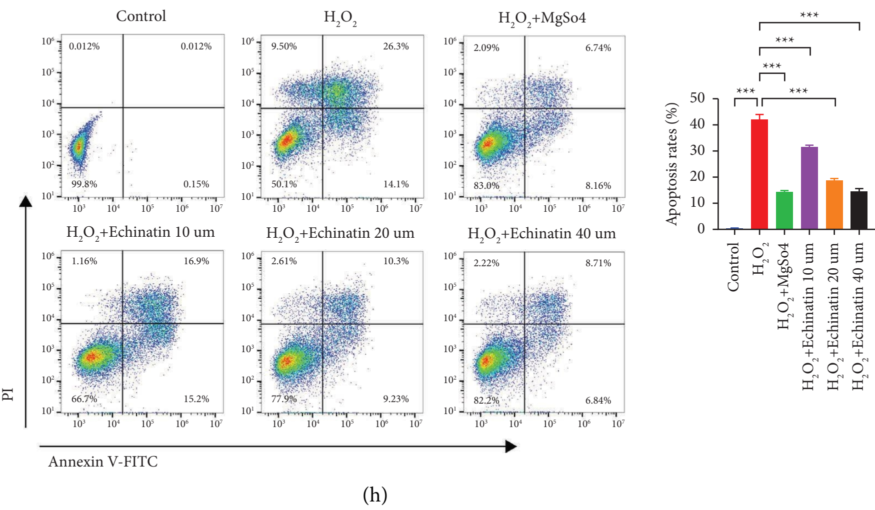
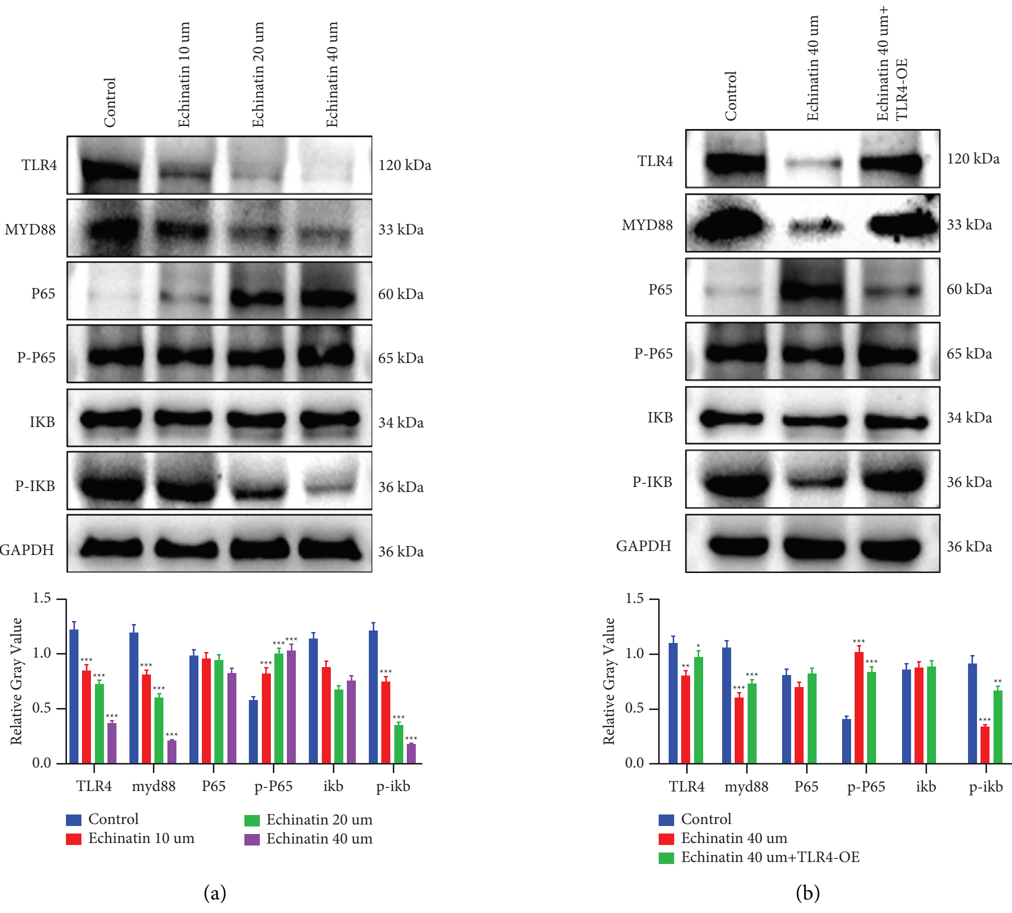


