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PVP-Mediated Synthesis of CeO_2 Nanoparticles: Characterization and Application in AKI Serum Modulation

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Abstract

In this study, cerium oxide nanoparticles (CeO_2 NPs) were successfully synthesized using a PVP-assisted co-precipitation method to evaluate their clinical efficacy on the biochemical markers of Acute Kidney Injury (AKI). The use of Polyvinylpyrrolidone (PVP) as a capping agent during synthesis was critical in controlling particle growth and preventing agglomeration. In structural characterization of CeO_2 NPs, the average crystallite size of XRD found to be 30 nm and the average particle size of FESEM found to be 35 nm, while the average diameter in TEM analysis shows (mean±SD) value about 6.913±1.644 nm. The therapeutic potential was assessed in vitro using serum samples from 60 male participants (30 AKI patients and 30 healthy controls). The results demonstrated that CeO_2 NPs in AKI samples significantly reduced elevated levels of serum urea (p=0.108), creatinine (p=0.083), and Glutathione S-transferase pi (GSTpi) (p=0.019). The used of CeO_2 NPs as potent antioxidant mimetics due to the reversible Ce^{3+}/Ce^{4+} redox cycle and high surface-to-volume ratio. By mitigating oxidative stress and inflammatory markers, CeO_2 NPs show significant promise as a renal-protective agent. These findings highlight the potential of engineered nanoceria in treating renal complications, though further in vivo validation is warranted.

Keyword: CeO_2 nanoparticles • polyvinylpyrrolidone (PVP) • AKI • XRD • SEM • TEM • oxidative stress.

Introduction

The revolution in biomedical research has made by the nanotechnology that it has allow for exact control over material properties at the nanoscale in size, surface chemistry, and redox activity[1, 2]. Nanomaterials have been investigated more and more potential therapeutic uses in the past few years, especially in conditions where traditional pharmacological therapies are unable to molecular systems and effectively address intricate cellular[3]. Among these disorders, acute kidney injury (AKI), which is defined by a fast decline in renal function after ischemia episodes, exposure to nephrotoxic substances, or significant systemic inflammation, continues to be a serious clinical issue[4]. Excessive oxidative stress, mitochondrial damage, endothelial dysfunction, and inflammatory signaling are all directly linked to the pathological course of AKI, which eventually results in tubular epithelial cell destruction and apoptosis. The dependent mechanisms of kidney injury need to new methods for therapeutic ways that can change[5, 6].

The cerium oxide nanoparticles (CeO_2 NPs) have become a unusual class of nanomaterials due to the reversible transition from the oxidation states of Ce^{3+} and Ce^{4+} and that is leads to use CeO_2 NPs as self-regenerating antioxidants like natural enzymes (superoxide dismutase and catalase)[7]. The conventional antioxidants have a low stability and short biological half-lives, but CeO_2 NPs has sustain redox activity in oxidative microenvironments, maintain renal tubular architecture, attenuate renal oxidative stress, and reduce inflammatory mediator release[8-11]. The surface of CeO_2 NPs has contain oxygen vacancies that leads to increase with contact with damaged renal tissues, promotes cellular absorption, and may offer targeted renal protection in case of acute oxidative assault[12-14]. Despite these encouraging results, there are still significant unanswered concerns about the ideal dosage, renal distribution, and safety profile of CeO_2 NPs in models of acute kidney damage. Furthermore, variability in nanoparticle synthesis, size distribution, and surface modification may influence biological outcomes; necessitating systematic evaluation under controlled experimental conditions[15, 16], with a focus on their effects on renal function markers, oxidative stress parameters, and inflammatory responses. The aim of this work synthesis and characterization of CeO_2 NPs via Polyvinylpyrrolidone (PVP)-assisted modified co-precipitation method with discuss the therapeutic potential of CeO_2 NPs in human models of acute kidney injury, and assist in the creation of the treatment of acute kidney failure by fusing nanotechnology with renal pathophysiology.

