

Combined Action of Cerium Oxide Nanoparticles and Ciprofloxacin Against *Staphylococcus aureus*: Driving Mechanisms

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ABSTRACT

The rise of antimicrobial resistance has intensified the need for alternative strategies to enhance antibiotic efficacy. Cerium oxide nanoparticles (CeO₂ NPs) have shown promise as antimicrobial agents; however, their potential in combination with antibiotics remains underexplored. To elucidate the mechanisms underlying the synergistic antibacterial effects of CeO₂ NPs with ciprofloxacin against *Staphylococcus aureus*, we conducted detailed mechanistic investigations. CeO₂ NPs were synthesized using a non-isothermal precipitation method and characterized via scanning electron microscopy (SEM), X-ray diffraction (XRD), and dynamic light scattering (DLS). Antibacterial activity was evaluated through minimum inhibitory concentration (MIC) assays. Mechanistic studies included bacterial growth curve analysis, ROS quantification, and SEM imaging of treated bacterial cells. CeO₂ NPs exhibited an additive effect with ciprofloxacin, reducing its MIC by threefold. Mechanistic investigations revealed that CeO₂ NPs disrupted bacterial membrane integrity, induced oxidative stress through ROS overproduction, and significantly prolonged bacterial doubling time. SEM analysis confirmed extensive structural damage, including cell wall disruption and cytoplasmic leakage. These findings highlight the dual antibacterial role of CeO₂ NPs: direct bacterial damage and enhancement of ciprofloxacin's efficacy. CeO₂ NPs hold potential as antibiotic adjuvants to combat resistant Gram-positive infections. Further studies should focus on optimizing nanoparticle formulations, evaluating *in vivo* efficacy, and assessing their clinical translation to address the global challenge of antimicrobial resistance.

Keywords: Antibacterial activity, Cerium oxide nanoparticles (CeO₂ NPs), Ciprofloxacin, Synergy

1. INTRODUCTION

The synergistic activity of different antibacterial agents presents a novel avenue for combating bacterial infections. Gram-positive bacteria, in particular, present unique challenges due to their ability to adapt and evade conventional treatments, leading to both community-acquired and nosocomial infections [1]. Although gram-negative bacteria

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possess a dual-membrane architecture comprising a thinner cell wall and lipopolysaccharide-rich outer membrane that contributes to their antibiotic resistance, gram-positive bacteria lack this outer membrane, while constituting a cell wall and thick peptidoglycan layer. However, gram-positive bacteria exhibit significant mechanisms of antibacterial resistance, including modifications to target sites and efflux systems [2]. The Gram-positive bacteria presents a challenge to clinicians treating community-acquired and nosocomial-acquired infections [3]. In recent year, ciprofloxacin resistance in *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), has emerged rapidly. Resistance rates increased dramatically from 7% to 83% within a few years of ciprofloxacin introduction [4–7]. Ciprofloxacin resistance is often associated with resistance to other antibiotics, limiting treatment options [7]. The rapid emergence of resistance suggests that ciprofloxacin has limited usefulness against *S. aureus*, especially MRSA [5,6]. To address this challenge, researchers are exploring ciprofloxacin hybrids as potential solutions to overcome drug resistance [8]. However, resistance rates vary geographically, with some regions reporting lower prevalence [9], emphasizing the need for ongoing surveillance and development of new treatment strategies.

A promising strategy entails augmenting the effectiveness of antibiotics via combination therapies. These strategies not only enhance antibacterial efficacy but also assist in overcoming resistance mechanisms by utilizing the synergistic effects of various agents [10]. In recent years, nanomaterials have emerged as a novel strategy [11]. Recent advancements in combination therapy for bacterial infections have used antibiotic combinations and nanoparticles conjugated with antibiotics NPs can infiltrate the cellular membranes of pathogenic organisms and impede critical molecular pathways, demonstrating distinctive antibacterial actions [12]. These compounds work by inhibiting antibiotic resistance, either by deactivating enzymes or blocking efflux pump systems [13,14]. When used with suitable pharmaceuticals, NPs exhibit synergy and may aid in mitigating the overall risk of emerging bacterial resistance. During the last decade the exploitation of NPs in combination with antibiotics are indicated as favorable approach with the intent of using lower doses of antibiotics in antimicrobial therapy which is especially important to combat the global threat of antimicrobial resistance [15,16].