(h)

Figure 2: Echinatin alleviates inflammation and oxidative stress induced by H_2O_2 in HTR-8/SVneo cells. (a)–(h) The HTR-8/SVneo cell line was treated with H_2O_2 at a concentration of 200 μM, as well as different concentrations (10, 20, and 40 μM) of echinatin. (a) ROS production was quantified using the DCFH-DA technique. Intracellular concentrations of MDA (b), SOD activity (c), and GSH-Px activity (d) were determined. ELISA experiments were conducted to quantify the concentrations of interleukin-18 (e), TNF-α (f), and interleukin-6 (g). The apoptotic rate of the cells was assessed using flow cytometry (h). The statistical results are reported as the mean ± standard deviation (SD). Significance levels are denoted as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.



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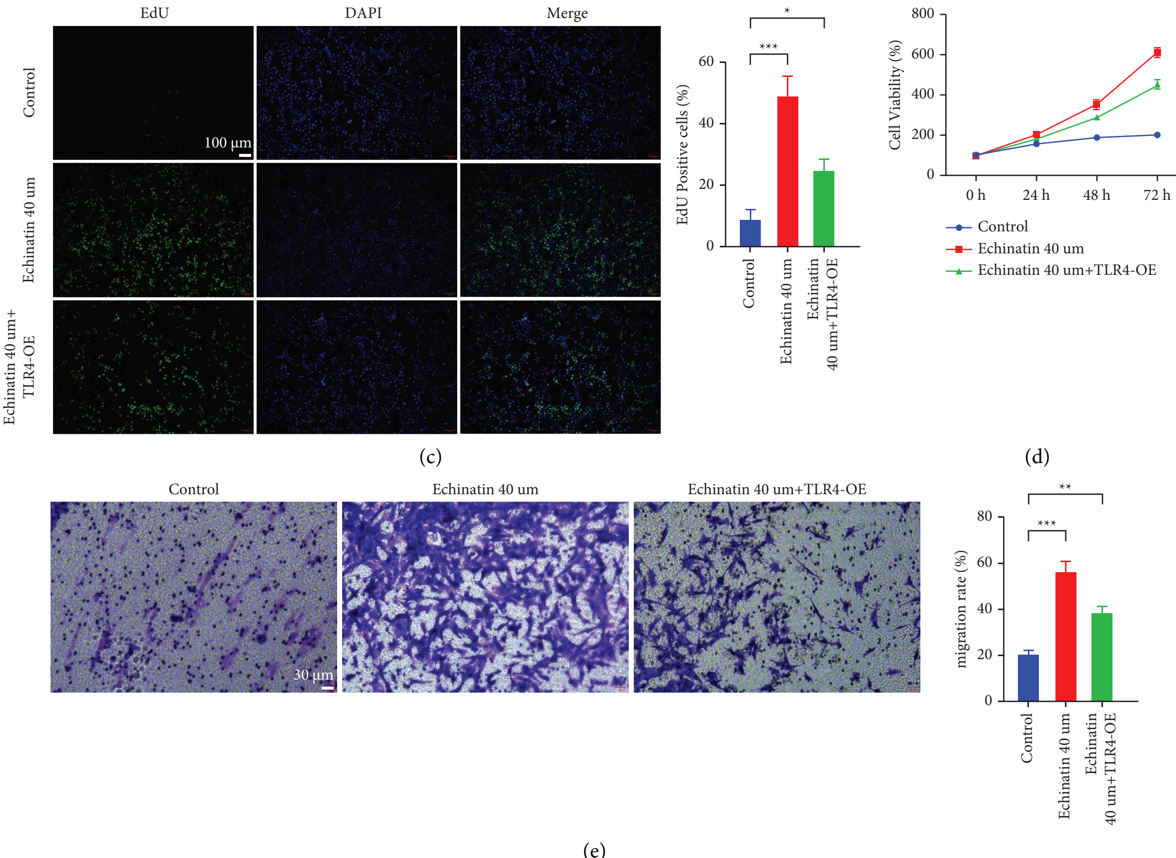
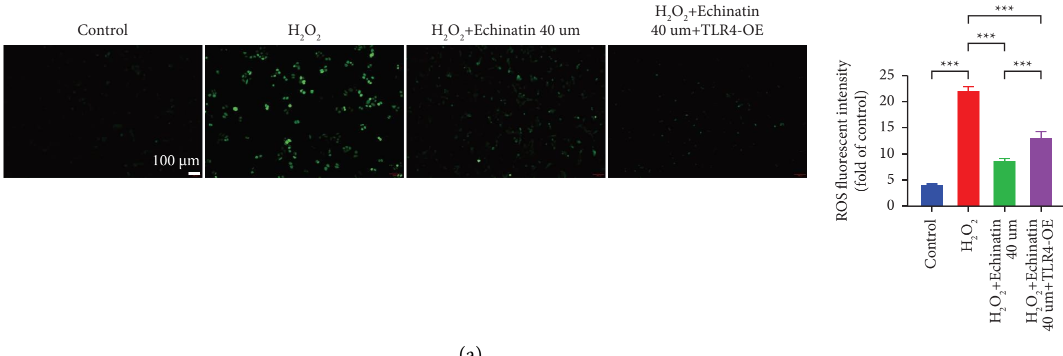


