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# Changes in intestinal flora in preeclampsia rats and effects of probiotics on their inflammation and blood pressure

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**Abstract. – OBJECTIVE: The aim of this study was to investigate the changes in intestinal flora in preeclampsia rats and the effects of probiotics on their inflammation and blood pressure.**

**MATERIALS AND METHODS: A total of 40 Specific Pathogen Free (SPF) Wistar rats were randomly selected in this study. Abdominal operation was performed to reduce uterine blood perfusion, so as to establish the model of preeclampsia in rats. All rats were randomly divided into two groups, namely, observation group (treated with probiotics, n=20) and control group (not treated with probiotics, n=20). Subsequently, the changes in serum endotoxin level during intervention, the 24-h urinary 99mTc-diethylene triamine pentaacetic acid (DTPA) excretion rate, and intestinal flora colonization ability after intervention were compared between the two groups. The distribution of intestinal flora after intervention was recorded in the two groups. Meanwhile, vascular endothelial function and blood pressure following intervention were compared between the two groups as well. In addition, the changing trend of inflammatory cytokines during intervention in the two groups, and the correlation of colonization ability of intestinal flora with changes in systolic blood pressure and high-sensitivity C-reactive protein (hs-CRP) level in patients were analyzed.**

**RESULTS: At 3 days and 1 week after intervention, serum endotoxin level in the observation group was significantly lower than that in the control group at the same period ($p$<0.05). Following intervention, observation group exhibited remarkably higher excretion rate of 24-h urinary 99mTc-DTPA ($p$<0.05), stronger colonization ability of intestinal flora ($p$<0.05), higher levels of Bifidobacteria and Lactobacillus in intestinal flora ($p$<0.05), lower level of endothelin-1 (ET-1) ($p$<0.05) and higher level of nitric oxide (NO) ($p$<0.05) than the control group. In addition, the systolic blood pressure and diastolic blood pressure in the observation group were basically normal, which were both notably lower than those in the control group ($p$<0.05). At 3 days and 1 week after intervention, the levels of serum inflammatory cytokine hs-CRP in the observation group was markedly lower than that in the control group ($p$<0.05) at the same period. Furthermore, the colonization ability of intestinal flora was negatively associated with the changes in systolic blood pressure and hs-CRP level in patients ($p$<0.05).**

**CONCLUSIONS: Treating preeclampsia rats with probiotics can effectively reduce the level of serum endotoxin, improve the body's capacity to eliminate metabolites and the colonization ability of intestinal flora, maintain the stability of intestinal flora, enhance vascular endothelial function, and reduce blood pressure and the body's inflammatory responses.**

*Key Words:*
Preeclampsia, Rats, Intestinal flora, Probiotics, Inflammatory response, Changes in blood pressure.

## Introduction

Preeclampsia is a special disease during pregnancy, which can affect all organs and systems of the whole body. It may lead to poor prognosis and even clinical death of pregnant women and fetuses[1]. Recently, with the popularization of prenatal care, as well as clinical diagnosis and treatment methods, the incidence rates of preeclampsia have been greatly reduced. However, preeclampsia remains a high-risk factor severely influencing the prognosis of pregnant women and fetuses[2]. Li et al[3] have revealed that over 100,000 pregnant

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women worldwide annually die of preeclampsia or eclampsia. Therefore, much more attention should be paid to the diagnosis and treatment of preeclampsia.

Due to diverse physiological changes of the mother during pregnancy, great physiological and immune functional changes will happen in the intestinal flora[4]. Intestinal flora may be dysfunctional, thus resulting in maternal maladjustment, fetal development restriction, spontaneous abortion, as well as pregnancy-induced hypertension[5]. Sun et al[6] have indicated that the changes in intestinal flora are related to the occurrence and development of preeclampsia to some extent. In this study, intervention with probiotics was carried out for preeclampsia rats. Meanwhile, its effects on changes in the intestinal flora, inflammatory cytokines, and blood pressure were analyzed. Our findings aimed to better investigate the correlation between preeclampsia and changes in the intestinal flora of pregnant women.