Recent studies have highlighted the potential of CeO₂ NP to act either alone or in conjunction with traditional antibiotics [17]. This is supported by the CeO₂ NP exhibit antibacterial potential against both gram-positive and gram-negative bacteria [18]. Controversial issues exist in the literature that CeO₂ NPs could act as antibiotic adjuvants to increase the effectiveness of antimicrobials [18]. Another study revealed that the introducing of CeO₂ NPs alongside ciprofloxacin can significantly reduce the antibacterial activity for biofilms however the activity in cellular level was not investigated [19].

In our study we evaluated CeO₂ NPs emerged as potential nanostructure capable of enhancing the activity of conventional antibiotics. We investigated the additive effects of CeO₂ NPs in combination with ciprofloxacin through a series of biochemical assays to determine their potential in combating *S. aureus* infections. By elucidating the fundamental

interactions underlying this synergy, our findings contribute to the expanding evidence for the use of CeO₂ NPs in antimicrobial therapies. This approach offers a promising strategy to lower antibiotic concentrations while maintaining efficacy, addressing the increasing challenge of antibiotic resistance in Gram-positive bacteria.

2. MATERIALS AND METHODS

2.1. Preparation and Characterization of CeO₂ Nanoparticles

CeO₂ NPs were produced by non-isothermal precipitation method as described in our previous study [20]. Morphology of CeO₂ NP was examined by SEM (Carl Zeiss 300VP) at 200 kV. XRD analysis of CeO₂ NP crystal structure was conducted at 40 mA, 40 kV, CuK α radiation $\lambda = 1.5405 \text{ \AA}$, room temperature (Empyrean, Malvern PANalytical). DLS analysis (Litesizer™ 500, Anton Paar) was used to measure the hydrodynamic size, polydispersity index (PDI), and zeta potential value of CeO₂ NP dispersion (0.25 mg/mL in HEPES buffer solution (25 mM, pH 7.2)). The total cerium content of the synthesized CeO₂ nanoparticles was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). Briefly, 200 $\mu\text{g/mL}$ CeO₂ nanoparticles were dispersed and digested in 1 N nitric acid (HNO₃) for 24h. to ensure complete dissolution of the nanoparticles. The resulting solutions were analyzed by ICP-OES, and the cerium concentration was quantified using external calibration [20].

2.2. *In vitro* Antibacterial Activity Investigation of CeO₂ NP, Ciprofloxacin, and CeO₂-Ciprofloxacin Treatments

The antibacterial activity of CeO₂ NP alone and combined with ciprofloxacin was determined by using *Staphylococcus aureus* (*S. aureus*, ATCC 29213) as the Gram-positive model organism. We examined the interaction between CeO₂ NPs and ciprofloxacin at different concentrations using a checkerboard assay to assess synergy. Following the identification of MIC and synergistic dosing, the corresponding concentrations were employed to evaluate the impacts on bacterial growth rate and doubling time. The interaction mechanism between bacteria cells and CeO₂ NP was resolved by considering the hydrophobicity index (HI) of CeO₂ NP and bacterial cells. The bacterial cells disruption mechanism was evaluated by bacterial membrane permeability and protein leakage examinations. Furthermore, we performed the production of reactive oxygen species (ROS) (e.g., hydrogen peroxide, hydroxyl radicals, superoxide anions) which known as inflicting oxidative damage on cellular components, including lipids, proteins, and nucleic acids, ultimately resulting in cell death.

Antibacterial synergism: A checkerboard assay was conducted to evaluate the MIC values of concurrent CeO₂ NP and ciprofloxacin treatments, as well as their individual treatments.