(c) (d)



(e)

Figure 3: Echinatin induces viability and migration of HTR-8/SVneo cells through the TLR4-MyD88-NF-κB pathway. (a) After a 24 h exposure of HTR-8/SVneo cells to echinatin, the Western blotting technique was used to assess the levels of TLR4, MyD88, NF-κB, p-NF-κB, IkB, and p-IkB protein expressions. (b)–(e) After treating HTR-8/SVneo cells overexpressing TLR4 with echinatin for 24 h. (b) The expression of TLR4, MyD88, NF-κB, p-NF-κB, IkB, and p-IkB was detected using Western blotting. (c) Cell viability of HTR-8/SVneo cells was assessed using the EdU assay. (d) Cell viability of HTR-8/SVneo cells was assessed using the CCK-8 assay. (e) Cell migration of HTR-8/SVneo cells was analyzed using the Transwell assay. Mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

(a)

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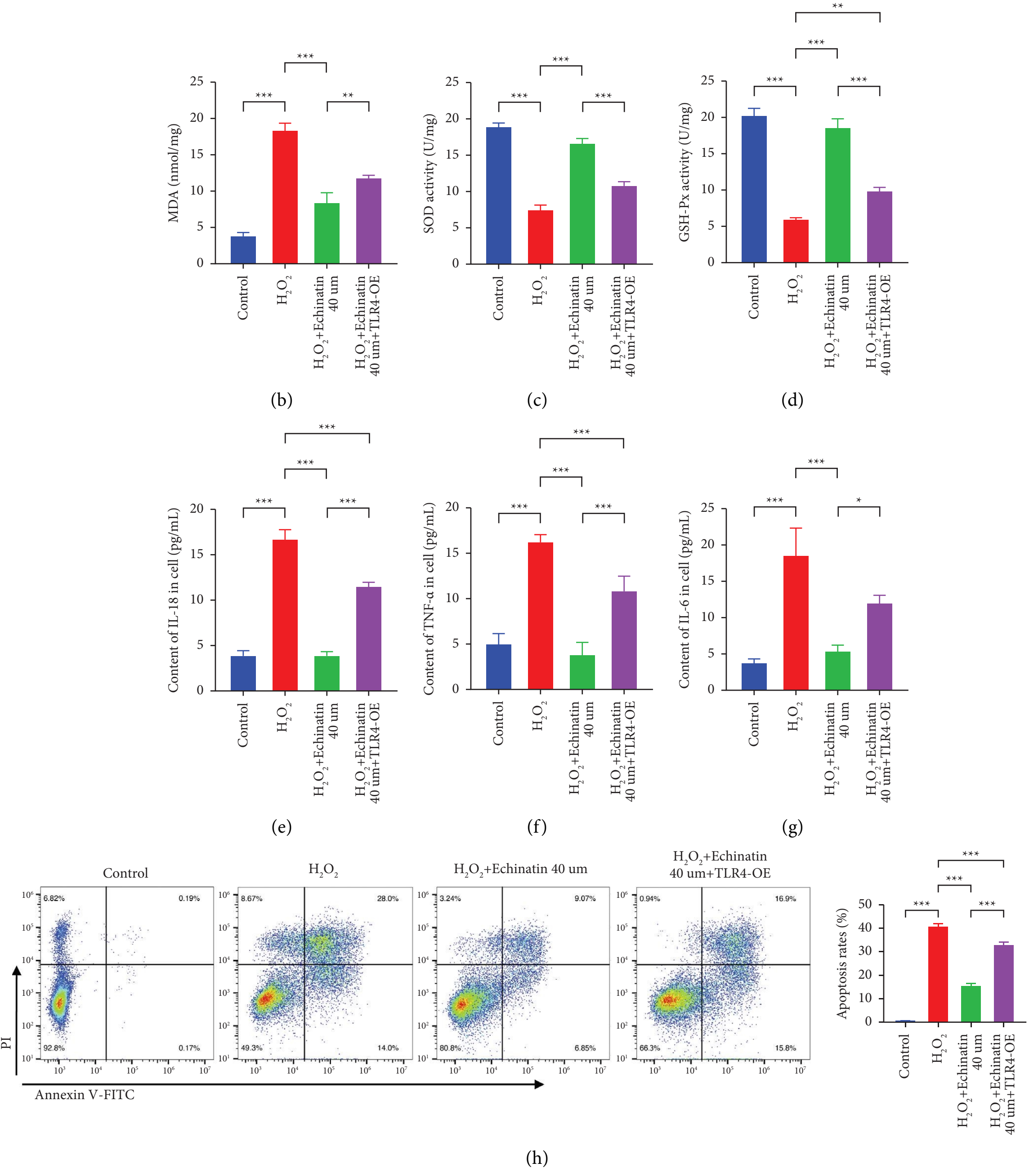

FIGURE 4: Echinatin alleviates H_2O_2-induced inflammation, oxidative stress, and cell apoptosis through the TLR4-MyD88-NF-κB pathway. (a)–(h) Treating HTR-8/SVneo cells with 200 μM H_2O_2 or 40 μM echinatin in combination with overexpressing TLR4. (a) ROS generation was measured using the DCFH-DA method. Intracellular levels of MDA (b), SOD activity (c), and GSH-Px activity (d). (e)–(g) ELISA experiments were performed to measure the levels of IL-18 (e), TNF-α (f), and IL-6 (g). (h) Flow cytometry was used to detect the apoptosis rate of the cells. Mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

4. Discussion

Preeclampsia is a prevalent and significant problem in the field of obstetrics, presenting a substantial risk to the well-being of both the pregnant individual and the developing baby [28]. Moreover, the condition of preeclampsia changes rapidly, and the prevention and treatment of preeclampsia are still important topics in clinical research in obstetrics and gynecology [29]. In the past few years, there has been an increasing acknowledgement of the unique advantages associated with traditional Chinese medicine (TCM) in the areas of disease prevention and management. Furthermore, TCM has shown significant promise for extensive use in many circumstances [30]. Nonetheless, adherence to the principles of TCM regarding syndrome differentiation and treatment philosophy remains imperative. Additionally,