## Materials and Methods

### General Data

A total of 40 Specific Pathogen Free (SPF) Wistar rats (provided by the Laboratory Animal Center of our hospital, No.: SCXK2018-0011) with a body mass of (250±15) g were randomly selected in this study. This research was approved by the Ethics Committee of Linyi Central Hospital. All laboratory rats were adaptively fed in the SPF Laboratory Animal Center of our hospital for 1 week. During the process, natural lighting and normal circadian rhythm were guaranteed, and the rats were given free access to food and water. Meanwhile, the temperature of the feeding center was kept at 24°C, with a humidity of 40-50%. Female rats and the sexually mature male rats were cooped up at a ratio of 2:1-3:1 overnight, and the vaginal secretions of female rats were taken regularly for examination. If sperms were found, the pregnancy would start and be observed. Using the random number table method, all rats were assigned into two groups, namely, observation group (treated with probiotics, n=20) and control group (not treated with probiotics, n=20).

### Preeclampsia Rat Modeling Method

The rats were given abdominal operation to reduce uterine blood perfusion on the $14^{th}$ day after pregnancy. Specifically, the rats received general anesthesia induction with isoflurane on the $14^{th}$ day after pregnancy. 3% pentobarbital sodium (4 mL/kg) was injected into the abdominal cavity. Next, the abdominal cavity was cut along the midline of the lower abdomen to fully expose the distal abdominal aorta and uterine and ovarian arteries. Then, titanium clips were applied to the bifurcation of the proximal iliac artery at the distal abdominal aorta to reduce uterine blood supply. The resulting placental ischemia and anoxia changes in rats might lead to pregnancy-induced hypertension. Thereafter, the abdominal cavity was closed, and invasive carotid catheterization was conducted at 9:00 a.m. On the $14^{th}$ day after pregnancy, continuous blood pressure was measured by connecting transducers. Blood pressure was measured again on the $19^{th}$ day after pregnancy. The elevation of arterial pressure by more than 30% indicated successful modeling.

### Preparation of Gavage Probiotic Solution

Probiotic powder (provided by Guangdong HuanKai Microbial Co., Ltd., Batch No.: 201802037, Guangzhou, China) freeze-dried in a refrigerator at -80°C was taken and activated for later use. At 1 day before gavage, the activated third-generation probiotic culture solution was centrifuged at 4500 r/min for 15 min. The solution was then taken out. 0.9% of normal saline was utilized for cleaning and re-suspension twice to form the probiotic suspension with the number of viable probiotics in paper cups $\geq 10^8$ cfu/mL. Thereafter, the solution was stored in the refrigerator at 4°C for subsequent application. In the observation group, 1 mL/100 g probiotic suspension was given to rats according to their body weight, and blood pressure was measured on the $14^{th}$ day after pregnancy. Following continuous intervention for 1 week, the rats were killed by neck dissection.

### Observation Indicators

The changes in serum endotoxin level during intervention, the 24-h urinary 99mTc-diethylene triamine pentaacetic acid (DTPA) excretion rate, and intestinal flora colonization ability after intervention were compared between the two groups. Next, the distribution of intestinal flora after intervention was recorded in the two groups. Vascular endothelial function and blood pressure following intervention were compared between the two groups as well. In addition, the changing trend of inflammatory cytokines during intervention, and the correlation of colonization ability of intestinal flora with changes in the systolic blood pressure and high-sensitivity C-reactive protein (hs-CRP) level in patients were analyzed.

### Evaluation Standards

Endotoxins in intestinal flora were examined using azo color development method, with reference value <0.1 EU/mL. Subsequently, the colonization ability of intestinal flora was evaluated based on the Bifidobacterium/Enterobacterium (B/E) level, with normal value >1. The distribution of intestinal flora was determined according to the distribution of *Bifidobacteria*, *Escherichia coli*, *Lactobacillus* and *Enterococcus*. Later, intestinal mucosa permeability was detected by radiation chromatography, and 24-h urinary excretion of macromolecular substance 99mTc-DTPA was calculated. Examined indicators related to vascular endothelial function included endothelin-1 (ET-1, normal value = 43.5 ng/L-58.4 ng/L, nitric oxide (NO, normal value = 13.8 μmol/L-34.6 μmol/L), and the inflammatory cytokine hs-CRP (normal value ≤10 mg/L) were also detected.

### Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was adopted for statistical analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x}$±s). The comparison of the mean and rate between two groups was conducted *via* *t*-test and $\chi^2$-test, respectively. $p<0.05$ was considered statistically significant.

## Results

### Changes in the Serum Endotoxin Level During Intervention in the two Groups

Before and at 3 days and 1 week after intervention, serum endotoxin level in the observation group was (1.31±0.30) EU/mL, (0.56±0.13) EU/mL, and (0.06±0.01) EU/mL, respectively.

