

Research Article

Anti-Inflammatory Effects of Cerium Dioxide Nanoparticles on Peritonitis in Rats Induced by *Staphylococcus epidermidis* Infection

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Objective. To investigate the effects of cerium dioxide (CeO₂) nanoparticles on the inflammatory response of peritonitis rats induced by *Staphylococcus epidermidis* infection. **Methods.** Green tea polyphenol CeO₂ nanoparticles were synthesized and characterized by transmission microscopy, ultraviolet-visible spectroscopy, FT-IR, and powder diffractometer. 40 male adult SD rats were randomly divided into 4 groups ($n = 10$ each): a control group, a model group, a CeO₂ group, and a CeO₂ + model group. *Staphylococcus epidermidis* solution was injected intraperitoneally with 10⁷ CFU/ml of bacterial solution in the model group, while the control group was injected intraperitoneally with the same amount of normal saline, and the CeO₂ and CeO₂ + model groups were injected with 0.5 mg/kg CeO₂ nanoparticles through the tail vein for 2 h and then injected with saline or bacterial solution for 2 h, respectively. After 0 h, 3 h, 12 h, 24 h, and 48 h of model construction, rats were sacrificed, and serum and peritoneal lavage fluid were collected. The total number of leukocytes and the percentage of each type of leukocytes in the peritoneal lavage fluid were determined. Enzyme-linked immunosorbent assay (ELISA) was used to detect the level of inflammatory factor TNF- α in serum and peritoneal lavage fluid, and myeloperoxidase (MPO) activity in peritoneal tissue was also measured. In addition, real-time fluorescence quantitative PCR (RT-PCR) was used to measure the expression of TLR2 and TLR4 in peritoneal tissue, and western blotting was used to detect the expression of TLR2, TLR4, and the activation of NF- κ B signaling pathways as well. **Results.** The CeO₂ has an average size of 37 \pm 3 nm with binding activity to proteins, phenolic compounds, and alkaloids. After counting the white blood cells in the peritoneal lavage fluid, it was found that the total number of white blood cells and the percentage of neutrophils in the model group were significantly increased (both $P < 0.05$), and CeO₂ treatment significantly reversed the above changes (both $P < 0.05$). The ELISA results showed that compared with the control group, the TNF- α in the peritoneal lavage fluid and serum of the model group increased in a time-dependent manner (all $P < 0.05$); however, there was no significant change in the CeO₂ group ($P > 0.05$); at the same time in the CeO₂ + model group, the TNF- α content was significantly reduced (all $P < 0.05$). Detection of MPO activity in peritoneal tissue revealed that MPO activity was significantly increased under peritonitis (all $P < 0.05$), and CeO₂ treatment could mitigate that increase (all $P < 0.05$). RT-PCR results showed that compared with the control group, the expression of TLR2 and TLR4 mRNA levels in the peritoneum of the model group were increased in a time-dependent manner (all $P < 0.05$), and there was no significant change in the CeO₂ group ($P > 0.05$); however, TLR2 and TLR4 mRNA levels were significantly reduced in the CeO₂ + model group (all $P < 0.05$). Western blotting test was performed on the peritoneal tissue collected after 48 h of the model establishment. Compared with the control group, the levels of TLR2, TLR4, p-NF- κ B, and p-I κ B α protein in the model group were significantly increased (all $P < 0.05$), while CeO₂ group showed no significant changes ($P > 0.05$) and administration of CeO₂ before model construction can significantly reverse the above protein activation (all $P < 0.05$). **Conclusion.** CeO₂ nanoparticles have anti-inflammatory effects in peritonitis caused by *Staphylococcus epidermidis* infection.

1. Background

Although the diagnosis and treatment of diseases have progressed with the development of medicine, the incidence of deaths associated with celiac infection and severe peritonitis remains high. Peritonitis can occur when infectious pathogens/microorganisms or their toxic metabolites are present in the peritoneum [1]. If the infection is aggravated due to improper handling and other reasons, it often develops into diffuse peritonitis and organ dysfunction. Peritoneal dialysis is also an important cause of peritonitis. It is often referred to as peritoneal dialysis-related peritonitis and among which, *Staphylococcus epidermidis* infection is the most common type [2]. It is reported that most of the organ damage in peritonitis is caused by the development of systemic inflammatory response syndrome (SIRS). In the occurrence and development of SIRS, there is an increase in inflammatory cytokines, tissue reactive oxygen levels, and an increase of monocytes, macrophages, and neutrophils infiltration in different organs; all of these combined together can cause tissue damage [3].

Cerium is a lanthanide series metal that can undergo a redox cycle from Ce^{4+} (oxidation) to Ce^{3+} (reduction) state and often exists in the form of cerium dioxide (CeO_2). Previous studies have confirmed that CeO_2 nanoparticles can act as superoxide dismutase mimics [4] and catalase mimics [5] and can reduce pulmonary hypertension [6] and reduce hypoxia-induced oxidative stress [7] as well as inhibiting cyclophosphamide-induced apoptosis [8]. In the study of peritonitis, CeO_2 nanoparticles have been reported to reduce peritonitis-related SIRS [2], and other studies have found that CeO_2 nanoparticles can improve diaphragmatic dysfunction caused by peritonitis [9]. In addition, green tea polyphenols, as the main ingredients of antioxidants, are well known for their antioxidant, anti-inflammatory, anticancer, anticardiolipin, antimicrobial, antihyperglycemic, and antiobesity properties [10].

