

Effect of Baicalin on Placental Tissue Damage in Preeclampsia Rats via the IL-33/ST2 Pathway

Shuyun Ding¹, Li Li^{1,*}, Feiyun Jiang², Yifan Mao²

¹Department of Obstetrical, Wuhu Second People's Hospital, Jinghu, Wuhu, Anhui, CHINA.

²Department of Gynaecology, Wuhu Second People's Hospital, Jinghu, Wuhu, Anhui, CHINA.

ABSTRACT

Objectives: To investigate the effect of baicalin on placental tissue injury in Preeclampsia (PE) rats and its possible mechanism. **Materials and Methods:** The caudal arterial blood pressure of Ed 15 and Ed 21 rats was detected. Bradford method was used to detect the 24 hr urinary protein content. The serum levels of Tumor Necrosis Factor- α (TNF- α), Interferon- γ (IFN- γ), Interleukin-4 (IL-4), Interleukin 10 (IL-10), Interleukin-33 (IL-33) and soluble growth Stimulating expression gene 2 protein (sST2) were determined by Enzyme-Linked Immunosorbent Assay (ELISA). The number and survival rate of pups in each group were recorded. The placental tissue was separated and weighed. The histopathological changes of placenta were detected by HE staining. The apoptosis index of placental trophoblast cells was detected by TUNEL staining. The relative expression of IL-33 and transmembrane growth Stimulation expression gene 2 protein (ST2L) in placental tissues of rats were detected by Western blotting. **Results:** The looser arrangement, more obvious inflammatory infiltration, more severe apoptosis and higher serum TNF- α , IFN- γ and sST2 levels were observed. Meanwhile, the levels IL-4, IL-10 and IL-33 decreased, the relative expression level of IL-33 protein in placenta decreased, but the relative expression level of ST2L protein increased ($p < 0.05$). Compared with the model group, the tail artery blood pressure, the 24 hr urinary protein content decreased, the number and survival rate of offspring, the pathological changes of placental tissue structure and cell apoptosis of the 4 administration groups increased. The levels of TNF- α , IFN- γ , sST2, IL-4, IL-10 and IL-33 increased. The relative expression level of IL-33 protein in placenta increased, while that of ST2L protein decreased ($p < 0.05$). The improvement effect of baicalin on placental tissue injury was enhanced in LD, MD, HD and PD groups ($p < 0.05$). **Conclusion:** Baicalin can improve the placental tissue injury in PE rats, level of bait receptor ST2, promoting the binding of IL-33 and ST2L and regulating immune imbalance.

Keywords: Baicalin, Interleukin-33, Placental tissue, Preeclampsia.

Correspondence:

Dr. Li Li

Department of Obstetrical, Wuhu
Second People's Hospital, No. 259 Jiu Hua
Middle Road, Jinghu District, Wuhu City,
Anhui-241000, CHINA.
Email: lili220620@163.com

Received: 05-03-2025;

Revised: 13-05-2025;

Accepted: 21-08-2025.

INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific syndrome characterized by insufficient placental perfusion and placental dysfunction caused by hypoxia. It manifests as abnormally elevated blood pressure, local edema and proteinuria and represents a major cause of morbidity and mortality in premature infants, pregnant women and neonates.¹ The pathogenesis of PE is complex, involving potential factors such as endothelial dysfunction,² oxidative stress,³ immune imbalance and inflammatory responses.⁴ Interleukin-33 (IL-33), released by cells as an "alarm" cytokine or endogenous signal in active or passive damage, interacts with its receptors, including growth Stimulation-expressed gene

2 protein (ST2) and Interleukin-1 (IL-1) receptor accessory protein, forming a dimer that participates in various pathological processes of PE.⁵ Relevant studies suggest that baicalin can effectively improve PE-induced excessive activation of stress and immune imbalance,⁶⁻⁸ demonstrating the potential of baicalin in preventing and treating PE. This experiment established a PE rat model and used baicalin intervention to observe the therapeutic effect of baicalin on PE, as well as its effect on the IL-33/ST2 signaling pathway, to provide experimental verification and basic research data for baicalin to become a clinical therapeutic drug for PE.

MATERIALS AND METHODS

Instruments

BP-98A/B Noninvasive Tail-Cuff Blood Pressure System for Rats and Mice (Softron Biotechnology, China), Multiskan FC Microplate Photometer (Thermo Fisher Scientific, USA) and FluorChem M MultiFluor System (ProteinSimple, USA).