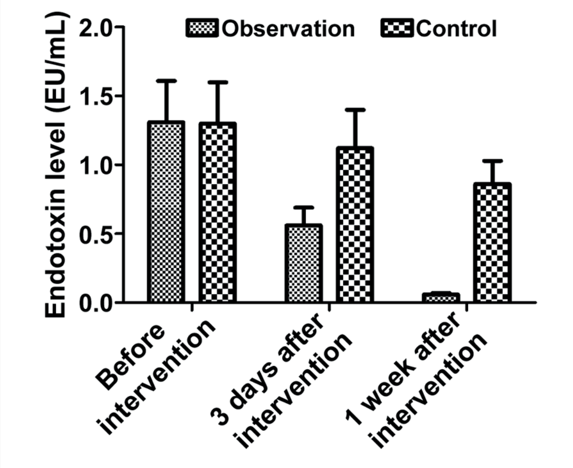


**Figure 1.** Changes in serum endotoxin level during intervention in the two groups.

Meanwhile, serum endotoxin level in the control group was (1.30±0.30) EU/mL, (1.12±0.28) EU/mL, and (0.86±0.17) EU/mL, respectively. The results showed that at 3 days and 1 week after intervention, the serum level of endotoxin in observation group was notably lower than that in control group at the same period ($t$=11.473 and 29.711, $p$=0.000) (Figure 1).

### Comparison of 24-h Urinary 99mTc-DTPA Excretion Rate and Intestinal Flora Colonization Ability Between the two Groups

Following intervention, the 24-h urinary 99mTc-DTPA excretion rate ($p<0.05$) was remarkably higher and the intestinal flora colonization ability ($p<0.05$) was markedly stronger in the observation group than those in the control group (Table I).

### Distribution of Intestinal Flora After Intervention in the two Groups

Observation group exhibited significantly higher levels of *Bifidobacteria* and *Lactobacillus*

**Table I.** Comparison of the 24-h urinary 99mTc-DTPA excretion rate and the colonization ability of intestinal flora after intervention between the two groups ($\bar{x} \pm s$).

| | 24-h urinary 99mTc-DTPA excretion rate (%) | Colonization ability of intestinal flora |
|---|---|---|
| Observation group | 12.1 ± 1.5 | 1.3 ± 0.2 |
| Control group | 9.1 ± 0.7 | 0.8 ± 0.1 |
| *t* | 11.462 | 14.142 |
| *p* | 0.000 | 0.000 |

**Table II.** Distribution of intestinal flora after intervention in the two groups (LgN/g, $\bar{x} \pm s$).

| | *Bifidobacteria* | *Lactobacillus* | *Escherichia coli* | *Enterococcus* |
|---|---|---|---|---|
| Observation group | 9.3 ± 0.9 | 8.1 ± 0.8 | 9.1 ± 0.8 | 7.3 ± 0.6 |
| Control group | 7.6 ± 0.5 | 6.9 ± 0.6 | 9.0 ± 0.8 | 7.3 ± 0.5 |
| *t* | 10.443 | 7.589 | 0.559 | 0.000 |
| *p* | 0.000 | 0.000 | 0.578 | 1.000 |

in intestinal flora than the control group ($p<0.05$). However, no evident differences were observed in the levels of *Escherichia coli* and *Enterococcus* between the two groups ($p>0.05$) (Table II).

### Comparison of Vascular Endothelial Function Between the two Groups

After intervention, ET-1 level in the observation group was remarkably lower than that in the control group ($p<0.05$). However, NO level in observation group was higher than control group ($p<0.05$) (Table III).

### Comparison of Blood Pressure After Intervention Between the two Groups

Systolic blood pressure and diastolic blood pressure were basically normal after intervention in observation group, which were both markedly lower than those in the control group ($p<0.05$) (Table IV).

### Changing Trend of Inflammatory Cytokines During Intervention in the two Groups

Before and at 3 days and 1 week after intervention, hs-CRP in observation group was (23.3±1.8) mg/L, (10.3±0.2) mg/L, and (6.8 0.2) mg/L, respectively. However, hs-CRP in the control group was (23.4±1.8) mg/L, (18.5±1.3) mg/L, and (13.3±0.8) mg/L, respectively. These results suggested that the level of serum inflammatory cytokine hs-CRP in observation group was prominently lower than that in control group at 3 days and 1 week after intervention ($t$=39.429 and 49.853, $p$=0.000) (Figure 2).