In this study, a rat model of *Staphylococcus epidermidis*-induced peritonitis was established to investigate the effects of biosynthetic CeO_2 nanoparticles on the inflammation of this model.

2. Materials and Methods

2.1. Plant Collection and Extraction. Green tea leaves were collected and dried to make a powder. 10 g of dried tea powder was added to a 250 ml beaker, and 100 ml of distilled water was added for continuous boiling for 10 min. The boiling solution was filtered through filter paper, and the filtrate was collected and stored at 4°C.

2.2. Synthesis of Polyphenol CeO_2 Nanoparticles. 20 ml of the above filtrate was added to the conical flask, heated to boiling, and when it was cooled to 50°C, ammonium cerium nitrate solution (1 g in 50 ml of distilled water) was mixed with the filtrate and stirred until the solution turned to milky yellow. The solution was then transferred to a clean centrifuge tube and was centrifuged (12,000 rpm/min) at 4°C for

10 minutes. The supernatant was then discarded, and the milky solid precipitate at the bottom of the centrifuge tube was transferred to a quartz crucible and placed in a muffle furnace (calcined at 600°C for 1 h). A yellow-white solid was obtained after 1 h, and CeO_2 nanoparticles were obtained by further grinding [11].

2.3. Characterization of Synthetic CeO_2 Nanoparticles. The synthesized CeO_2 nanoparticles were observed using a transmission microscope (JEM-2010, JEOL, Japan) and characterized and identified by UV-visible spectroscopy (125 spectrophotometers, PerkinElmer, Germany), and an FT-IR instrument (JASCO, USA) was used for infrared spectrum analysis. In addition, a powder diffractometer (D8, BRUKER-AXS, Germany) was used to analyze the XRD pattern.

2.4. Experimental Design and Establishment of the Rat Model of Peritonitis. 40 male Sprague Dawley rats (10-week-old, 250–300 g) were purchased from Changzhou Cavins Experimental Animal Co., Ltd. The rats were housed in a cage with an indoor temperature of 20°C–24°C and alternating light and dark cycles (12 h each) within 2 weeks of the experiment. The rats had free access to standard rodent food and water. This study was reviewed and approved by the ethics committee of our hospital, and all surgical procedures were performed in accordance with the guidelines. The rats were randomly divided into 4 groups ($n = 10$ each): the control group, the model group, the CeO_2 group, and the CeO_2 + model group. For the preparation of *Staphylococcus epidermidis* solution, please refer to the related literature [12]. Specifically, *Staphylococcus epidermidis* strain (ATCC 35984) was inoculated into 300 ml of conventional culture medium and shaken at 250 rpm/min at 37 °C overnight. The next day, it was centrifuged at 10,000 rpm/min for 5 minutes at 4°C. The supernatant was discarded and the pellet was collected. The pellet was then suspended in 50 ml of prechilled normal saline, centrifuged, and washed at 5000 rpm/min for 5 minutes, which was repeated three times. The final pellet was then resuspended in prechilled normal saline to prepare *Staphylococcus epidermidis* solution with a concentration of 10^7 CFU/ml. The solution was stored at 4°C for subsequent experiments. Rats in the control group were injected intraperitoneally with saline; model group was injected intraperitoneally with *Staphylococcus epidermidis* solution 10^7 CFU/ml; CeO_2 group was injected with 0.5 mg/kg CeO_2 nanoparticles (dissolved in 200 ml sterilized water) via tail vein 2 h before intraperitoneal saline injection; CeO_2 + model group was intraperitoneally injected with 0.5 mg/kg CeO_2 nanoparticles as well (dissolved in 200 ml of sterilized water) via tail vein 2 h before model construction and *Staphylococcus epidermidis* solution injection. Rats were then sacrificed at 0 h, 3 h, 12 h, 24 h, and 48 h after model construction and whole blood was centrifuged to obtain serum, peritoneal lavage fluid, and peritoneal tissue for the subsequent experiment.

2.5. Counting the Number of White Blood Cells in the Peritoneal Lavage Fluid. The peritoneal lavage fluid was centrifuged at 1,000 rpm for 10 min at room temperature, and the supernatant was collected and stored at -80°C for subsequent detection of inflammatory factors. The pellet was collected and resuspended in $200\ \mu\text{l}$ PBS containing 0.6 mM EDTA. Density gradient centrifugation and Wright-Giemsa staining were used to count the white blood cells.

2.6. Detection of Inflammatory Factors in Serum and Peritoneal Lavage Fluid by ELISA. The concentration of TNF- α in peritoneal lavage fluid and serum was determined using the ELISA kit (R&D, USA) according to the manufacturer's instructions.

2.7. Detection of Myeloperoxidase Levels in Peritoneal Tissue. 3 ml of 0.05 M K-phosphate buffer (pH = 6.0) containing 0.5% cetyltrimethylammonium bromide was added to a total of 200 mg peritoneal sample and sonicated for 20 s to prepare a homogenate. Homogenate was then freeze-thawed for 3 times and centrifuged at 10,000 rpm for 15 min. Then, 0.1 ml supernatant was added and mixed to a 2.9 ml 0.05 M K-phosphate buffer (pH = 6.0) containing 0.53 mM o-diphenylamine and 0.15 mM H_2O_2 . A spectrophotometer was used to measure the solution absorbance at 460 nm every 15 s continuously for 5 min. The MPO activity is expressed in U (units)/mg protein, and 1 U is defined as the degradation of $1\ \mu\text{M}$ peroxide/min at 25°C [13].