DOI: 10.5530/ijper.20271063

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Reagents and drugs

Baicalin (Sigma, USA); Aspirin (Bayer); N-Nitro L-Arginine Methyl Ester (L-NAME) (Sigma, USA); Tumor Necrosis Factor α (TNF- α), Interleukin-4 (IL-4) and Interleukin-10 (IL-10) (AmyJet Scientific, China); Interferon-gamma (IFN- γ) (Yuanmu Biological Technology, China); soluble ST2 (sST2) Enzyme-Linked Immunosorbent Assay (ELISA) Kit (EK-Bioscience Biotechnology, China); IL-33 ELISA Kit, rabbit anti-IL-33 monoclonal antibody and rabbit anti-ST2 monoclonal antibody (Abcam, UK).

Experimental animals

Sixty SPF-grade female and male Wistar rats, aged 7 weeks, weighing (200 $\pm$ 10) g, were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd., (license number: SCXK(L) 2019-0003).

Methods

Modeling, grouping and drug administration

Healthy adult female and male Wistar rats were housed under standard conditions at a constant temperature of 22°C to 24°C with a relative humidity of 50% to 60%, following a 12 hr light-dark cycle. After a one-week acclimatization period, female and male rats were paired at a ratio of 1:1 and daily vaginal smears were conducted. The presence of sperm was recorded as Day 0 of pregnancy (Ed 0).⁹ 7 non-pregnant rats and 3 pregnant rats were excluded. The remaining pregnant rats were randomly assigned to the Normal group, Model group, Low-Dose baicalin (LD) group, High-Dose baicalin (HD) group and Aspirin (ASA) group, with 10 rats in each group. Except for the Normal group, pregnant rats in the other groups were subcutaneously injected with 50 mg/kg L-NAME from Ed 13 to Ed 21 to induce PE models, while the Normal group was given an equivalent volume of physiological saline via the subcutaneous route. The LD, HD,¹⁰ and ASA¹¹ groups were treated from Ed 15 to Ed 21, with oral gavage doses of 40, 160 and 2 mg/kg, respectively. The Normal and Model groups were administered equivalent volumes of physiological saline by oral gavage once daily.

Sample collection and processing

Noninvasive blood pressure measurements were taken using a tail-cuff blood pressure system on Ed 15 and 21 for each group. On Ed 20, pregnant rats were individually placed in metabolic cages and 24-hr urine samples were collected to assess 24 hr urine protein levels. An intraperitoneal injection of 80 mg/kg pentobarbital sodium was performed to anesthetize pregnant rats in each group. Blood was drawn from the heart, allowed to stand overnight at 4°C and the serum was harvested. After centrifugation at 1500 rpm for 10 min at 4°C, the supernatant was obtained and used for ELISA testing. The number and survival rate of offspring in each group were recorded. Euthanasia was

performed on both rats and pups. Placental tissues were collected, weighed and preserved separately in 40 g/L Paraformaldehyde (PFA) and liquid nitrogen for tissue histopathology staining and Western Blotting (WB).

Quantification of 24 hr urine protein using the Bradford assay

Controls and sample wells were set up as follows: the protein control was diluted to 0.5 μ g/ μ L and different volumes were aspirated into standard wells. The phosphate-buffered solution was added to each well to a total volume of 20 μ L. For the reaction wells, 20 μ L of urine sample diluted 20 times was added. Subsequently, 200 μ L of Coomassie Brilliant Blue G250 was added to both control and sample wells, mixed with the samples and allowed to stand at room temperature for 5 min. Absorbance (A) values at a wavelength of 570 nm were measured and a standard curve was plotted to calculate the 24 hr protein concentration in the urine samples.

Histopathological examination of placental tissues via Hematoxylin and Eosin (H&E) staining

Placental tissues were fixed in 40 g/L PFA for 24 hr, embedded in paraffin and sectioned into 5 μ m paraffin slices in a standard manner. Deparaffinization was performed with xylene before rehydration. The nuclei were stained with hematoxylin for 5 min, followed by differentiation in 1% hydrochloric acid in ethanol for 10 sec, bluing in ammonia solution for 3 seconds and counterstaining with eosin for 30 sec. After washing and dehydration, the slides were cleared, mounted with neutral resin and observed under a microscope. Images were captured for analysis.

Detection of apoptosis index of trophoblasts via TUNEL staining

Paraffin sections were deparaffinized in xylene, hydrated in a graded ethanol series, treated with DNase-free proteinase K solution for 20 min and then incubated with 50 μ L TUNEL reaction solutions for 1 hr. After peroxidase blocking, color development was achieved by reacting with a mixture of substrate and diaminobenzidine. The sections were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene and mounted with neutral resin. Brown particles in the cytoplasm or cell nucleus indicated positive staining. The apoptosis index was calculated as (number of TUNEL-positive cells/total number of cells) $\times$ 100%.