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Materials and Methods

Preparation of CeO_2 nanoparticles

Cerium oxide (CeO_2) nanoparticles were synthesized via a co-precipitation method based on the research of Mohammed et al.[17], with specific modifications. Initially, 0.1 M of $Ce(NO_3)_3{\cdot}6H_2O$ (≥99.0%, Fluka) was dissolved with 0.02 g of Polyvinylpyrrolidone (PVP) (≥99.0%, Merck) in 50 ml of

deionized water. The resulting mixture of Ce(III) nitrate and PVP was stirred at 25 °C. Subsequently, a 1 M K_2CO_3(⩾ 99.0%, Fluka) solution was added dropwise from a burette to the mixture to obtain the precipitate (reached a pH of 11). After multiple washes with deionized water, the precipitate was dried for one hour at 80 °C. Finally, the calcination process was conducted in a muffle furnace at 500 °C for 3 hours to obtain pure CeO_2 nanoparticles **Figure1.**

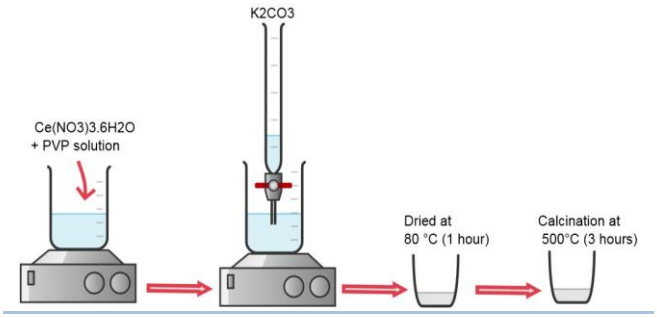

Figure 1. Scheme of synthesis CeO_2 nanoparticles via PVP-Assisted modified co-precipitation method.

Samples collection and preparation samples

About 3 milliliters of venous blood were extracted from 60 samples, which were divided into 30 male patients with AKI, the duration of the AKI was less than three months, and it was caused by high blood pressure and 30 male controls, all diseases were ruled out except for high blood pressure. Every group's age fell between 30 and 70. To investigate the effect of cerium oxide nanoparticles on the serum of patients with acute kidney injury in vitro, prepared 50 ppm of cerium oxide nanoparticles that dissolved in D.I.W, and mixed of 25% of CeO_2 NP with 75% of the serum of a patient with AKI, with incubation for one hour at 25 °C. The final result is multiplied by the dilution factor. The Abbott Architect c-4000 autoanalyzer was used to detect serum urea and creatinine, and the ELISA was used to measure glutathione S-transferases (GSTpi).

Statistical test

The research clinical data were analyzed using Microsoft Office's SPSS version 26 statistical program. One-way ANOVA was used to evaluate the data.

Results and Discussion

The formation of CeO_2 Nanoparticles via PVP-Assisted through the following reactions:

First, $Ce(NO_3)_3{\cdot}6H_2O$ dissolves and dissociates into ions in the aqueous medium. To stabilize the Ce^{3+} ions and prevent their premature agglomeration, PVP is added as a capping agent.

$$Ce(NO_3)_3.6\,H_2O \rightarrow [Ce(H_2O)_n]^{3+} + 3NO_3^- \quad \ldots (1)$$

Second, K_2CO_3 is added, which reacts with the Ce^{3+} ions to form a white precipitate of $Ce_2(CO_3)_3$:

$$2Ce^{3+}_{(aq)} + 3K_2CO_3 \rightarrow Ce_2(CO_3)_{3(s)} \downarrow + 6K^+_{(aq)} \quad \ldots (2)$$

Third, after being dried for 1 hour at 80°C, the white $Ce_2(CO_3)_3$ precipitate is heated in a muffle furnace at 500 °C. During this process, the carbonate decomposes and reacts with O_2 to form the final stable cerium(IV) oxide (CeO_2):

$$Ce_2(CO_3)_{3(s)} + \frac{1}{2}O_{2(g)} \rightarrow 2CeO_{2(s)} + 3CO_{2(g)} \quad \ldots (3)$$

The PVP coating provides a steric barrier (steric hindrance) that physically prevents Ce^{3+} ions from coming into close contact. the PVP ensures uniform nanoparticles and high surface-to-volume ratios by inhibiting premature agglomeration and uncontrolled grain growth. This is essential for their after antioxidant activity in AKI serum samples **Figure 2.**

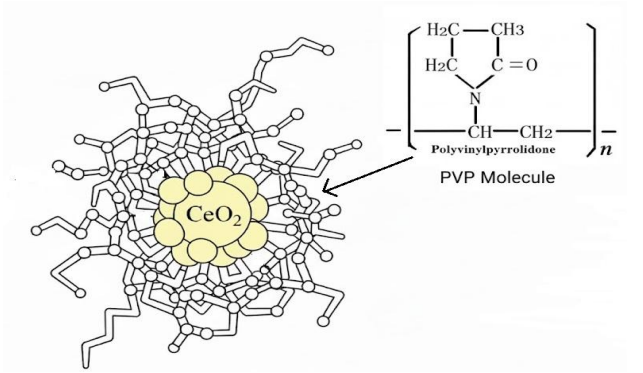

Figure 2. PVP-stabilized CeO_2 nanoparticles preventing premature agglomeration.