The investigations were performed by employing Alamar Blue assay. CeO₂ NP was prepared in HEPES buffer (pH 7.2, 25 mM) and diluted in TSB to final concentrations of 0, 25, 50, 100, 200, 250, 400, and 600 µg/mL. Ciprofloxacin was diluted similarly to concentrations of 0, 0.05, 0.1, 0.15, 0.3, 0.6, and 1.2 µg/mL [21]. 33.3 µL of CeO₂ NP and ciprofloxacin solutions were added horizontally and vertically to each well, followed by 33.3 µL of *S. aureus* culture (3 x 10⁵ CFU/mL). The plate was incubated at 37°C for 24 h. Afterward, 10% (v/v) Alamar Blue was added, and the plate was incubated at 37°C in the dark and fluorescence measurements were carried out at excitation and emission values of 540 and 590 nm when a color change occurs in the control group. The combination of CeO₂ NP with ciprofloxacin was evaluated by calculating the fractional inhibitory concentration (FIC) index using the following equation (2.1) [22]:

$$FIC\ index = FIC_A + FIC_B = \frac{A}{MIC_A} + \frac{B}{MIC_B} \quad (2.1)$$

where MIC_A and MIC_B are the individual MIC of CeO₂ NP and ciprofloxacin while A and B are the MIC value in simultaneous CeO₂ NP and ciprofloxacin treatments in a single well. The FIC values were utilized to investigate the interaction between CeO₂ NP and ciprofloxacin. The results were interpreted as synergism if FIC index ≤ 0.5, additive if 0.5 < FIC index < 2, indifference if 2 < FIC index < 4, and antagonism if FIC index > 4.

The HI of CeO₂ NP was measured following previously reported protocols [23–25]. The HI values of CeO₂ NP (400 µg/mL) and CeO₂ NP + ciprofloxacin (200 µg/mL + 0.05 µg/mL) were evaluated by first dispersing the samples in water and subsequently incubating them in TSB medium for 24 h under experimental conditions. After incubation, the suspensions were centrifuged, and the resulting pellets were resuspended in water before hydrophobicity analysis. After that 1 mL of toluene was added to the suspension and vortexed, the mixture was then left for 30 minutes until the phase separation. The optical density of the aqueous phase was measured after phase separation, and the HI was calculated using the equation given below [24]:

$$HI\% = \frac{A_i - A_f}{A_i} \times 100 \quad (2.2)$$

where HI is the hydrophobicity index, A_i is the initial optical density and A_f is the final optical density. The same investigation was performed for *S. aureus* bacteria in TSB. In the HI investigations, the dosing obtained from the checkerboard assay were evaluated to understand whether the hydrophobic and hydrophilic interactions take place between the bacterial cells and treatment materials [26].

Exploring the growth rate, doubling time and regrowth of bacterial cell cultures: *S. aureus* culture was adjusted to 3 x 10⁵ CFU/mL and CeO₂ NP (400 µg/mL), ciprofloxacin (0.15 µg/mL), and CeO₂ NP + ciprofloxacin (200 µg/mL + 0.05 µg/mL) treated and non-treated bacterial culture were taken for investigations. The OD₆₀₀ measurements were performed at 37°C for 18 hours by 30 min intervals. The effect of CeO₂ NP, ciprofloxacin, and CeO₂

NP- ciprofloxacin on the growth rate (μ) and doubling time (g) of bacterial cells were investigated by applying the following equations (2.3) and (2.4) [27]:

$$\mu = \frac{\ln OD_2 - \ln OD_1}{t_2 - t_1} \quad (2.3)$$

$$a = \frac{\ln 2}{\mu} \quad (2.4)$$

where μ is the growth rate constant, g is the doubling time, OD_1 and OD_2 are the optical density values corresponding to the starting and ending of the exponential growth phase, and t_1 and t_2 are the time points of the recordings. Furthermore, the regrowth of the treated bacterial cells was investigated by resuspending the cells in fresh culture media to ascertain whether the detrimental effects of the treatment could be reversed by the bacterial cells. The bacteria cells were precipitated by centrifugation at 2500 rpm, and cells were resuspended in fresh TSB media and incubated for additional 24 hours and Alamar Blue cell viability assay was performed.