Determination of the serum levels of relevant markers via ELISA

Control and sample wells were set up and 50 μ L of differently concentrated control and 10-fold diluted test samples were added. After 1 hr of incubation with the enzyme-labeled working solution, the plate was washed. Substrates A and B were added

sequentially, followed by incubation in the dark for 15 min. The reaction was terminated by adding the stop solution and the A values at 450 nm were measured. A linear regression curve was plotted using the control sample concentrations as the x-axis and corresponding A values as the y-axis. Serum levels of TNF- α , IFN- γ , IL-4, IL-10, IL-33 and sST2 were calculated for each group.

Determination of IL-33 and ST2 protein relative expression levels

The placental tissues from each group of rats were retrieved from liquid nitrogen and crushed in a grinding bowl. The tissue homogenates were lysed using a pre-cooled radio immuno precipitation assay buffer. After centrifugation, the supernatant was collected. The protein concentration was determined, and the proteins were denatured by heating. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis was performed (60 V for 25 min for stacking gel, 120 V for 62 min for separating gel), followed by transfer of proteins to the PVDF membrane (200 mA for 110 min). The PVDF membrane was then blocked with 50 mg/mL defatted milk (diluted in 1×TBST) at room temperature for 2 hr. Subsequently, the membrane was incubated with the primary antibodies (diluted at 1:1000) overnight at 4°C. The next day, the membrane was washed 6 times. The secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (diluted at 1:8000), was incubated at room temperature for 2 hr. After washing the membrane 6 times, the Electrochemiluminescence (ECL) reaction was performed, and the bands were observed and recorded using an ECL gel imaging system. Image J software was used to analyze the grayscale values of the bands and the relative expression levels of the target proteins IL-33 and ST2 were represented as the ratio of their grayscale values to the internal reference GAPDH.

Statistical methods

Statistical analysis was conducted using the software SPSS 22.0. Measurement data were presented as mean±standard deviation (Mean±SD). The normality and homogeneity of variance of the data distribution were analyzed using Kolmogorov Smirnov test and Levene test, respectively. When comparing multiple groups that follow a normal distribution and have homogeneous

variance, one-way analysis of variance was used and LSD-t test was used for pairwise comparisons between groups; when the variance is uneven, choose Dunnett-t3 test; for those who do not follow a normal distribution, rank sum test is used. $p < 0.05$ was considered statistically significant.

RESULTS

Rat tail arterial pressure at Ed 15 and Ed 21

The results of tail arterial pressure measurements showed no statistically significant differences among all groups of rats at Ed 15 (all $p > 0.05$), while statistically significant differences were observed at Ed 21 ($p < 0.05$, respectively). At Ed 21, tail arterial pressure in the Model group was significantly increased compared to the Normal group ($p < 0.05$). In comparison to the Model group, all three treatment groups showed a significant decrease in Ed 21 tail arterial pressure ($p < 0.05$, respectively). When compared to the LD-B group, the HD-B and ASA groups exhibited a significant decrease in Ed 21 tail arterial pressure ($p < 0.05$, respectively), with the ASA group showing the lowest values ($p < 0.05$) See Table 1.

24 hr urine protein

The Bradford assay results demonstrated statistically significant differences in 24-hr urine protein levels among the five groups ($p < 0.05$, respectively). In comparison to the Normal group, the Model group exhibited a significant increase in 24-hr urine protein ($p < 0.05$). When compared to the Model group, all three treatment groups showed a significant decrease in 24-hr urine protein ($p < 0.05$, respectively). When compared to the LD-B group, the HD-B and ASA groups exhibited a significant decrease in 24-hr urine protein ($p < 0.05$, respectively), with the ASA group showing the lowest level ($p < 0.05$) See Table 2.

Number and survival rate of offspring

There were statistically significant differences in the number and survival rate of offspring among the five groups ($p < 0.05$, respectively). Compared to the Normal group, the Model group exhibited a significant decrease in the number and survival rate of offspring ($p < 0.05$). Compared to the Model group, all three treatment groups showed a significant increase in the number

Table 1: Blood pressure of Ed 15 and Ed 21 caudal arteries of rats (Mean±SD).

	n	Ed 15/mmHg	Ed 21/mmHg
Normal	10	108.50±1.75	111.51±2.19
Model	10	110.26±1.65	146.84±2.98***
LD	10	109.45±2.41	136.74±2.74#
HD	10	108.98±3.06	124.51±2.49##,&
PD	10	109.09±1.79	115.98±2.07###,&&.@
F		0.805	279.510
P		0.619	<0.001

Note: ***, $p < 0.001$, vs. Normal; #, $p < 0.05$, ##, $p < 0.01$, ###, $p < 0.001$, vs. Model; &, $p < 0.05$, &&, $p < 0.01$, vs. LD; @, $p < 0.05$, vs. HD.

and survival rate of offspring ($p < 0.05$, respectively). Compared to the LD-B group, the HD-B and ASA groups exhibited a significant increase in the number and survival rate of offspring ($p < 0.05$, respectively), with the ASA group showing the highest values ($p < 0.05$, respectively) See Table 3.