The distinctive diffraction peaks corresponding to the (111) 28.5°, (200) 33.1°, (220) 47.5°, (311) 56.3°, (222) 59.17o, (400) 69.35o, and (331) 76.77oplanes in the XRD pattern verify the creation of crystalline CeO_2 nanoparticles with a cubic structure, (JCPDS PDF 00-034-0394). Peak broadening indicates that the produced material is nanocrystalline, whilst the lack of impurity peaks indicates good phase purity, this agrees with Yasmin et al.[18] in **Figure 3**.

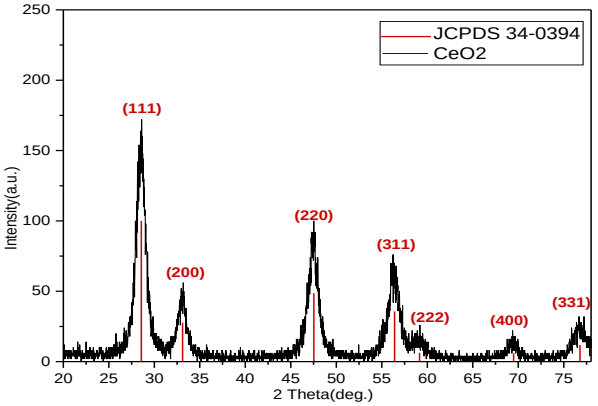

Figure 3. XRD of black line (CeO_2 NPs) and red line (JCPDS 34-0394).

The average size of crystallite calculated according to Debye-Scherrer formula was 30 nm, as in equation (1):

$$D = \frac{K\lambda}{\beta COS\theta} \quad \ldots (4)$$

Where, k is the Scherrer constant, D is the crystallite size, β is the full width at half maximum (FWHM), θ is the diffraction angle, and λ is the X-ray wavelength (1.54 Å).

These particles can be modified to suit a wide range of medical applications such as drug delivery, medical imaging,

and thermotherapy, making them a powerful tool in the fight against cancer[19].

Furthermore, Silver nanoparticles have many advantages due to their unique chemical and physical properties, such as highly tunable, stable optical properties, and easily variable surface chemistry[20]. These properties make it a good candidate for wide-ranging medical applications such as drug delivery, diagnosis, and treatment of various diseases. The unique physicochemical properties of silver nanoparticles have allowe The field emission scanning electron microscope (FESEM) was used to examine the surface morphology of the produced cerium oxide nanoparticles (CeO_2 NPs). The CeO_2 nanoparticles have a mostly quasi-spherical to irregular granular shape with observable particle aggregation, according to the FESEM micrographs, this can be explained by the high surface energy and powerful nanoscale interparticle interactions **Figure 4**. The average particle size is approximately 35 nm, indicating that the production of nanostructured CeO_2 was successful. Overall, the FESEM results support the synthesis of nanocrystalline CeO_2 with appropriate shape and particle size, indicating its potential use in oxidative stress-related biological research and nanomedicine.