2.3. Mechanistic Evaluation of Antibacterial Activity in Cellular Level

Bacterial cell membrane permeability and cellular component leakage: The propidium iodide (PI) staining was performed to observe *S. aureus* membrane disruption due to the treatments [28]. *S. aureus* was cultured at a concentration of 10^8 CFU/mL in TSB and subsequently suspended in PBS. CeO_2 NP (400 μ g/mL), ciprofloxacin (0.15 μ g/mL), and CeO_2 + ciprofloxacin (200 μ g/mL + 0.05 μ g/mL) solutions were combined with the *S. aureus* suspension in each well of a 96-well plate at a 1:1 ratio and incubated for 24 h at 37°C. Subsequently, 50 μ L of 15 μ M PI solution was introduced to each well, and the plate was incubated for an additional 15 min in the dark on an orbital shaker. Following incubation, the fluorescence intensity of samples was quantified using a Synergy HTX multimode plate reader at excitation/emission wavelengths of 540/600 nm. The treatment suspensions without bacteria were prepared as a blank to eliminate fluorescence interference during calculations. The protein concentration due to the cellular component leakages were analyzed using BCA assays [29]. For this assay, the treated bacteria pellets were collected by centrifugation at 5000 rpm for 5 minutes. Supernatants were then used in a BCA assay as per manufacturer's protocol for quantification of protein leakage by performing absorbance measurement at 562 nm.

Intracellular ROS production: The change in intracellular ROS production of *S. aureus* was performed after the exposure of the bacterial cells by CeO_2 NP (400 μ g/mL), ciprofloxacin (0.15 μ g/mL), and CeO_2 NP-ciprofloxacin (200 μ g/mL + 0.05 μ g/mL) via fluorogenic dye 2,7- dichlorofluorescein diacetate (DCFH-DA) [26]. In this investigation prior to treatments the bacteria culture was treated with the DCFH-DA dye and the excess

dye was removed by centrifugation. The treatment conditions were kept the same as in the cellular leakage investigations. At the end of incubation time, fluorescence measurement was performed (excitation/emission 485/528 nm). *S. aureus* culture treated with 0.33 mM hydrogen peroxide solution was used as positive control.

Experiments were performed in six replicates and data are represented as mean $\pm$ standard deviation (SD). Statistical analyses were performed in GraphPad Prism software (Version 8.2) using ordinary one-way ANOVA followed by Tukey's multiple comparison test with the level of significance (α) set to 0.05. The degree of significance was * $p < 0.03$, ** $p < 0.002$, *** $p < 0.0002$, **** $p < 0.0001$ for all statistical tests.

3. RESULTS AND DISCUSSION

3.1. Morphological and Structural Properties of Nanoparticles

CeO₂ NP with a diameter of approximately 20 nm was observed (Figure 1A). The XRD pattern of the CeO₂ NP shows the characteristic peaks of the cubic fluorite structure where the peaks were observed at $2\theta = 28.5, 33.1, 47.5, 56.2$, and 59.0° (Figure 1B). The analysis confirmed the cubic lattice of pure CeO₂ and good agreement with JCPDS DATA (898436). No identifiable diffraction peaks Ce₂O₃ crystalline was observed and indicates a single-phase nature [30] (Figure 1A). ICP-OES analysis revealed a cerium concentration of 13.23 mg/L in the digested CeO₂ NP suspension, corresponding to approximately 67 μ g of detectable cerium per mg of nanoparticles under the applied digestion conditions. The hydrodynamic diameter and net surface charge of CeO₂ NP in physiological pH (7.2) were measured as 611.2 ± 17.6 nm and -8.06 ± 0.23 mV, respectively. In this study, CeO₂ NP were synthesized, yielding cubic structured nanoaggregates with close to net neutral surface charge values of -8.06 ± 0.23 mV which foreseen as the potential interaction with bacteria could be driven by hydrophobic/hydrophilic interactions different than the literature findings of electrostatic interactions [18]. The high HI value of CeO₂ decreased after being introduced in TSB led the adsorption from broth media, allowing interaction with hydrophilic *S. aureus* bacterial cells with a low HI value of 22.00 ± 3.58 . CeO₂ NP features and potential hydrophilic-hydrophilic interactions were confirmed by SEM images of treated *S. aureus* as shown in Figure S1 [31].