**Table III.** Comparison of vascular endothelial function after intervention between the two groups ($\bar{x} \pm s$).

| | ET-1 (ng/L) | NO (µmol/L) |
|---|---|---|
| Observation group | 37.4 ± 2.8 | 62.3 ± 3.9 |
| Control group | 62.6 ± 5.2 | 36.5 ± 1.8 |
| *t* | 26.986 | 37.988 |
| *p* | 0.000 | 0.000 |

**Table IV.** Comparison of blood pressure after intervention between the two groups (mmHg, $\bar{x} \pm s$).

| | Systolic blood pressure | Diastolic blood pressure |
|---|---|---|
| Observation group | 134.5 ± 3.9 | 80.6 ± 3.1 |
| Control group | 155.8 ± 5.1 | 98.8 ± 5.3 |
| *t* | 20.982 | 18.747 |
| *p* | 0.000 | 0.000 |

### Correlation Analyses of Intestinal Flora Colonization Ability with Changes in Systolic Blood Pressure and hs-CRP Level in Patients

Intestinal flora colonization ability was found negatively associated with changes in systolic blood pressure and hs-CRP level in patients ($p<0.05$) (Figure 3-4).

## Discussion

The species and quantity of intestinal flora in mammals are generally in a relatively balanced state. They may vary with changes in diet, en-

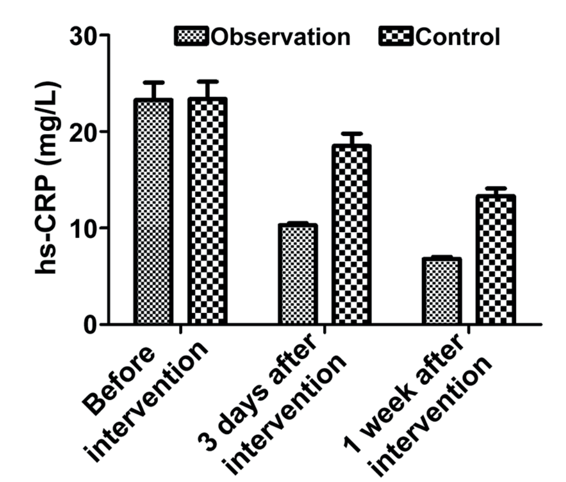


**Figure 2.** Changing trend of inflammatory cytokines during intervention in the two groups.

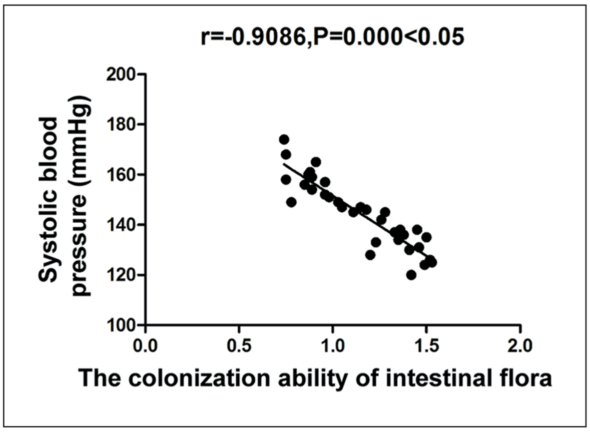


**Figure 3.** Correlation analysis of the colonization ability of intestinal flora with systolic blood pressure in patients.

docrine status, sanitary conditions, diseases, and age[7]. The composition of intestinal flora in pregnancy stage will change significantly. In particular, the diversity of maternal intestinal flora in the third trimester of pregnancy will be evidently reduced. This may be accompanied by increased actinomycetes and deformities, decreased levels of *Lactobacillus* and *Bifidobacteria*, as well as the appearance of physiological enteritis and dyspepsia[8]. Safari and Girard[9] have illustrated that the number of soft-walled bacteria increases significantly in the intestinal flora of the Asian population in the second and third trimester of pregnancy. However, pregnant women with preeclampsia have enhanced vascular tension and permeability, weakened body immunity and increased aseptic inflammatory responses due to changes in the neurotransmitter level in the autonomic nervous system. Ultimately, this may increase pathogenic

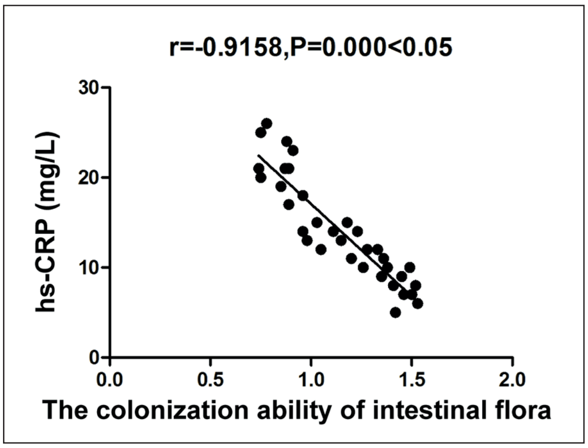


**Figure 4.** Correlation analysis of the colonization ability of intestinal flora with hs-CRP level.

microorganisms, such as *Clostridium perfringens* and *Brefeldin* in the intestinal tract, and distinctively reduce probiotics, especially *Coprococcus*, *Lactobacillus* and *Bifidobacteria*[10].