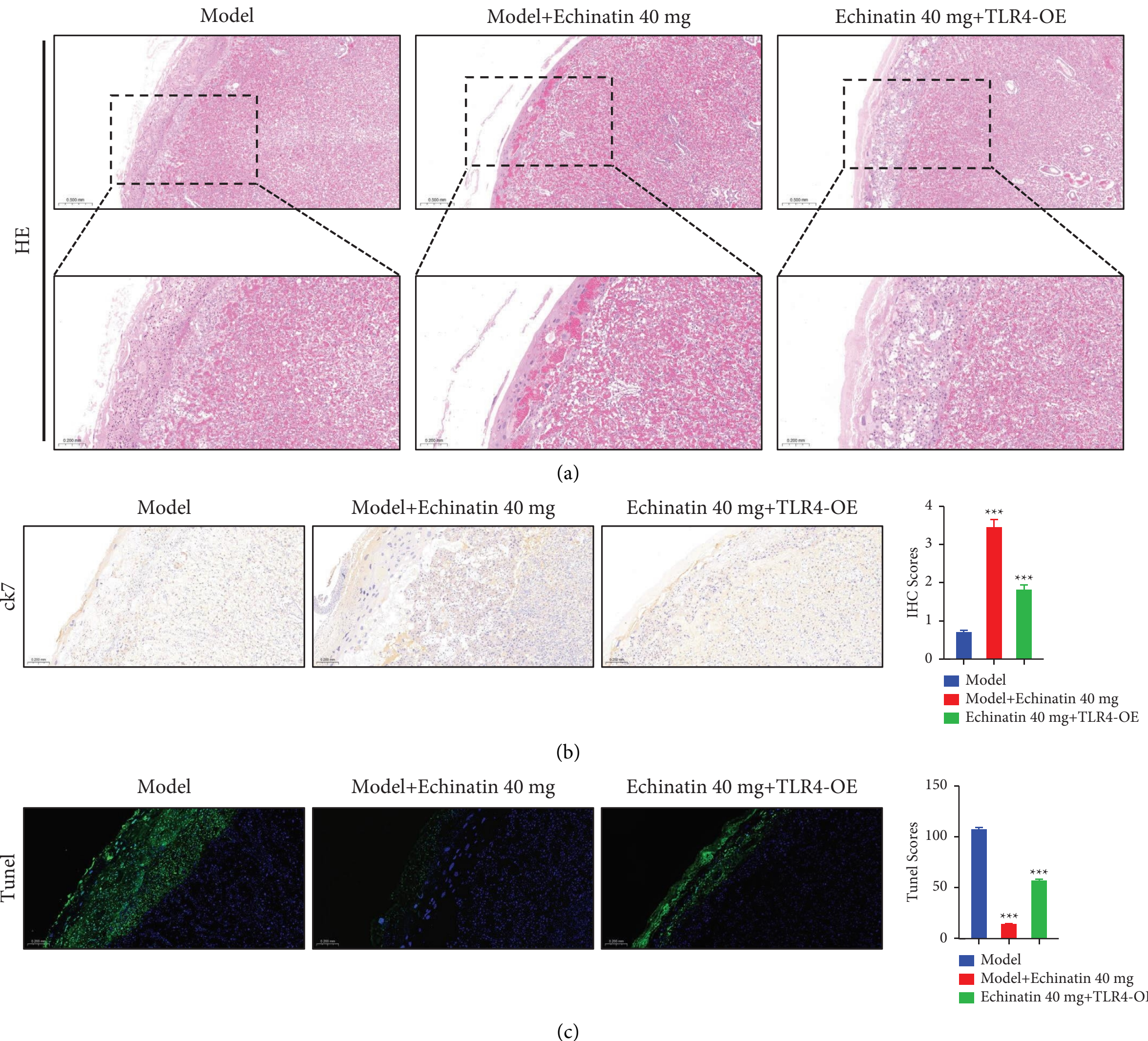

Figure 5: The effect of echinatin and TLR4 overexpression on apoptosis in the placental tissue from rats with preeclampsia. (a) HE staining was performed. (b) CK7 immunohistochemistry and quantitative analysis were conducted. (c) TUNEL staining and quantitative analysis were carried out on rat placental tissue. Mean ± SD; $^{***}P < 0.001$.