2.8. TLR2 and TLR4 mRNA Levels in Peritoneal Tissue Detected by Real-Time Fluorescence Quantitative PCR (RT-PCR). Total RNA was extracted from peritoneal tissue using an RNA extraction kit (DP420, Tiangen, China), and the concentration and purity were determined by a spectrophotometer. $1\ \mu\text{g}$ of RNA was reverse transcribed into cDNA, followed by SYBR Green real-time fluorescence quantitative amplification. The primer sequences are shown in Table 1. β -Actin was used as the internal reference gene, and the relative expression of the target gene was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method.

2.9. Expression of TLR2, TLR4, and NF- κ B Signaling Pathway Proteins in Peritoneal Tissue Determined by Western Blotting. After washing the tissue with prechilled PBS, a certain amount of RIPA strong lysate and protease inhibitor (both purchased from Biyuntian, China) was added, and the tissue was completely lysed under ultrasound. The total protein from the peritoneal tissue was then extracted and the BCA protein concentration determination kit (Biyuntian, China) was used for protein quantification before protein was heated to denature. A certain amount of protein was added to the SDS-PAGE gel wells for electrophoresis before transferring to membranes. The membranes were then blocked, and primary antibodies were added. After incubation, membrane washing, secondary antibody incubation, membrane washing, and other steps, ECL luminescent solution was dropped on the membrane for fluorescence

development. TLR2, TLR4, NF- κ B p65, phosphorylated NF- κ B p65 (p-NF- κ B p65), I κ B α , p-I κ B α , and β -actin were purchased from Cell Signaling Technology, USA.

2.10. Statistical Analysis. Measurement data in this study were expressed in the form of mean \pm standard deviation. *t* test was used for comparisons between the two groups and ANOVA analysis was used for comparison within groups under univariate conditions. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Transmission Electron Microscopy and UV-Visible Spectrum Analysis of CeO₂ Nanoparticles. The transmission electron microscope image of CeO₂ nanoparticles is shown in Figure 1(a). The UV-visible spectrum analysis of polyphenol CeO₂ nanoparticle mixtures was performed every 30 minutes. As shown in Figure 1(b), the surface plasmon resonance peak observed at 267 nm indicates the formation of CeO₂ nanoparticles.

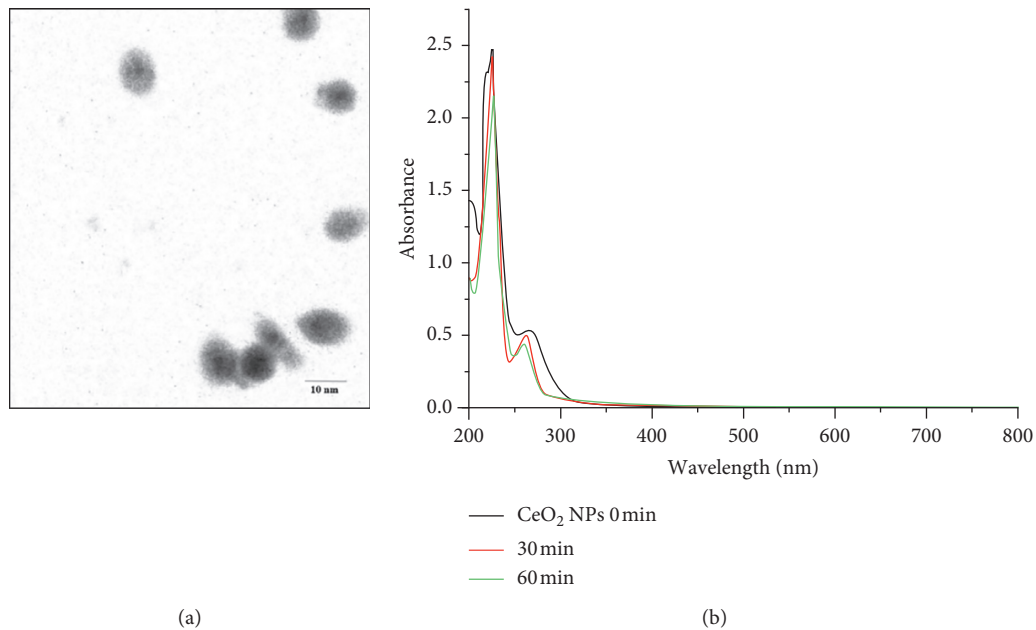
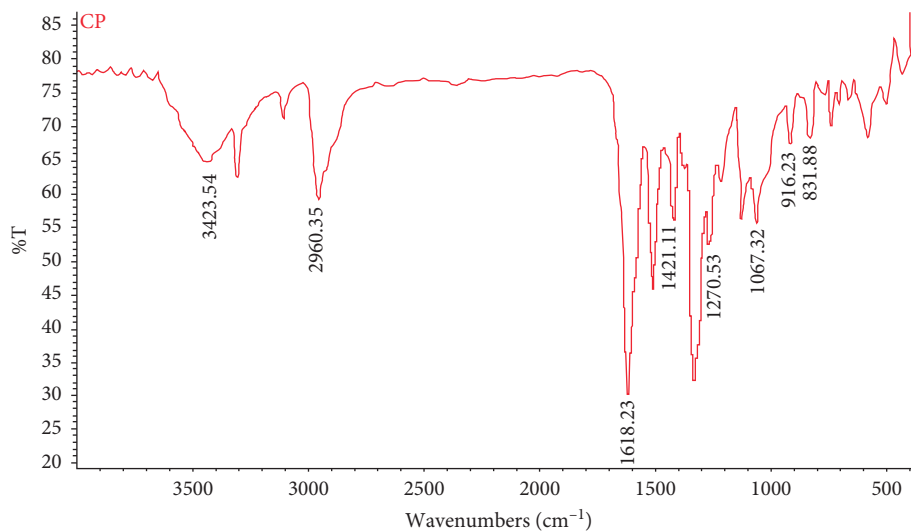
3.2. FT-IR Analysis of CeO₂ Nanoparticles. As shown in Figure 2, the functional group wavelengths of polyphenol CeO₂ nanoparticles were from $400\ \text{cm}^{-1}$ to $4000\ \text{cm}^{-1}$. And at $3423\ \text{cm}^{-1}$ (COOH-OH), $2960\ \text{cm}^{-1}$ (CH), $1618\ \text{cm}^{-1}$ (amide II band or N-diamine), $1421\ \text{cm}^{-1}$ (COH), $1270\ \text{cm}^{-1}$ and $1067\ \text{cm}^{-1}$ (thio and vinyl), $916\ \text{cm}^{-1}$, and $831\ \text{cm}^{-1}$ (organic sulfur compounds), CeO₂ reached the peak, confirming the existence of multiple functional groups in tea polyphenols. FT-IR analysis of CeO₂ nanoparticles synthesized by polyphenols showed that CeO₂ nanoparticles can be combined with compounds such as proteins, phenolic compounds, and alkaloids. These chemicals play a capping reagent role in the synthesis of nanoparticles.