Quality of placental tissue

Statistically significant differences in placental tissue quality were observed among the five groups ($p < 0.05$, respectively). Compared to the Normal group, the Model group exhibited a significant reduction in the quality of rat placental tissue ($p < 0.05$). In contrast, the three treatment groups showed a significant improvement in the quality of rat placental tissue compared to the Model group ($p < 0.05$, respectively). Moreover, when comparing the LD-B group with the HD-B and ASA groups, there was a significant increase in the quality of rat placental tissue in the HD-B and ASA groups ($p < 0.05$, respectively) and the improvement was more evident in the ASA group ($p < 0.05$) See Table 4.

Pathological changes in pregnant rat placental tissue

H&E staining results revealed that the Normal group exhibited well-organized cell arrangement, intact and regular structure and even distribution of capillaries in rat placental tissue, without any signs of inflammatory infiltration. In comparison, the Model group displayed disrupted tissue structure, reduced capillaries, increased intercellular spaces, loose arrangement and evident

inflammatory infiltration. However, the pathological conditions of rat placental tissue in all three treatment groups improved when compared to the Model group (See Figure 1).

Apoptosis of trophoblasts in pregnant rat placental tissues

TUNEL staining results demonstrated that the Normal group had a lower number of apoptotic trophoblasts, whereas the Model group showed a significantly elevated apoptosis index ($p < 0.001$). When compared to the Model group, the apoptosis index decreased significantly in all three treatment groups ($p < 0.05$, respectively). Compared to the LD-B group, the HD-B and ASA groups showed a significant decrease in the apoptosis index ($p < 0.05$, respectively), with the ASA group exhibiting a more pronounced decrease ($p < 0.05$) (See Table 5 and Figure 2).

Serum levels of relevant markers

The ELISA results revealed statistically significant differences in the serum levels of TNF- α , IFN- γ , IL-4, IL-10, IL-33 and sST2 among the five groups (all $p < 0.05$). Compared to the Normal group, the Model group exhibited elevated levels of serum TNF- α , IFN- γ and sST2, along with decreased levels of IL-4, IL-10 and IL-33 ($p < 0.05$, respectively). In contrast, the three treatment groups showed decreased serum levels of TNF- α , IFN- γ and sST2, while IL-4, IL-10 and IL-33 levels were significantly increased compared to the Model group ($p < 0.05$, respectively). Furthermore, when comparing the HD-B and ASA groups with the LD-B group, there is a significant decrease in the serum levels of TNF- α , IFN- γ and sST2 and a significant increase in the serum levels of IL-4, IL-10 and IL-33 ($p < 0.05$, respectively). Notably, changes in these markers were more evident in the ASA group ($p < 0.05$, respectively) See Table 6.

Protein expression levels in pregnant rat placental tissues.

The WB results indicated statistically significant differences in the relative expression levels of IL-33 and ST2 proteins among the five groups (all $p < 0.05$). Compared to the Normal group, the Model group exhibited a significant decrease in the relative expression level of IL-33 protein and a significant increase in

Table 2: 24 hr urinary protein content in rats (Mean $\pm$ SD).

	n	24 hr UP (mg/d)
Normal	10	21.16 $\pm$ 2.51
Model	10	48.11 $\pm$ 2.79***
LD	10	41.87 $\pm$ 2.61#
HD	10	32.98 $\pm$ 3.01##,&
PD	10	23.46 $\pm$ 2.46###,&&,@
F		195.51
P		<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs. HD.

Table 3: Number of pregnant offspring and survival rate of offspring (Mean $\pm$ SD).

	n	Offspring mice number	Offspring mice survival rate (%)
Normal	10	6.50 $\pm$ 0.89	94.65 $\pm$ 6.55
Model	10	2.20 $\pm$ 0.55***	6.65 $\pm$ 12.51***
LD	10	3.50 $\pm$ 0.54#	50.65 $\pm$ 21.53#
HD	10	4.80 $\pm$ 0.95##,&	70.53 $\pm$ 10.35##,&
PD	10	5.60 $\pm$ 0.75###,&&,@	85.56 $\pm$ 16.84###,&&,@
F		45.615	56.878
P		<0.001	<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs. HD.