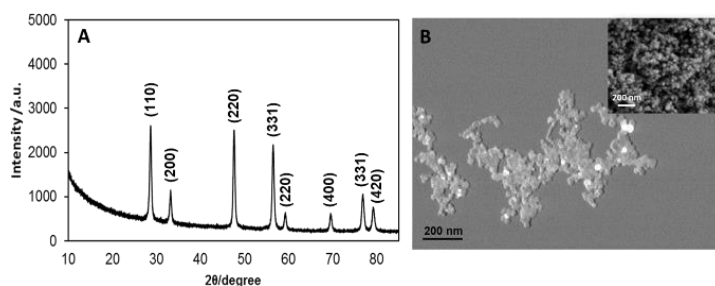


Figure 1. A) XRD pattern, and B) STEM image CeO₂ NP with an inset figure of SEM micrograph.

3.2. Additive Effects of CeO₂ NPs and Ciprofloxacin Against *S. aureus*

The checkerboard assay identified MIC values against *S. aureus* for CeO₂ (400 µg/mL) and ciprofloxacin (0.15 µg/mL) and in combination treatments 200 µg/mL CeO₂ and 0.05 µg/mL ciprofloxacin (Figure 2). In combination treatments inhibition was achieved with an additive effect (FIC = 0.83). The findings highlight that CeO₂ or ciprofloxacin reduces the other's MIC, almost 70% enabling lower antibiotic concentrations in treatment. Nevertheless, the literature presents inconclusive findings regarding the antibacterial efficacy of CeO₂ NPs. CeO₂ NPs have been found to enhance antibiotic efficacy against multidrug-resistant pathogens [32], but some studies found them as interfering the antibiotics activity [33]. CeO₂ alone has also been reported to increase bacterial outer membrane permeability [18] and exhibit antibacterial activity against ESKAPE pathogens [34].

In this study we evaluate the adjuvant activity of CeO₂ NP by comparing simultaneous administration with ciprofloxacin and individual administration, providing a comprehensive assessment of the combinatory antibacterial synergy mechanism. Results demonstrate the CeO₂ NPs promote the vulnerability of *S. aureus* bacteria to be exploited for antibacterial activity. The growth curve CeO₂ NP treated *S. aureus* showed a bacteriostatic effect whereas the antibacterial action prone to bactericidal effect when CeO₂ and ciprofloxacin were combined. (Figure S1). The growth rate decreased 3.3- fold with concurrent treatment, while the doubling time of *S. aureus* also showed a decline.

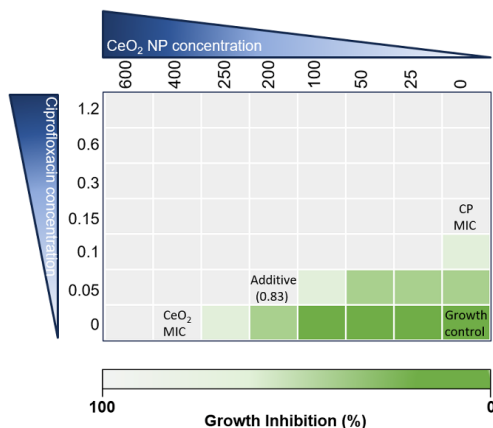


Figure 2. Heat plot of checkerboard assay of CeO₂ NP in combination with ciprofloxacin against *S. aureus*.

The HI index values for CeO₂ NP in water and after incubating in TSB was determined as $74.03 \pm 2.92\%$ and $8.75 \pm 0.99\%$ respectively. In water mixture of 200 $\mu\text{g/mL}$ CeO₂ NP and 0.05 $\mu\text{g/mL}$ ciprofloxacin yielded with HI value of $4.43 \pm 0.40\%$ accepted as in hydrophilic range, however the preincubation of mixture in TSB and resuspending in water yielded with sedimentation and HI values could not be determined. The HI value of *S. aureus* after incubating in TSB and resuspending in water was yielded with value of $22.00 \pm 3.5\%$. After treating *S. aureus* with 400 $\mu\text{g/mL}$ CeO₂ NP the value decreased to $3.97 \pm 0.002\%$ which means the hydrophobicity of the *S. aureus* bacterial cells was diminished due to the treatments with the CeO₂ NP. However, the treatment with 200 $\mu\text{g/mL}$ CeO₂ NP and 0.05 $\mu\text{g/mL}$ ciprofloxacin of *S. aureus* yielded with the sedimentation and the HI values could not be determined.