3.3. XRD Analysis of CeO₂ Nanoparticles. As shown in Figure 3, the intensity of XRD was recorded from 20° to 80° at two angles. All the peaks obtained from the diffraction pattern were well characterized by CeO₂ nanoparticles. The intensities of the narrow-tip diffraction peaks appearing at 2θ were 28.02, 33.08, 47.01, 57.45, 59.37, 69.39, and 78.43, corresponding to the lattice planes 111, 200, 220, 311, 222, 400, and 331 of CeO₂ nanoparticles. The above results showed that CeO₂ nanoparticles are spherical. The Debye-Scherrer equation was adopted to calculate the average size of the nanoparticles that was $37 \pm 3\ \text{nm}$ [13].

3.4. Number of Leukocytes in Peritoneal Lavage Fluid of Rats in Each Group. As shown in Figure 4(a), compared with the control group, the total number of white blood cells in the peritoneal lavage fluid of the model group was significantly increased (all $P < 0.05$) and was time-dependent (all $P < 0.05$); however, there was no significant difference in the CeO₂ group ($P > 0.05$), whereas compared with the model group as shown in Figure 4(b), 4(c), and 4(d), pretreatment with CeO₂ significantly reduced the total number of white

TABLE 1: Primer sequence.

| Primer | Forward (5'-3') | Reverse (5'-3') |
|----------------|-----------------------|----------------------|
| TLR2 | TGGAGGTCTCCAGGTCAAATC | TTCCGCTGGACTCCAATGTC |
| TLR4 | CCGCTCTGGCATCATCTTCA | TGGGTTTTAGGCGCAGAGTT |
| β -actin | CGCGAGTACAACCTTCTTGC | CGTCATCCATGGCGAACTGG |

FIGURE 1: Transmission electron microscopy and UV-visible spectrum analysis of CeO₂ nanoparticles.FIGURE 2: FT-IR analysis of CeO₂ nanoparticles.

blood cells (all $P < 0.05$). The changes in the percentage of neutrophils in each group were consistent with the changes in the total number of white blood cells, while the percentage changes in macrophages and lymphocytes were the opposite.

3.5. Expression of Inflammatory Factors in Peritoneal Lavage Fluid and Serum of Rats in Each Group.

As shown in Table 2,

compared with the control group, TNF- α levels in the peritoneal lavage fluid of the model group rats were increased in a time-dependent manner during the period of 3–48 h after *Staphylococcus epidermidis* stimulation ($P < 0.05$); however, compared with the control group, there was no significant difference in CeO₂ group ($P > 0.05$). On the other hand, compared with the model group, the administration of CeO₂ before infection significantly reduced

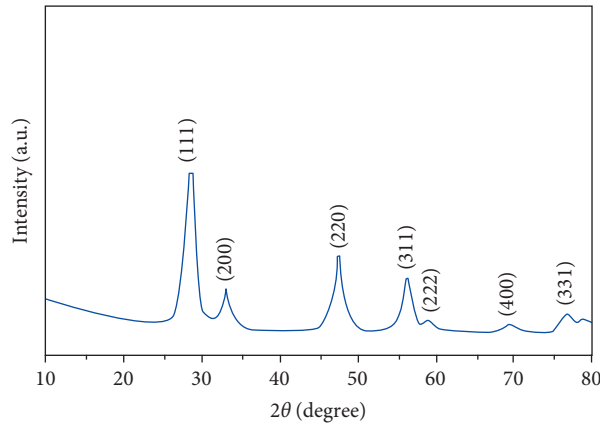


FIGURE 3: XRD analysis of CeO₂ nanoparticles.