To better maintain the balance of intestinal flora in pregnant women with preeclampsia, probiotics were mainly used for intervention in this study. The changes in the serum endotoxin level between the two groups during intervention were compared. The results revealed that serum endotoxin level in the observation group was remarkably lower than that in the control group at the same period (at 3 days and 1 week after intervention). This suggested that probiotic therapy for preeclampsia rats could efficaciously reduce the level of serum endotoxin in rats. The 24-h urinary 99mTc-DTPA excretion rate and intestinal flora colonization ability were compared between the two groups as well. Our results discovered that following intervention, the 24-h urinary 99mTc-DTPA excretion rate was evidently higher, and the intestinal flora colonization ability was notably stronger in the observation group than those in the control group. It can be inferred that probiotic therapy for preeclampsia rats can more effectively facilitate kidney metabolism and enhance the colonization ability of intestinal flora. In the meantime, the distribution of intestinal flora after intervention was compared between the two groups. The results demonstrated that the observation group exhibited significantly higher levels of *Bifidobacteria* and *Lactobacillus* in the intestinal flora than control group. The above findings indicated that treating preeclampsia rats with probiotics successfully upregulated the level of probiotics in the intestinal tract and reduced the number of pathogenic bacteria. According to the comparison of the changes in vascular endothelial function and blood pressure after intervention between the two groups, it was found that ET-1 level in observation group was remarkably lower than that in the control group. However, NO level in observation group was significantly higher. Besides, systolic blood pressure and diastolic blood pressure in the observation group were basically normal, which were both overtly lower than those in the control group. It could be inferred that probiotic treatment for preeclampsia rats could reduce vascular endothelial cell damage and vascular permeability to a certain extent. This treatment method was also valuable for stabilizing blood pressure of preeclampsia rats. At last, the changes in the level of the inflammatory cytokine hs-CRP during intervention were analyzed in the two groups. The results revealed that the level of serum inflammatory cytokine hs-CRP in the observation group was significantly lower than that in the control group at the same period (at 3 days and 1 week after intervention). Furthermore, the colonization ability of intestinal flora was discovered through correlation analyses to be negatively correlated with the changes in systolic blood pressure and hs-CRP level in patients. The above findings suggested that treating preeclampsia rats with probiotics was able to effectively enhance the colonization ability of intestinal flora, and reduce blood pressure and the body's inflammatory responses in rats.

In this study, preeclampsia rats in observation group were intervened by probiotics to efficaciously modulate the integrity of intestinal barrier in rats[11]. This may also contribute to the maintenance of intestinal flora balance in preeclampsia rats, and effectively change the metabolic abnormalities caused by pregnancy and preeclampsia-induced hypertension[12], eventually slowing down the progression of preeclampsia[13]. The reason may be that the applied probiotics promote the proteolysis and fermentation capability of probiotic strains, such as *Lactobacillus* and *Bifidobacteria*[14]. Ultimately, this can activate angiotensin-converting enzyme II to inhibit plasma activity and effectively lower blood pressure[15]. The potential phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signal transduction pathway of *Lactobacillus* is enhanced as well, which regulates vascular permeability[16] and improves vascular endothelial function[17]. Besides, utilized probiotics are also capable of decreasing the levels of pathogenic *Enterobacteriaceae* and *Enterococcus* in the intestinal tract of rats. This may further improve intestinal biological barrier capability[18], modulate Th1/Th2 cytokine level[19], reduce the body's inflammatory responses of preeclampsia, and enhance the immunity capability of the body[20].

## Conclusions

To sum up, the novelty of this study was that the application of probiotics for the treatment of preeclampsia rats can effectively reduce the level of serum endotoxin, improve the body's capacity to eliminate metabolites and the colonization ability of intestinal flora, maintain the stability of intestinal flora, enhance vascular endothelial function, and reduce blood pressure and the body's inflammatory responses.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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