Inhibited bacterial growth rate, doubling time and bacterial regrowth: The effect of CeO₂ NP alone, ciprofloxacin alone, and CeO₂-ciprofloxacin combination on bacteria growth rate and doubling time was investigated by following the growth curves of *S. aureus* culture overnight (Figure S2). The growth curves were generated using data from kinetic OD measurements and the linear region of the exponential growth phase (Figure S3) was used to calculate growth rate followed by doubling time. The concentrations of CeO₂ NP (400 $\mu\text{g/mL}$) and ciprofloxacin (0.15 $\mu\text{g/mL}$) were determined from the checkerboard assay to see the effect of individual components and their combination (200 $\mu\text{g/mL}$ for CeO₂ and 0.05 $\mu\text{g/mL}$ for ciprofloxacin) as shown in Figure 3.

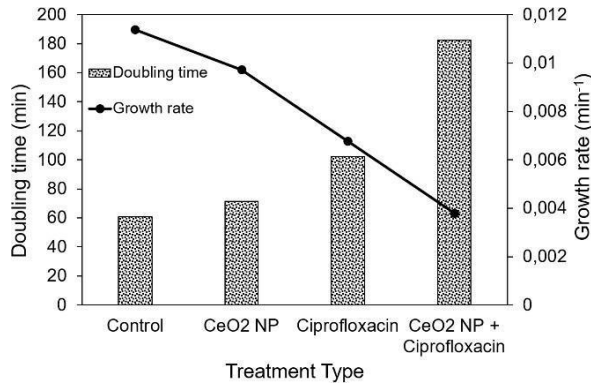


Figure 3. Doubling time and growth rate of *S. aureus* after treatment with CeO₂ NP, ciprofloxacin, and CeO₂ NP + ciprofloxacin combination.

To observe whether bacterial growth occurred after the regrowth experiment, the wells were subjected to fluorescent intensity measurements. The regrowth of *S. aureus* was statistically evaluated. The results presented in Figure 4 showed that the presence of CeO₂ NP or ciprofloxacin had a highly inhibitory effect on regrowth during antibacterial treatment. Therefore, metal oxide NPs have concentration-dependent antibacterial properties, leading to cellular destruction and prolonging the bacterial lag phase [21,22].

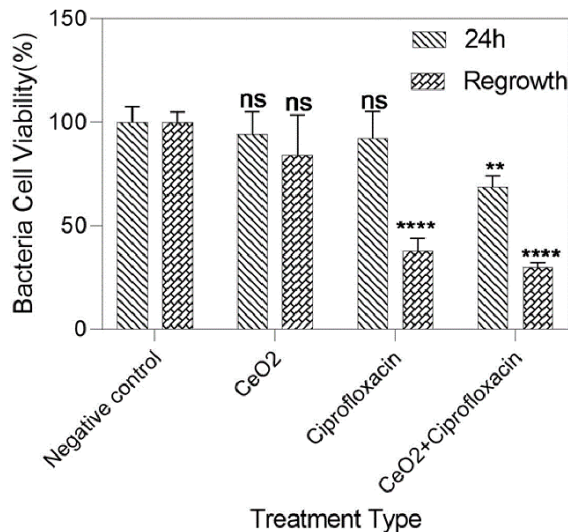


Figure 4. Regrowth capacity of *S. aureus* following treatment with CeO₂ NPs, ciprofloxacin, or their combination. After antibacterial treatment, bacterial cells were transferred into fresh TSB medium and incubated for an additional 24 h to evaluate their recovery potential. Cell viability was subsequently determined using the Alamar Blue assay. Data are presented as mean ± SD. ns, not significant; **p < 0.0021; ****p < 0.0001.