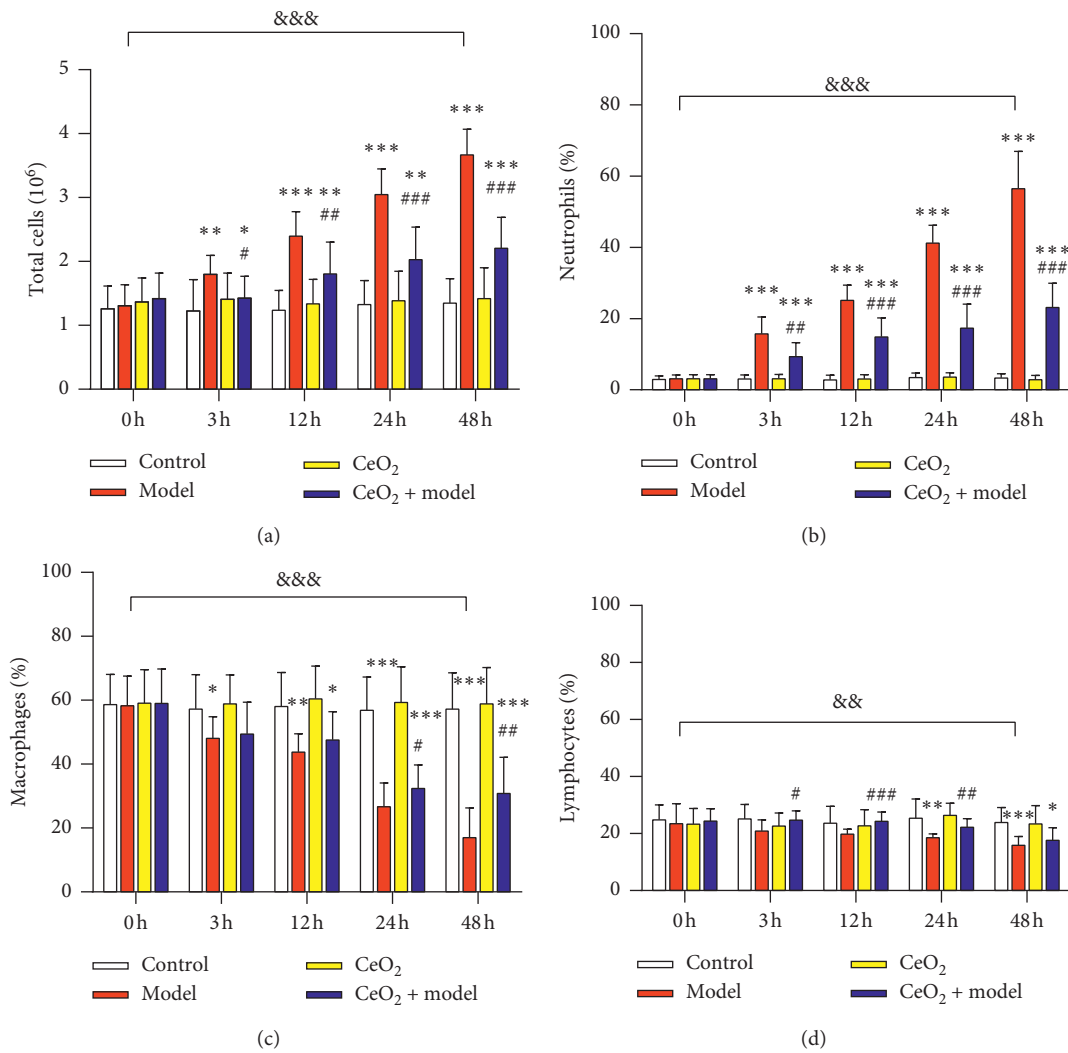


FIGURE 4: Differential counting of leukocytes in the peritoneal lavage fluid of each group of rats: (a) white blood cell count, (b) percentage of neutrophils, (c) percentage of macrophages, and (d) percentage of lymphocytes. Compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; differences within the model group, && $P < 0.01$, &&& $P < 0.001$; compared with the model group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$.

TABLE 2: TNF- α levels in peritoneal lavage fluid of each group of rats (pg/ml).

| Groups | 0 h | 3 h | 12 h | 24 h | 48 h |
|--------------|------------------|------------------------|-------------------------|------------------------|------------------------|
| Control | 47.78 \pm 3.17 | 44.02 \pm 2.81 | 49.47 \pm 5.11 | 43.01 \pm 8.21 | 46.28 \pm 4.01 |
| Model | 42.84 \pm 6.38 | 130.28 \pm 10.34**&& | 289.33 \pm 19.23***&& | 331.90 \pm 22.24**&& | 377.84 \pm 25.34**&& |
| CeO2 | 48.29 \pm 2.34 | 41.02 \pm 7.81 | 49.65 \pm 2.33 | 47.01 \pm 4.82 | 49.52 \pm 3.11 |
| CeO2 + model | 49.88 \pm 2.88 | 77.12 \pm 5.34*### | 87.32 \pm 11.34*### | 100.24 \pm 12.54*### | 130.23 \pm 14.23*### |

Note. Compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; differences within the model group, && $P < 0.01$; compared with the model group, ## $P < 0.01$, ### $P < 0.001$.

TNF- α levels ($P < 0.05$). Table 3 shows that the serum results were consistent with the results obtained from peritoneal lavage fluid.

3.6. MPO Activity in Peritoneal Tissue of Rats in Each Group. As shown in Figure 5, compared with the control group, the MPO activity in the peritoneal tissue of the model group was significantly higher (all $P < 0.05$) and was time-dependent (all $P < 0.001$). No significant difference in the CeO2 group ($P > 0.05$) was observed compared to the control group. However, compared with the model group, the administration of CeO2 before infection significantly reduced MPO activity (all $P < 0.05$).