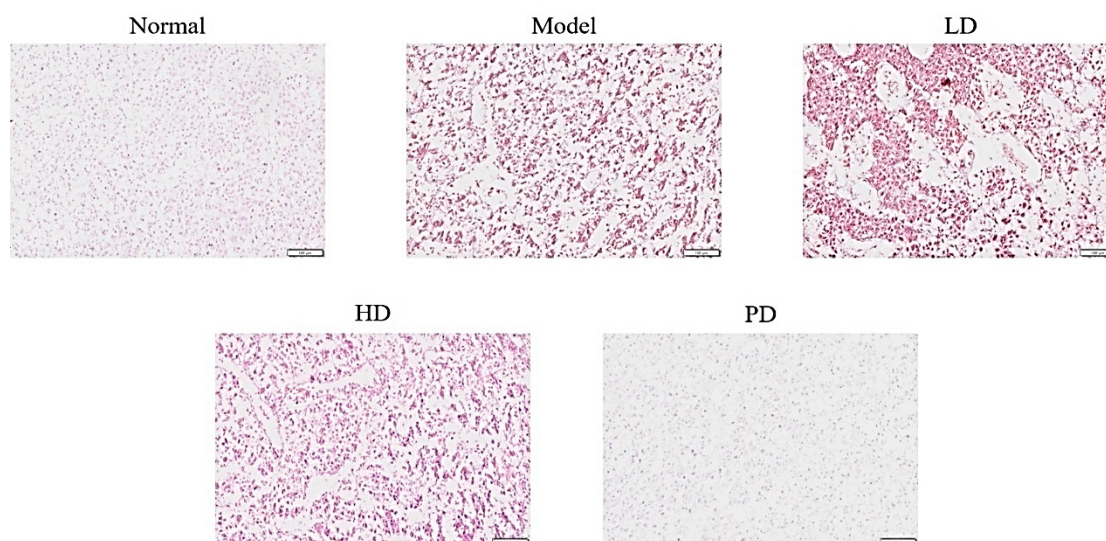


Figure 1: He staining results of placental tissue of rats in each groups (100×).

Table 4: The weight of rat placental tissue (Mean±SD).

	<i>n</i>	Placental tissue quality (g)
Normal	10	0.70±0.05
Model	10	0.41±0.03***
LD	10	0.48±0.04#
HD	10	0.55±0.03##,&
PD	10	0.62±0.02###,&&,@
F		172.516
P		<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs HD.

the relative expression level of ST2 protein in placental tissues ($p < 0.05$, respectively). In comparison to the Model group, the three treatment groups showed significantly increased relative expression levels of IL-33 protein and significantly decreased levels of ST2 protein in placental tissues ($p < 0.05$, respectively). In addition, when comparing the HD-B and ASA groups with the LD-B group, there was a significant increase in the relative expression levels of IL-33 protein and a significant decrease in the relative expression levels of ST2 protein ($p < 0.05$, respectively) and these changes were more pronounced in the ASA group ($p < 0.05$, respectively) (See Figure 3 and Table 7).

DISCUSSION

Nitric oxide is crucial for spiral artery remodeling and pregnancy and its deficiency can lead to PE-associated pathological changes in the uterus and placenta of pregnant mice.¹² L-NAME, an inhibitor of nitric oxide synthase, is commonly used to induce PE rat models.¹³ In the initial stages of subcutaneous injection of L-NAME in the present study, there were no statistically significant differences in tail arterial pressure among all groups. However, after several injections, the Model group showed a

significant increase in tail arterial pressure and 24-hr urine protein as compared to the Normal group. This indicates that L-NAME can induce PE syndrome-manifested by hypertension and proteinuria-in the mid-to-late stages of pregnancy.¹⁴ Meanwhile, compared with Normal group, there were There were significant pathological changes in the placental tissue of the Model group by HE staining, the appearance of hypertension, proteinuria and pathological changes suggested the successful construction of the PE rat model.

Aspirin is currently the only recommended pregnancy intervention drug for the prevention of PE.¹⁵ It can improve PE symptoms and trophoblast damage through pro-inflammatory pathways.¹⁶ The results of this study showed that, compared to the Model group, the baicalin-treated rats had reduced levels of tail artery pressure and 24-hr urine protein. This suggests that baicalin can lower early pregnancy hypertension in rats and alleviate symptoms of proteinuria. The placenta serves as an organ for the transport of respiratory gases, nutrients and waste products between the mother and the fetus. Therefore, its growth and development are crucial for the healthy growth and survival of the fetus. Inflammation and excessive activation of trophoblasts in the placenta can lead to abnormalities.¹⁷ Fetal growth restriction is mainly attributed to inadequate remodeling of uterine spiral arteries in early pregnancy and a decrease in vascular distribution in the placenta.^{18,19} The resulting inflammatory response and placental tissue damage can lead to a decrease in neonatal survival rates. Studies have found a reduction in the number and survival rate of offspring in PE rats.²⁰ In comparison to the Normal group, the Model group exhibited decreased placental tissue quality, structural disarray, decreased capillaries, enlarged cell gaps, noticeable inflammatory infiltration and severe cell apoptosis. The number and survival rate of offspring in the Model group were significantly reduced. However, treatment with baicalin at low and high doses was shown to improve placental