3.3. Antibacterial Effect of the Treatments in Cellular Level

The effects of CeO₂ NP, ciprofloxacin, and their combination on the membrane permeability of *S. aureus* were assessed by quantifying PI uptake. The fluorescence intensity, indicative of membrane disruption, was significantly higher in all treatment groups compared to the untreated control (Figure 5A). The data indicate that ciprofloxacin displayed a more pronounced rise in PI fluorescence compared to CeO₂ NP. The combination of CeO₂ NP and ciprofloxacin yielded the maximum fluorescence intensity, suggesting a synergistic impact on membrane disruption. This indicates that the combined therapy enhances the disruption of bacterial membrane integrity more significantly than each individual treatment, consistent with the observed synergistic antibacterial effects in previous experiments. We observed intracellular protein leakage in *S. aureus* bacterial cells treated with CeO₂ NP, ciprofloxacin, and their combination, indicating cytoplasm leakage. Additionally, bacterial cell membrane damage was observed due to exposure to the MIC [23,24]. This is also in line with the SEM images of the bacterial cells after the CeO₂ NP treatments (Figure S1) [25,26]. Our results reveal that CeO₂ NPs can cause a progression from sublethal to lethal effects, involving alterations in outer membranes, cytoplasmic membrane damage, and intracellular content leakage [35]. As demonstrated in Figure 5B, CeO₂ NP and ciprofloxacin resulted in $96.39 \pm 1.09\%$ and $97.80 \pm 3.96\%$ intracellular protein leakage from *S. aureus*, respectively. However, the combination of CeO₂ NP and ciprofloxacin in a single treatment has resulted in intracellular protein leakage of $77.8 \pm 4.5\%$. The obtained results thus reveal that the concurrent treatment of *S. aureus* with CeO₂ NP and ciprofloxacin had a lower membrane disruption activity than that of either CeO₂ NP or ciprofloxacin.

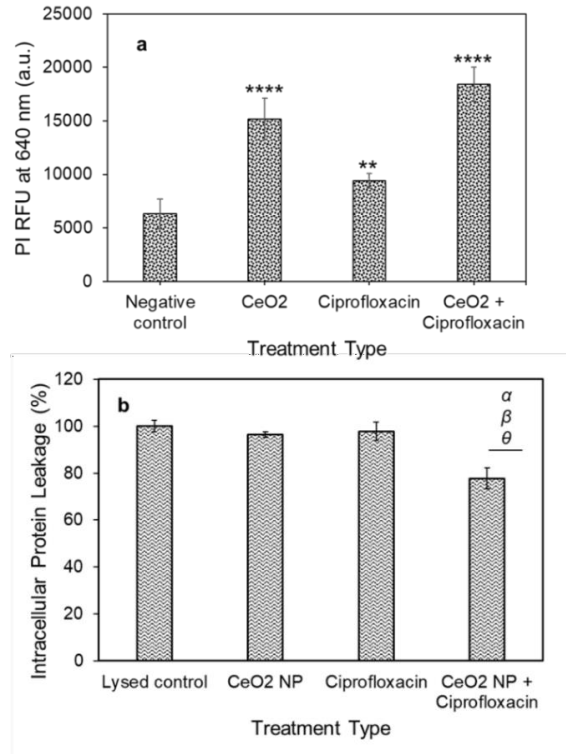


Figure 5. A) Membrane permeability alterations in *S. aureus* treated with CeO₂ NP, ciprofloxacin, and their combination **B)** Intracellular protein leakage of *S. aureus* after treatments in comparison to lysed control measured by BCA assay. The significance level is **A)** **** $p < 0.0001$, ** $p < 0.0021$, **B)** α , β , and θ were $p < 0.0001$ for CeO₂ NP + ciprofloxacin compared to lysed control, CeO₂ NP, and ciprofloxacin, respectively.