3.7. mRNA Levels of TLR2 and TLR4 in the Peritoneal Tissue of Rats in Each Group. As shown in Figure 6, RT-PCR results showed that compared with the control group, mRNA expression levels of TLR2 and TLR4 in the peritoneal tissue of the model group rats increased gradually after infection with *Staphylococcus epidermidis* (all $P < 0.05$), but there was no significant difference in CeO2 group ($P > 0.05$). However, compared with the model group, mRNA expression levels of TLR2 (Figure 6(a)) and TLR4 (Figure 6(b)) in the CeO2 + model group were reduced, and the difference was significant (all $P < 0.05$). ELISA and RT-PCR results suggested that with the establishment of the model, the inflammatory response gradually increased with time. Therefore, in the western blotting experiment, 48 h was chosen as the final detection time point.

3.8. Expression of TLR2, TLR4, and NF- κ B Signaling Pathway Proteins in the Peritoneal Tissue of Each Group of Rats. As shown in Figure 7, western blot results showed that compared with the control group, the levels of TLR2, TLR4, p-NF- κ B, and p-I κ B α protein in the model group were significantly increased (all $P < 0.05$). However, no significant changes were found in the CeO2 group ($P > 0.05$). Compared with the model group, the levels of TLR2, TLR4, p-NF- κ B, and p-I κ B α protein in the CeO2 + model group were significantly reduced (all $P < 0.05$).

4. Discussion

Peritonitis is a major complication of peritoneal dialysis, a technique used to treat end-stage renal disease [14]. Among others, *Staphylococcus epidermidis* is an important pathogenic bacterium in the peritonitis with extremely strong

drug resistance, so it is very difficult to completely remove them. In the occurrence of peritonitis, pathogenic bacteria can enter the abdominal cavity through the catheter or catheter outlet, activating peritoneal mesothelial cells and macrophages, which in turn releases a series of products including pro-inflammatory mediators to cause an inflammatory response [15].

Nanotechnology has shown great potential in improving the quality of daily life and has been widely used in the fields of new material production, industry, consumer goods, and medical applications. Studies have found the positive effects of CeO2 nanoparticles in antioxidant therapy, neuroprotection, radiation protection, and eye protection [16]. Meldrum et al. have confirmed that CeO2 nanoparticles can affect the occurrence of allergic airway diseases by regulating the pulmonary inflammation caused by exhaust particles and allergens [17]. With the in-depth study of nanomedicine, the organic combination of nanoparticles and bioactive drugs has gradually become a research hotspot. Green tea polyphenols, as the main component of antioxidants, have anti-inflammatory properties [10]. In view of the anti-inflammatory advantages of CeO2 nanoparticles, and no related reports on the effects of CeO2 nanoparticles on peritonitis, in this study, CeO2 nanoparticles were first biosynthesized based on the literature, and the nanoparticles were further characterized and identified by transmission microscope observation, ultraviolet-visible spectrum, infrared spectrum, and XRD pattern and then peritonitis induced by *Staphylococcus epidermidis* in rats was established to explore the role of biosynthesized CeO2 nanoparticles on the inflammatory response to peritonitis.

The NF- κ B pathway is a typical proinflammatory signaling pathway, which plays an important role in the pathogenesis of inflammatory reactive diseases. NF- κ B can be activated through the activation of Toll-like receptors (TLRs) to upregulate proinflammatory genes including cytokines, chemokines, and adhesion molecules [18]. TLRs are single, type I transmembrane glycoprotein that plays an important defensive role by activating immune cell responses in both innate and adaptive immune responses [19]. When the ligand binds to TLRs, it can trigger the myeloid differentiation primary response protein 88-TNF α receptor-related factor 6-transforming growth factor β -activated kinase 1-I κ B kinase protein phosphorylation, leading to activation of NF- κ B light chain enhancer, thereby promoting the production of inflammatory factors and inducing an inflammatory response. In this study, by detecting the mRNA and protein expression levels of TLR2 and TLR4, we found that the expression of the two receptors increased to

TABLE 3: TNF- α levels in serum of each group of rats (pg/ml).

| Groups | 0h | 3h | 12h | 24h | 48h |
|--------------------------|-----------------|--|--|---|--|
| Control | 7.74 \pm 1.26 | 7.90 \pm 2.36 | 8.14 \pm 2.77 | 9.04 \pm 1.17 | 8.22 \pm 1.77 |
| Model | 7.10 \pm 1.87 | 15.24 \pm 3.44 ^{&&} | 23.22 \pm 5.34 ^{**&&} | 27.99 \pm 7.14 ^{**&&&} | 30.22 \pm 5.88 ^{***&&&} |
| CeO ₂ | 6.95 \pm 2.70 | 6.15 \pm 2.66 | 7.23 \pm 3.01 | 7.29 \pm 1.99 | 6.90 \pm 1.73 |
| CeO ₂ + model | 7.04 \pm 2.01 | 10.12 \pm 4.24 ^{***###} | 14.23 \pm 4.99 ^{***###} | 16.20 \pm 4.33 ^{***###} | 18.11 \pm 5.39 ^{***###} |

Note. Compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; differences within the model group, &&& $P < 0.001$; compared with the model group, ## $P < 0.01$, ### $P < 0.001$.

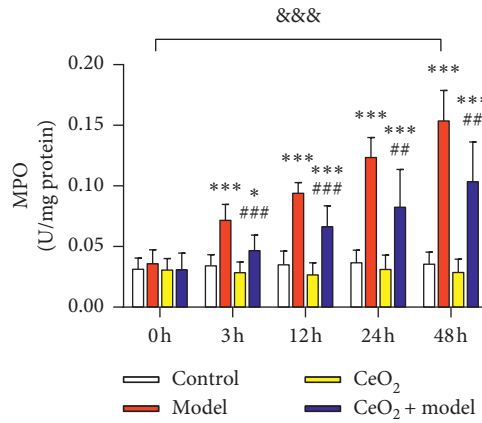


FIGURE 5: MPO activity changes in peritoneal tissue of rats in each group. Compared with the control group, * $P < 0.05$, *** $P < 0.001$; differences within the model group, &&& $P < 0.001$; compared with the model group, ## $P < 0.01$, ### $P < 0.001$.