tissue pathology and offspring survival, indicating the potential of baicalin to ameliorate PE symptoms, possibly through its association with the inflammatory response. Our present study also found High concentration of baicalin's treatment effects were similar as Aspirin, which was PD group, treatment effects. These results provided a new treatment plan for the future clinical treatment of PE.

Normal pregnancy is associated with mild systemic inflammation and a T helper 2 (Th2)-biased immune response. When there is an imbalance in the immune cell population, a T helper 1 (Th1)-biased immune occurs and promotes chronic immune activation. The imbalance between anti-inflammatory and pro-inflammatory cytokines leads to a robust inflammatory response, causing placental tissue damage and inducing the occurrence of PE.²¹ TNF- α is a key mediator of the maternal immune system. It can increase vascular permeability, impair endothelial cell function, induce trophoblast apoptosis and complicate maternal inflammation.²² Many studies have shown that TNF- α has an increased level in PE, while IL-4 and IL-10, as anti-inflammatory cytokines secreted by Th2 cells, are negatively correlated with pro-inflammatory cytokines in PE.²³ IFN- γ , secreted by Th1

cells, effectively induces endothelial activation and excessive inflammation in PE.²⁴ In this study, the Model group had elevated serum levels of the pro-inflammatory cytokines TNF- α and IFN- γ and decreased levels of the anti-inflammatory cytokines IL-4 and IL-10 as compared to the Normal group. This indicates that the Th1/Th2 immune imbalance during normal pregnancy can trigger a pro-inflammatory state in the body, promoting the development and progression of PE. After treatment with low and high doses of baicalin, the levels of TNF- α and IFN- γ

Table 5: The apoptosis of rat placental trophoblast cells (Mean $\pm$ SD).

	<i>n</i>	Apoptosis index (%)
Normal	10	3.51 $\pm$ 1.59
Model	10	28.69 $\pm$ 2.51***
LD	10	21.59 $\pm$ 2.31#
HD	10	13.74 $\pm$ 2.69##,&
PD	10	8.91 $\pm$ 2.06###,&&,@
F		152.749
P		<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs. HD.

Table 6: The level of serum related factors in rats (Mean $\pm$ SD).

	<i>n</i>	TNF- α (pg/mL)	IFN- γ (pg/mL)	IL-4(pg/mL)	IL-10(pg/mL)	IL-33(pg/mL)	sST2(pg/mL)
Normal	10	111.66 $\pm$ 10.98	42.38 $\pm$ 3.69	130.00 $\pm$ 6.79	118.16 $\pm$ 7.09	52.26 $\pm$ 6.51	14.92 $\pm$ 2.63
Model	10	218.18 $\pm$ 20.74***	99.42 $\pm$ 6.98***	60.88 $\pm$ 5.53***	52.61 $\pm$ 3.09***	26.31 $\pm$ 4.09***	33.49 $\pm$ 3.95***
LD	10	182.19 $\pm$ 13.41#	87.16 $\pm$ 4.09#	70.09 $\pm$ 4.51#	65.79 $\pm$ 6.84#	32.16 $\pm$ 4.67#	26.41 $\pm$ 3.55#
HD	10	155.69 $\pm$ 11.09##,&	65.71 $\pm$ 4.51##,&	88.19 $\pm$ 5.19##,&	80.95 $\pm$ 7.09##,&	42.18 $\pm$ 4.87##,&	19.09 $\pm$ 4.15##,&
PD	10	131.95 $\pm$ 7.51###,&&,@	50.79 $\pm$ 5.16###,&&,@	109.51 $\pm$ 6.98###,&&,@	106.58 $\pm$ 8.91###,&&,@	50.14 $\pm$ 3.81###,&&,@	15.59 $\pm$ 1.65###,&&,@
F		96.416	219.941	248.584	171.415	51.168	54.984
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs. HD.