The intracellular ROS production in *S. aureus* was evaluated following the treatments. As shown in Figure 6, the positive control (0.33 mM hydrogen peroxide) exhibited the highest fluorescence intensity, confirming the assay's sensitivity. CeO₂ NPs and ciprofloxacin treatments individually led to an increase in ROS levels compared to the untreated control, indicating their ability to induce oxidative stress. We also found that *in situ* treatment caused the overproduction of ROS and oxidative stress increment in *S. aureus* suggests a synergistic interaction between CeO₂ NPs and ciprofloxacin, amplifying oxidative damage within *S. aureus* cells. This finding provides further evidence of the adjuvant potential of CeO₂ NPs, augmenting the antibacterial mechanism of ciprofloxacin through increased oxidative stress. It is known that the CeO₂ NPs can act as both antioxidants and pro-oxidants, and literature findings align with our results which states that the nano-sized cerium oxide particles promote the increment in ROS generation and membrane disruption

[36]. We highlight the underlying antibacterial mode of action of CeO₂ NPs action in combination therapy, offering a promising approach to improving antibacterial chemotherapy efficacy.

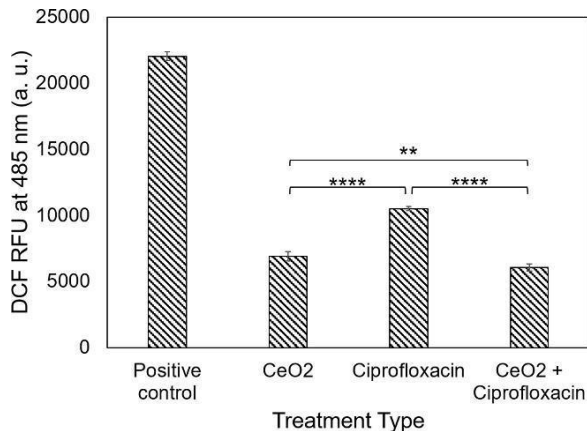
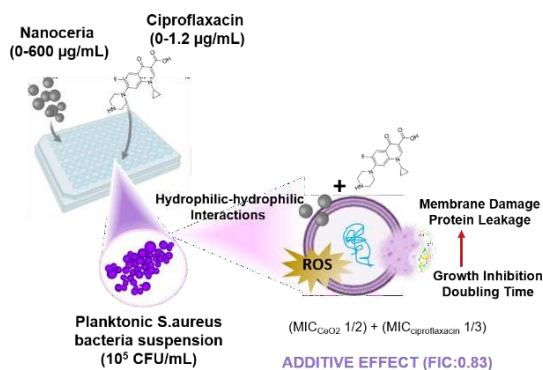


Figure 6. ROS alterations in *S. aureus* due to CeO₂ NP, ciprofloxacin, and their combination treatments. The significance level is ** $p < 0.0021$, **** $p < 0.0001$.

4. CONCLUSION

The present study demonstrated that cerium CeO₂ NP significantly enhanced the antibacterial activity of ciprofloxacin against *S. aureus*. The combined treatment reduced the MIC of ciprofloxacin threefold and shifted its action from bacteriostatic to bactericidal. CeO₂ NP induced membrane disruption, intracellular leakage, and ROS overproduction, confirming their dual role as both antimicrobials and antibiotic adjuvants as presented in Scheme 1.



Scheme 1. CeO₂ nanoparticle-mediated enhancement of ciprofloxacin antibacterial activity against *S. aureus*.

These findings highlight the potential use of CeO₂ NPs in combination therapies to improve antibiotic efficacy and reduce required dosages, offering a promising strategy against resistant Gram-positive pathogens. Future *in vivo* and translational studies are warranted to validate their clinical applicability and safety, aiming toward the development of next-generation nanoadjuvant systems in antibacterial chemotherapy.

Research and Publication Ethics Statement

The entire process of the manuscript was carried out in compliance with the research and publication ethics guidelines of the Celal Bayar University Journal of Science.

Author's Contributions

Ayşenur Pamukcu: Drafted and wrote the manuscript, performed the experiment and result analysis.

Emine Hilal Altıntop: Drafted and wrote the manuscript, performed the experiment and result analysis.

Didem Şen Karaman: Supervised the experiment's progress, interpreted the result and wrote the manuscript.

All authors contributed to the study conception. All authors read and approved of the final manuscript.

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