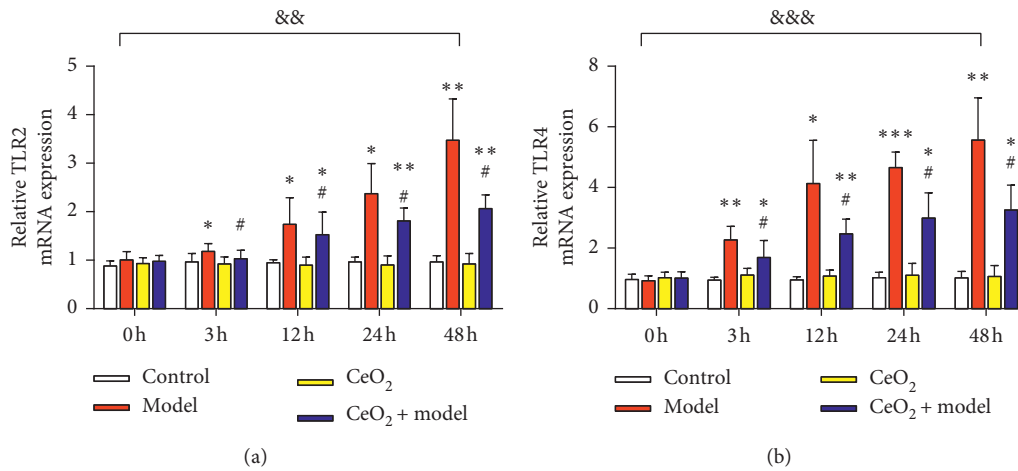


FIGURE 6: mRNA expression changes in the peritoneal tissue of each group of rats: (a) changes in mRNA expression levels of TLR2; (b) changes in mRNA expression levels of TLR4. Compared with the control group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; differences within the model group, &&& $P < 0.01$, &&&& $P < 0.01$; compared with the model group, # $P < 0.05$.

varying degrees under the condition of peritonitis. By further testing the downstream $I\kappa B\alpha$ and NF- κB signaling pathways, we found their phosphorylation levels were increased, suggesting that this signaling pathway was activated. In addition, the number of white blood cells in the peritoneal lavage fluid and the percentage of various types of white blood cells, the activity of myeloperoxidase, and the protein expression level of the inflammatory factor TNF- α were also measured in this study. The white blood cell count and the proportion of neutrophils can directly reveal the

inflammatory status of the cells; MPO is a heme protein synthesized during the myeloid differentiation process. As a special component in neutrophils, its concentration is related to the severity of inflammation; TNF- α is a proinflammatory factor secreted by monocytes and is known for its strong inflammatory effect. This study tested the above indicators to confirm that the peritonitis status can increase the total number of white blood cells and the proportion of neutrophils, enhance MPO activity, and induce a significant increase in TNF- α levels in peritoneal lavage fluid and

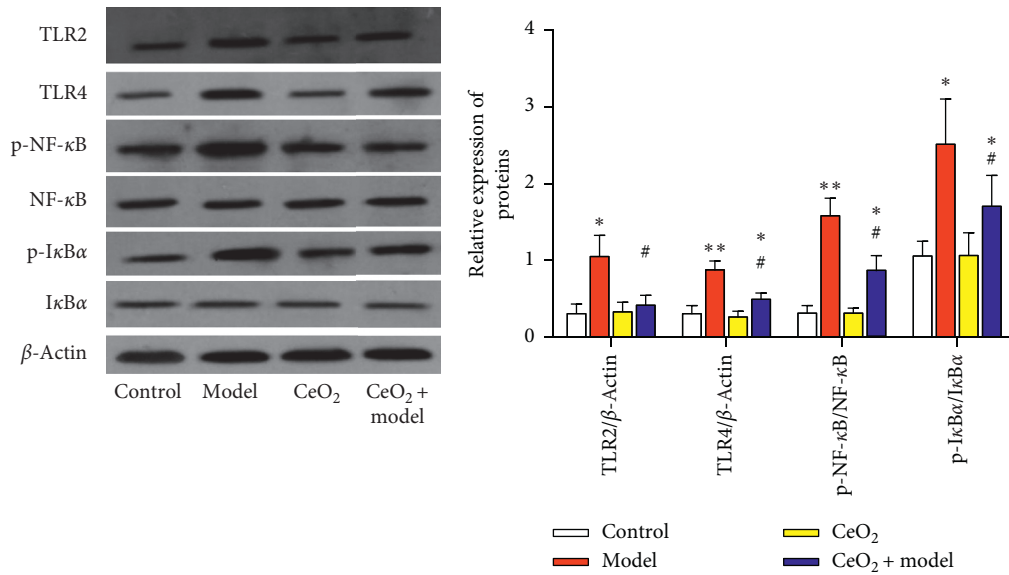


FIGURE 7: Changes of protein expression in peritoneal tissue of rats in each group. Compared with the control group, * $P < 0.05$, ** $P < 0.01$; compared with the model group, # $P < 0.05$.

serum. And CeO₂ nanoparticles can reduce the levels of the above indicators to different degrees and thus exert the anti-inflammatory effects.