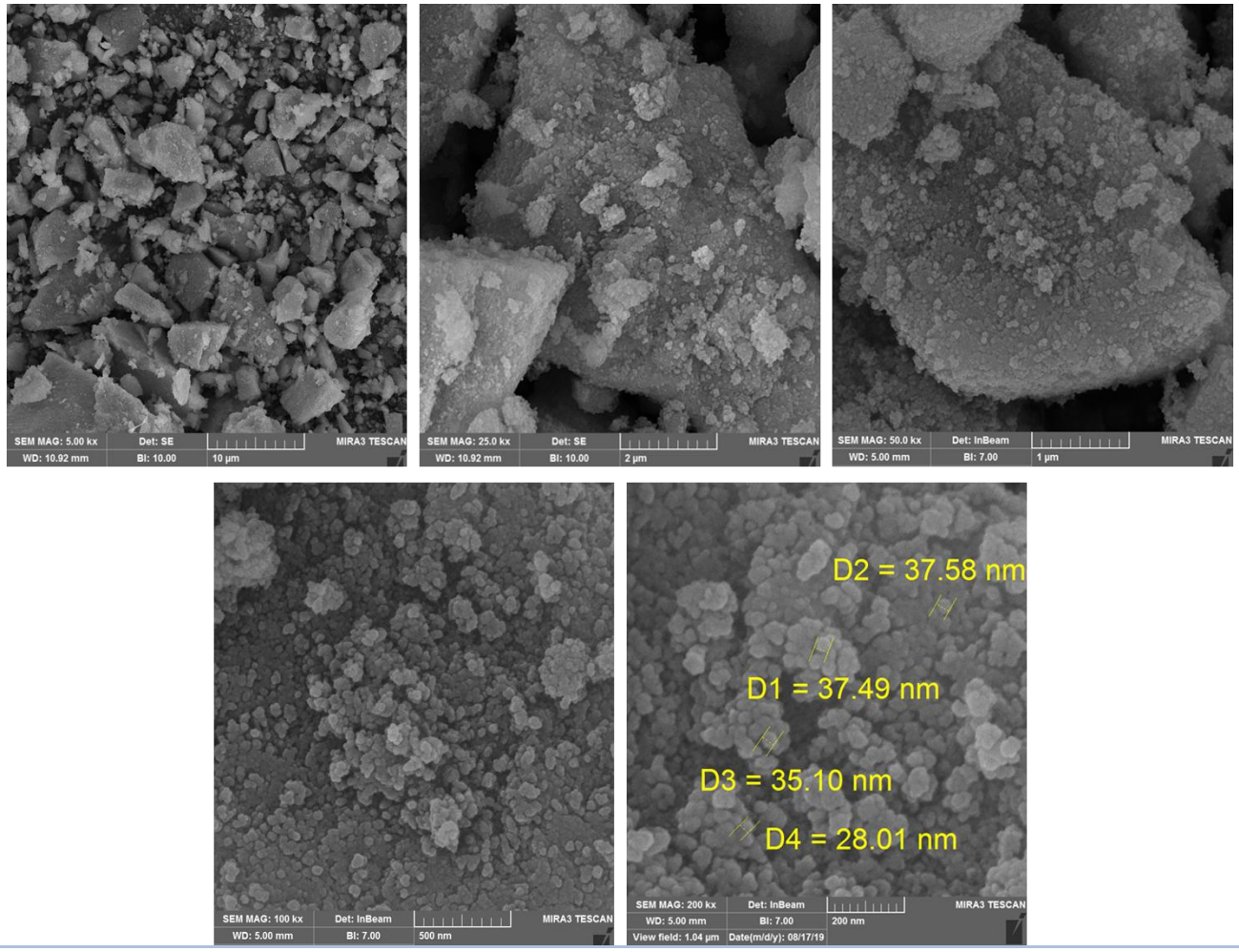

Figure 4. FESEM of CeO_2 NPs at different magnifications (10 µm, 2 µm, 1 µm, 500 nm, and 200 nm).

As shown in **Figure 5**. CeO_2 nanoparticles have a quasi-spherical to irregular shape with considerable aggregation, according to TEM examination. The effective creation of nanoparticles is confirmed by the primary particles staying within the nanoscale range. Interparticle interactions and high surface energy is responsible for the observed agglomeration. The histogram of the average diameter CeO_2 nanoparticles in TEM analysis shows (mean±SD) value about 6.913±1.644 nm.