incorporating contemporary medical research methodologies and techniques is essential. This includes leveraging cell and animal models, as well as conducting comprehensive clinical investigations. These efforts are crucial for unraveling the underlying material basis and mechanisms of action of TCM in the realm of disease prevention and treatment. Echinatin, a naturally occurring compound derived from the Chinese plant licorice, has hepatoprotective, anti-inflammatory, and antioxidant properties [31]. Many drug developers have long been committed to the medicinal development of echinatin [32]. The increased focus towards the significance of echinatin in the prevention and treatment of numerous illnesses may be attributed to its pharmacological properties and therapeutic uses, which have been extensively researched. The findings of this work demonstrate that echinatin has a noteworthy capacity to augment the activity and migratory potential of HTR-8/SVneo cells, while concurrently mitigating inflammation and oxidative stress produced by H_2O_2 in HTR-8/SVneo cells. The findings of this study indicate that echinatin has considerable therapeutic promise in the context of therapy for preeclampsia.

The TLR4 plays a crucial role as a pattern recognition receptor within the nonspecific immune system. It becomes activated upon encountering PAMPs or DAMPs present in the body. This activation triggers the MyD88-dependent signaling cascade, which, in turn, activates the NF-κB signaling pathway [33, 34]. During the state of rest, NF-κB exists in an inactive form where it is present as a dimer that is coupled to the inhibitor IκB [35]. The activation of the MyD88-dependent signaling pathway leads to the initiation of IκB kinase activation *via* the involvement of intermediate adapter molecules. The following consequence of this activation is the phosphorylation and degradation of IκB, which, in turn, enables the activation and translocation of NF-κB into the cellular nucleus. As a result, the process of transcribing and expressing NLRP3 and IL-1β precursors is

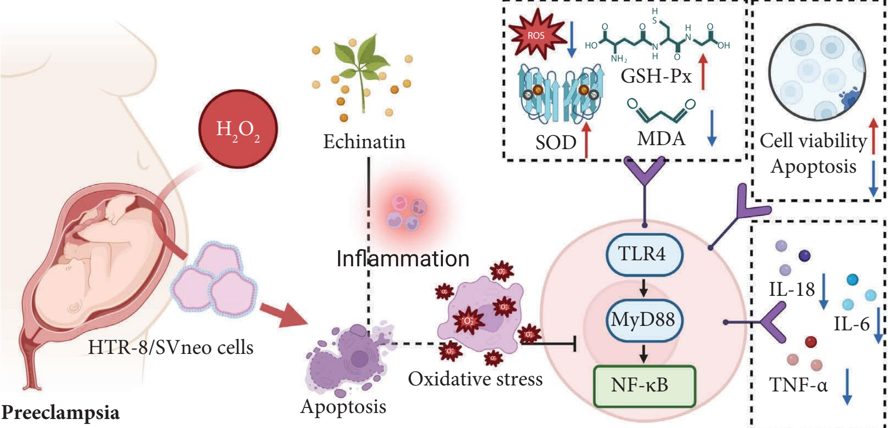

Figure 6: The mechanism diagram of echinatin in preeclampsia. Echinatin can enhance cell viability, reduce cell apoptosis, and decrease the expression of inflammatory factors such as IL-6, IL-18, and TNF-*α*. It promotes the activity of SOD and GSH-Px to inhibit inflammation while reducing the production of MDA and ROS to improve oxidative stress in H_2O_2-induced progression of preeclampsia by inhibiting the TLR4-MyD88-NF-*κ*B signaling pathway.

launched. Subsequently, the activation of the NLRP3 inflammasome is triggered by PAMPs or DAMPs, therefore launching a series of inflammatory cascades [33]. Hence, it is evident that the TLR4-MyD88-NF-*κ*B pathway assumes a crucial function in facilitating inflammatory reactions. Research has shown that several chemicals derived from natural sources, including hesperetin, gallic acid, and genistein A, have therapeutic potential in the treatment of disorders because of their ability to block the TLR4-NF-*κ*B pathway.