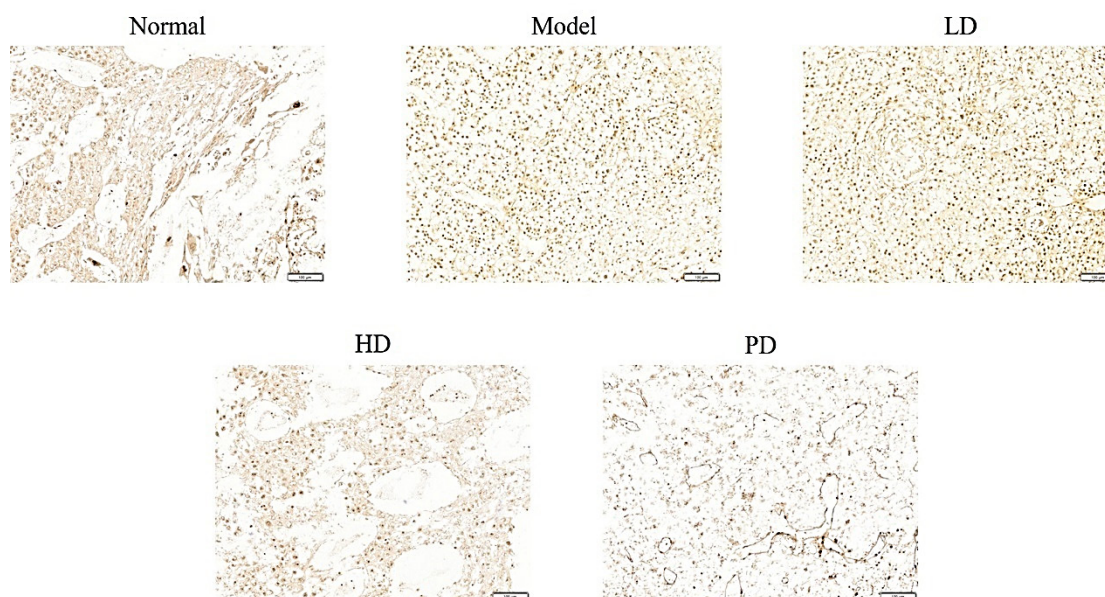


Figure 2: The TUNEL staining results of rat placental trophoblast cells in each group (100 $\times$).

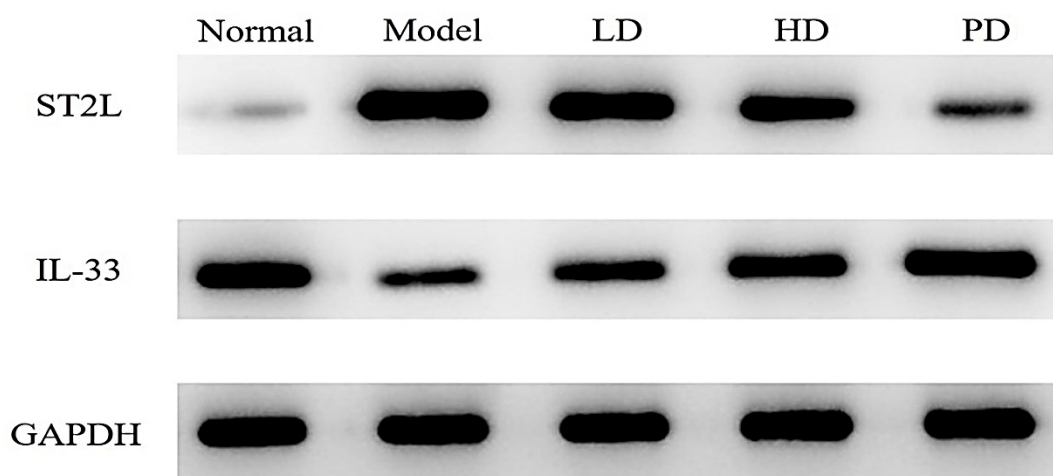


Figure 3: ST2L and IL-33 protein bands in placental tissue of rat in each group.

Table 7: The relative expression levels of IL-33 and ST2L proteins in rat placenta (Mean±SD).

	n	IL-33/GAPDH	ST2L/GAPDH
Normal	10	0.57±0.04	0.15±0.03
Model	10	0.12±0.03***	0.61±0.05***
LD	10	0.20±0.02#	0.55±0.05#
HD	10	0.37±0.03##,&	0.40±0.04##,&
PD	10	0.50±0.04###,&&,@	0.31±0.03###,&&,@
F		281.295	203.198
P		<0.001	<0.001

Note: ***: $p < 0.001$, vs. Normal; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, vs. Model; &: $p < 0.05$, &&: $p < 0.01$, vs. LD; @: $p < 0.05$, vs. HD.

decreased, while the levels of IL-4 and IL-10 increased, suggesting the regulatory role of baicalin in Th2-biased immune responses, promoting the recovery of immune imbalance, reducing inflammation and improving the pathological symptoms of PE.