In summary, this study found that in the occurrence and development of peritonitis caused by *Staphylococcus epidermidis*, CeO₂ inhibited the transcription of TLR2 and TLR4 genes, inhibited the activation of the NF- κ B signaling pathway, and further inhibited its downstream inflammatory factor TNF- α and reduced the number of white blood cells, thereby mitigating the occurrence of inflammatory reactions. Therefore, it is suggested that CeO₂ plays a protective role in *Staphylococcus epidermidis*-induced peritonitis and provides new ideas for the treatment of this disease.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

- [1] J. O. A. M. van Baal, K. K. Van de Vijver, R. Nieuwland et al., "The histophysiology and pathophysiology of the peritoneum," *Tissue and Cell*, vol. 49, no. 1, pp. 95–105, 2017.
- [2] N. D. P. K. Manne, R. Arvapalli, N. Nepal et al., "Therapeutic potential of cerium oxide nanoparticles for the treatment of peritonitis induced by polymicrobial insult in sprague-dawley rats," *Critical Care Medicine*, vol. 43, no. 11, pp. e477–e489, 2015.
- [3] M. Bosmann and P. A. Ward, "The inflammatory response in sepsis," *Trends in Immunology*, vol. 34, no. 3, pp. 129–136, 2013.
- [4] V. Baldim, N. F. Bedioui, and I. MargailleBerret, "The enzyme-like catalytic activity of cerium oxide nanoparticles and its dependency on Ce³⁺ surface area concentration," *Nanoscale*, vol. 10, no. 15, pp. 6971–6980, 2018.
- [5] B.-H. Chen and B. Stephen Inbaraj, "Various physicochemical and surface properties controlling the bioactivity of cerium oxide nanoparticles," *Critical Reviews in Biotechnology*, vol. 38, no. 7, pp. 1003–1024, 2018.
- [6] M. B. Kolli, N. D. P. K. Manne, R. Para et al., "Cerium oxide nanoparticles attenuate monocrotaline induced right ventricular hypertrophy following pulmonary arterial hypertension," *Biomaterials*, vol. 35, no. 37, pp. 9951–9962, 2014.
- [7] A. Arya, N. Sethy, S. Singh, and M. Das, "Cerium oxide nanoparticles protect rodent lungs from hypobaric hypoxia-induced oxidative stress and inflammation," *International Journal of Nanomedicine*, vol. 8, no. 8, pp. 4507–4520, 2013.
- [8] M. Hamzeh, S. J. Hosseinimehr, A. Karimpour, A. R. Mohammadi, and F. Talebpour Amiri, "Cerium oxide nanoparticles protect cyclophosphamide-induced testicular toxicity in mice," *International Journal of Preventive Medicine*, vol. 10, no. 1, p. 5, 2019.
- [9] S. Asano, R. Arvapalli, N. Manne et al., "Cerium oxide nanoparticle treatment ameliorates peritonitis-induced diaphragm dysfunction," *International Journal of Nanomedicine*, vol. 10, pp. 6215–6225, 2015.
- [10] M. Prasanth, B. Sivamaruthi, C. Chaiyasut, and T. Tencomnao, "A review of the role of green tea (camellia sinensis) in anti-photoaging, stress resistance, neuroprotection, and autophagy," *Nutrients*, vol. 11, no. 2, p. 474, 2019.
- [11] X.-Li Tencomnao, Li Sun, Yi Fan et al., "Effects of curcumin on expressions of toll-like receptor 2 and 6 in rat peritoneum during acute peritonitis," *Chinese Journal of Nephrology, Dialysis and Transplantation*, vol. 21, pp. 244–248, 2012.

- [12] G. Chen and Y. Xu, "Biosynthesis of cerium oxide nanoparticles and their effect on lipopolysaccharide (LPS) induced sepsis mortality and associated hepatic dysfunction in male sprague dawley rats," *Materials Science and Engineering: C*, vol. 83, pp. 148–153, 2018.
- [13] Y. Xia and J. L. Zweier, "Measurement of myeloperoxidase in leukocyte-containing tissues," *Analytical Biochemistry*, vol. 245, no. 1, pp. 93–96, 1997.
- [14] R. Mehrotra, O. Devuyst, S. J. Davies, and D. W. Johnson, "The current state of peritoneal dialysis," *Journal of the American Society of Nephrology*, vol. 27, no. 11, pp. 3238–3252, 2016.
- [15] C. C. Johnson and S. J. Jenkins, "The biology of serous cavity macrophages," *Cellular Immunology*, vol. 330, pp. 126–135, 2018.
- [16] S. Hussain, F. Al-Nsour, A. B. Rice et al., "Cerium dioxide nanoparticles do not modulate the lipopolysaccharide-induced inflammatory response in human monocytes," *International Journal of Nanomedicine*, vol. 7, pp. 1387–1397, 2012.
- [17] K. Meldrum, S. Robertson, I. Römer et al., "Diesel exhaust particle and dust mite induced airway inflammation is modified by cerium dioxide nanoparticles," *Environmental Toxicology and Pharmacology*, vol. 73, p. 103273, 2020.
- [18] S. Mitchell, J. Vargas, and A. Hoffmann, "Signaling via the NF κ B system," *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, vol. 8, no. 3, pp. 227–241, 2016.
- [19] A. M. Hosseini, J. Majidi, B. Baradaran et al., "Toll-like receptors in the pathogenesis of autoimmune diseases," *Advanced Pharmaceutical Bulletin*, vol. 5, no. 1, p. p605, 2015.