Echinatin has been shown to be a potent activator of Nrf2, as evidenced by prior research. *In vitro* investigations have further revealed its ability to effectively suppress the generation of PGE2, IL-6, ROS, and NO produced by LPS [17]. Nevertheless, the underlying molecular mechanism of this phenomenon remains elusive. The involvement of the TLR4-MyD88-NF-*κ*B pathway in the development of several human disorders has been established [33, 36, 37]. The present work aimed to elucidate the underlying mechanism by which echinatin exerts its activity on the TLR4-MyD88-NF-*κ*B pathway (Figure 6). Furthermore, a comprehensive investigation was undertaken to examine the effects of echinatin in detail. In the present study, we revealed the significant involvement of the TLR4-MyD88-NF-*κ*B signaling pathway in preeclampsia. The activation of TLR4, a pattern recognition receptor, through its MyD88-dependent signaling, results in NF-*κ*B activation, a key regulator of inflammatory responses. The cumulative evidence shows multiple natural compounds inhibit this pathway, offering therapeutic potential [38]. Moreover, our results indicate that echinatin exerts its beneficial effects by inhibiting this pathway, suppressing inflammatory mediators and oxidative stress markers in HTR-8/SVneo cells. Comparative studies have demonstrated that other natural compounds also target the TLR4-MyD88-NF-*κ*B pathway. For example, hesperetin, gallic acid, and genistein A have been reported to exert anti-inflammatory effects through inhibition of this pathway, highlighting the prospect of targeting TLR4-MyD88-NF-*κ*B for therapeutic intervention. Moreover, studies have also highlighted Nrf2 activation by echinatin, further aligning with our findings that echinatin can serve as an effective modulator of cellular stress responses [36]. Considering our results and the established role of the TLR4-MyD88-NF-*κ*B pathway in inflammatory and oxidative processes, we propose that echinatin's therapeutic action in preeclampsia likely involves the suppression of this signaling cascade. These insights contribute to a deeper understanding of the molecular mechanisms underpinning the protective effects of echinatin against preeclampsia, facilitating the further development and clinical application of echinatin-based treatments. Additionally, our study provides new targets and conceptual frameworks for the therapy for preeclampsia.

This study has several limitations that should be acknowledged. First, our experiments were conducted using the HTR-8/SVneo cell line, an *in vitro* model of trophoblast cells. While this model is valuable for mechanistic studies, it does not fully recapitulate the *in vivo* complexity of human placental development and function. Second, the effect of echinatin on the clinical outcomes of preeclampsia could not be established as *in vivo* or clinical studies were not conducted. Third, while our study investigated the TLR4-MyD88-NF-*κ*B pathway, other potential signaling pathways and molecular mechanisms might also be involved in the observed effects of echinatin, hence the need for further investigation. Lastly, while echinatin was compared with magnesium sulfate, a conventional treatment, the study lacked other positive controls and did not assess potential interactions with other treatments for preeclampsia.

5. Conclusions

Our study indicates that echinatin can ameliorate inflammation and oxidative stress in the context of

preeclampsia through the inhibition of the TLR4-MyD88-NF-κB signaling pathway. Given its pharmacological profile, echinatin emerges as a promising candidate for the therapeutic management of preeclampsia, potentially paving the way for novel interventions in obstetric care. To validate these findings, further clinical trials are warranted to assess the safety, efficacy, and optimal dosing of echinatin in pregnant individuals at risk of preeclampsia.

Data Availability

All data are provided in this study, and raw data can be requested to the corresponding author.

Ethical Approval

The study was approved by the Shengli Oilfield Central Hospital (No. 2022036).

Disclosure

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' Contributions

Xiangyun Deng and Qian Zhou conceived and designed the study; Hu Chen, Yang Zhang, and Fengmei Xu analyzed and interpreted the data; Hu Chen, Yang Zhang, and Fengmei Xu collected the data; Xiangyun Deng and Qian Zhou wrote the article; Xiangyun Deng and Qian Zhou critically revised the article; Yang Zhang and Fengmei Xu involved in statistical analysis; Qian Zhou had overall responsibility; and all authors read and approved the final manuscript. Xiangyun Deng and Hu Chen contributed equally to this work. These authors share the first authorship.

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