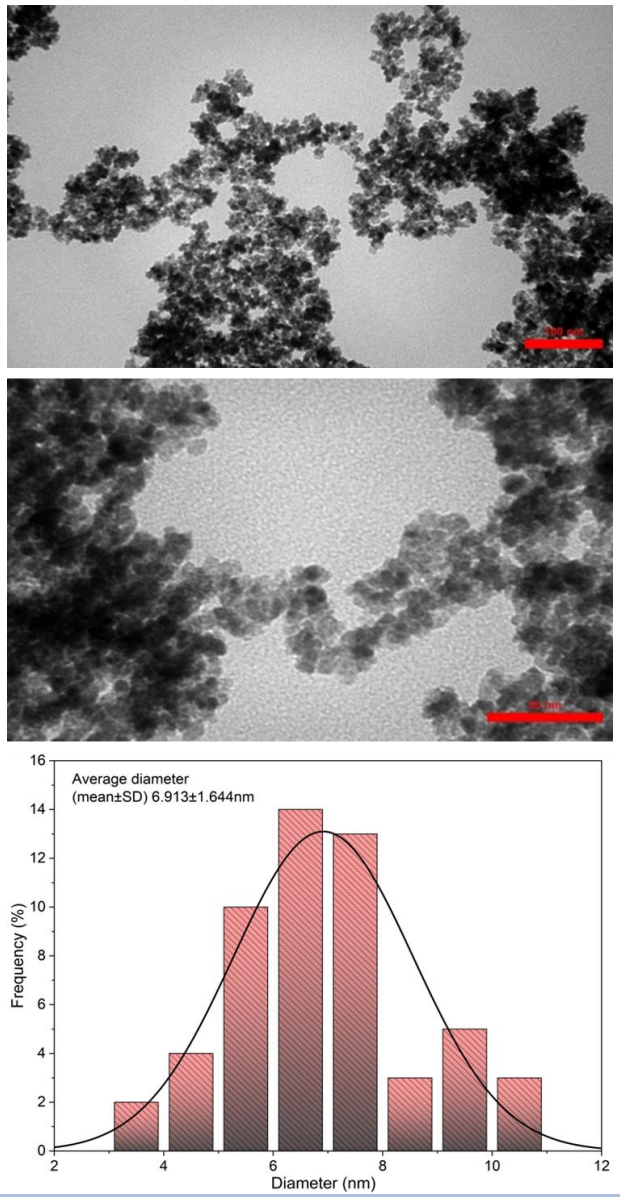

Figure 5. TEM images of CeO_2 NPs at different magnifications (100 nm and 50 nm) and particle-size distribution histogram.

FTIR Analysis of CeO2 Nanoparticle

The large absorption band at about 3327 cm^{-1} in the CeO_2 nanoparticles' FTIR spectra is ascribed to the O-H stretching vibration of the hydroxyl groups and adsorbed water molecules on the nanoparticle surface. The bending vibration of water molecules and residual surface species is associated with the bands observed at 1640 and 1539 cm^{-1}. The surface functional groups linked to C-O and C-N vibration are represented by the peaks that show up at 1343 and 1068 cm^{-1}. The successful production of cerium oxide nanoparticles is confirmed by the assignment of the distinctive absorption band at 712 cm^{-1} to the Ce-O stretching vibration. The production of CeO_2 nanostructures with surface functional groups that support nanoparticle stability is generally confirmed by the FTIR data **Figure 6**. In vitro study of CeO_2 NPs on levels of renal function test and oxidative stress parameters of patients with AKI.

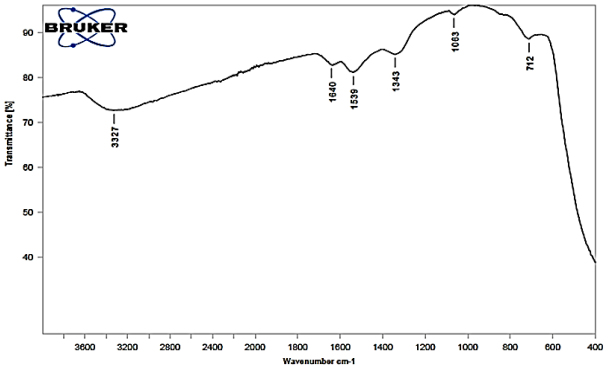

Figure 6. FTIR spectra of CeO_2.

Table 1. explains the effect of cerium oxide nanoparticles as an agent for treating or reducing the causes of kidney failure, where kidney function tests (urea and creatinine) were measured, in addition to measuring oxidative stress such as glutathione S-transferases (GSTpi). Parameters were measured before and after the addition of the nanoparticles, and compared with a control group.

Table 1. Effect of CeO_2 NPs on levels of renal function test and oxidative stress parameters of patients with p ⩾0.05(non-significant), P ⩽0.05 (significant).

	Number	S.urea (mg/dl) Mean+SD	p-value	S.creatinine (mg/dl) Mean+SD	p-value	GSTpi (U/mL) Mean+SD	p-value
Control	30	30.62±3.44	0.000	0.52±0.07	0.000	23.51±4.56	0.000
Patients with AKI without CeO_2 NP	30	54.45±3.56		1.55±0.24		76.48±9.87	
Control	30	30.62±3.44	0.065	0.52±0.07	0.092	23.51±4.56	0.004
Patients with AKI with CeO_2 NP	30	42.98±11.56		0.94±0.39		45.21±10.522	
Patients with AKI without CeO_2 NP	30	54.45±3.56	0.108	1.55±0.24	0.083	76.48±9.87	0.019