IL-33, when released into the extracellular space, can initiate and sustain Th2-type immune responses. When translocated to the cell nucleus, IL-33 can inhibit pro-inflammatory signals.⁵ SST2 is considered a decoy receptor for IL-33 and high levels of sST2 can competitively bind to IL-33, promoting Th1-type immune responses and favoring Th1 immune bias. On the other hand, ST2L can stimulate IL-33 to induce the production of Th2 cytokines and immune factors.²⁵ Our study results showed that the Model group had elevated serum levels of sST2 and decreased levels of IL-33. In placental tissue, there was an increase in the relative expression level of ST2L protein and a decrease in the relative expression level of IL-33 protein. This suggests that the high levels of circulating sST2 may competitively bind to IL-33 protein, triggering an increase in the relative expression levels of ST2L protein, promoting immune cell imbalance and disrupting the equilibrium of pro-inflammatory/anti-inflammatory states, ultimately leading to the development and progression of PE. After treatment with low and high doses of baicalin, the

circulating level of sST2 was reduced and the IL-33 level was elevated. Considering the binding of ST2 with IL-33, its relative expression level was decreased. This suggests that baicalin may lower the circulating level of the decoy receptor sST2, increase the IL-33 level in circulation and placental tissue, promote Th2-biased immune responses and restore the balance between pro-inflammatory and anti-inflammatory factors. Consequently, baicalin improves placental tissue damage in PE rats.

CONCLUSION

In conclusion, baicalin can promote the recovery of the pro-inflammatory/anti-inflammatory imbalance in circulation, reduce hypertension and 24-hr urine protein, alleviate pathological changes in placental tissue, mitigate trophoblast apoptosis and suppress the occurrence of symptoms in pregnant and offspring rats. This study first proposes that the mechanism by which baicalin improves PE may be related to reducing sST2 levels, promoting IL-33 expression and thereby regulating immune imbalance. Also, there were some limits in our present study; we did not used sST2 inhibitor effects to baicalin's treatment effects and Lack of in vitro cell experiments for validation. We will continue to our future study which will improve these limits.

ABBREVIATIONS

PE: Preeclampsia; **TNF- α :** Tumor necrosis factor- α ; **IFN- γ :** Interferon- γ ; **IL 4:** Interleukin-4; **IL 10:** Interleukin 10; **IL 33:** Interleukin-33; **sST2:** Soluble growth stimulating expression gene 2 protein; **ELISA:** Enzyme-linked immunosorbent assay.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by Ethics Committee of Wuhu Second People's Hospital (No. 2022060923).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

To investigate the effect of baicalin on placental tissue injury in Preeclampsia (PE) rats and its possible mechanism. **Materials and Methods:** The caudal arterial blood pressure of Ed 15 and Ed 21 rats was detected. Bradford method was used to detect the 24 hr urinary protein content. The serum levels of Tumor Necrosis Factor- α (TNF- α), Interferon- γ (IFN- γ), Interleukin-4 (IL 4), Interleukin 10 (IL 10), Interleukin-33 (IL 33) and soluble growth stimulating expression gene 2 protein (sST2) were determined by Enzyme-Linked Immunosorbent Assay (ELISA). The number and survival rate of pups in each group were recorded. The placental tissue was separated and weighed. The histopathological changes of placenta were detected by HE staining. The apoptosis index of placental trophoblast cells was detected by TUNEL staining. The relative expression of IL-33 and transmembrane growth Stimulation expression gene 2 protein (ST2L) in placental tissues of rats were detected by Western blotting. **Results:** The looser arrangement, more obvious inflammatory infiltration, more severe apoptosis and higher serum TNF- α , IFN- γ and sST2 levels were observed. Meanwhile, the levels IL-4, IL-10 and IL-33 decreased, the relative expression level of IL-33 protein in placenta decreased, but the relative expression level of ST2L protein increased ($p < 0.05$). Compared with the model group, the tail artery blood pressure, the 24 hr urinary protein content decreased, the number and survival rate of offspring, the pathological changes of placental tissue structure and cell apoptosis of the 4 administration groups increased. The levels of TNF- α , IFN- γ , sST2, IL-4, IL-10 and IL-33 increased. The relative expression level of IL-33 protein in placenta increased, while that of ST2L protein decreased ($p < 0.05$). The improvement effect of baicalin on placental tissue injury was enhanced in LD, MD, HD and PD groups ($p < 0.05$). **Conclusion:** Baicalin can improve the placental tissue injury in PE rats, level of bait receptor ST2, promoting the binding of IL-33 and ST2L and regulating immune imbalance.

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Cite this article: Ding S, Li L, Jiang F, Mao Y. Effect of baicalin on Placental Tissue Damage in Preeclampsia Rats via the IL-33/ST2 Pathway. *Indian J of Pharmaceutical Education and Research.* 2027;61(1):231-9.