That there was a significant increase in the serum level of urea in AKI patients without CeO_2 NPs and non-significant increase patients with AKI with CeO_2 NP compared to control group and non-significant decrease in AKI patients with CeO_2 NPs compared to patients without CeO_2 NPs. Addition to, significant increase in the serum level of creatinine in AKI patients without CeO_2 NPs and non-significant increase patients with AKI with CeO_2 NP compared to control group and non-significant decrease in AKI patients with CeO_2 NPs compared to patients without CeO_2 NPs. Significant increase in the serum level of GSTpi in AKI patients without CeO_2 NPs and in AKI patients with CeO_2 NPs compared to control group, and a significant decrease in the level GSTpi in AKI patients with CeO_2 NPs compared to patients without CeO_2 NPs.

The GSTpi is a useful marker for the diagnosis of AKI because renal tubules have significant concentrations. Elevated glutathione S-transferases, which aid in the detoxification of free radicals[19, 20]. Sheng-Jie et al.[21] research showed that cerium oxide nanoparticles may

successfully eliminate excess reactive oxygen species and treat metabolic diseases. The results of a study conducted by Adel et al.[22] on rats indicate that cerium oxide nanoparticles alleviate oxidative stress in the kidneys **Figure 7**.

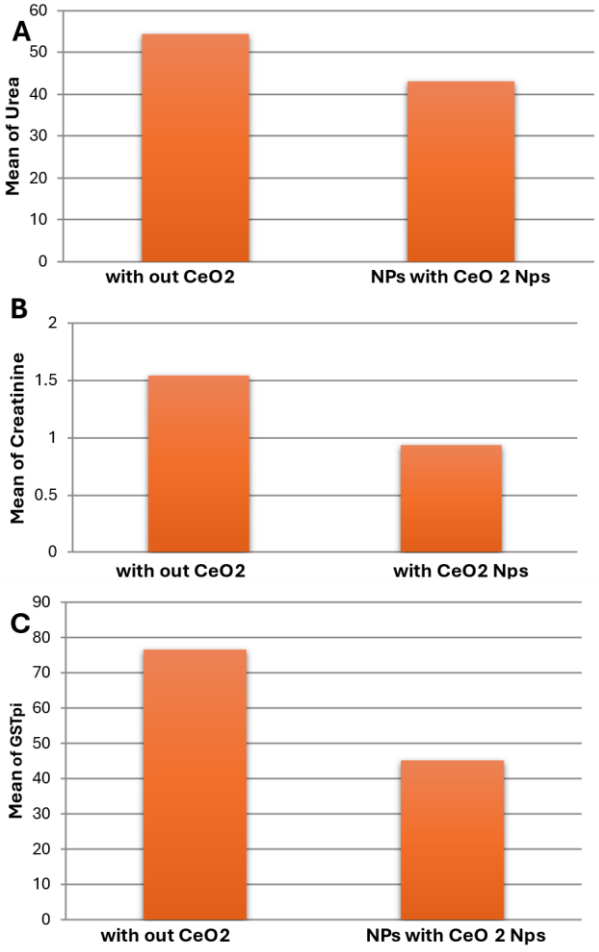

Figure 7. Affects CeO_2 NPs on (A) Urea, (B) Creatinine, and (C) GSTPi.

Conclusion

CeO_2 nanoparticles' high surface area and nanoscale size make them useful for biomedical applications. CeO_2 nanoparticles may function as potent antioxidants because of their reversible Ce^{3+}/Ce^{+} redox activity. These features imply that CeO_2 nanoparticles may have promise for renal protective applications given the importance of oxidative stress in renal diseases. To verify their therapeutic efficacy and safety, more in vitro and in vivo research is necessary. The results showed that cerium oxide nanoparticles are effective in treating or reducing the severity of the disease by mitigating its causes and complications, including inflammation and oxidative stress. Evidence of the nanoparticles' effectiveness was demonstrated by a decrease in kidney function parameters after elevations due to acute kidney disease, as well as a decrease in GSTpi enzyme levels after elevations caused by the disease. This indicates that the nanoparticles play a role in reducing oxidative stress.

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Authorship Contribution

The authors contributed equally to the article.

Declaration of Competing Interests

The authors declare that there are no conflicts of interest regarding this publication.

Ethical Statement

All participants agreed to provide the investigator with the specimens. The ethics committee of College of Science, Mustansiriyah University approved this work (Reference No.: BCSMU/0226/0049C, February 2026). Informed consent according to the Declaration of Helsinki was obtained from all participant.

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