Original Article

The physio-histological improvement of CeO_2NPs and calcitriol treatment on inflamed chronically kidneys in male laboratory rats

Asawer Kareem ALsadoon[1], Jafar Abass Issa Al-Maamori[2], Ahmed Mahdi Rheima[3]

[1]Department of Basic Science, College of Dentistry, University of Wasit, Kut, Iraq, [2]Department of Biology, College of Science, University of Wasit, Kut, Iraq, [3]Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq

Abstract

Background: Chronic kidney disease affects almost 10% of the world, according to current studies. The complications of chronic renal disease can lead to mortality. **Objective:** Using a safe treatment that improves kidney functions and tissues and prevents negative complications resulting from kidney inflammation. **Materials and Methods:** Cerium oxide nanoparticles (Ce_2O_3NPs) were synthesized using a sustainable approach that involves the utilization of basil leaf extract. Calcitriol is loaded by a physical adsorption mechanism onto the NPs. Subsequently, rats suffering from chronic renal illness induced by oral administration of adenine (100 mg/kg) for 60 days, then treated with calcitriol (0.16 mg/kg), Ce_2O_3NPs (200 mg/kg), and Ce_2O_3NPs loaded calcitriol for 28 days. **Results:** Urea and creatinine levels showed a substantial decrease in all treatment groups. In comparison to the CKD group, the G2 group exhibited an elevation in serum albumin levels, with no notable variation observed in the G3 and G4 groups. Conversely, the G2 group saw a large decrease in total protein levels, while no significant alteration was observed in the G3 and G4 groups. The histological analysis revealed that the group administered with CeO_2NPs loading calcitriol had the most favorable histological features, resembling the healthy control group the most. **Conclusion:** Loading calcitriol onto Ce_2O3NPs improves its chronic renal disease treatment.

Keywords: Adenine, calcitriol, cerium oxide nanoparticles, and chronic kidney disease

Introduction

A sustained deterioration in kidney function lasting more than three months indicates chronic kidney disease (CKD), the criteria include albuminuria, a GFR < 66 mL/min per 1.73 m^2, and kidney structural abnormalities.[1] CKD is expected to become the sixth chief cause of death by 2040. CKD prevalence is predicted to rise faster than other chronic illnesses. Detecting and treating chronic kidney disease (CKD) early is crucial to preventing ESRD, Each year, 1.5 million patients with ESRD (end-stage renal disease) require dialysis before receiving a kidney transplant. This strains people and healthcare systems financially.[2] In individuals with vitamin D deficiency and impaired VDR activation, 50% have normal PTH levels due to altered kidney function.[3,4]

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_995_24

Cerium, a rare earth metal, begins the lanthanide sequences, rare earth metals' 4f orbitals are protected by 5p and 4d electrons, charitable them fascinating catalytic capabilities for treating liver, ovarian, cardiomyopathy, sepsis, obesity, intestinal, and lung illnesses. Renowned therapeutic agents in regenerative medicine and tissue engineering, CeO_2NPs enhance cell proliferation *in vitro* and expedite lesion repair *in vivo*.[5,6] Nanotechnology has advanced nanoparticles greatly in recent decades. Nanoparticles give medications greater bioavailability,

Address for correspondence: Dr. Asawer Kareem ALsadoon, Department of Basic Science, College of Dentistry, University of Wasit, Kut 52001, Iraq.
E-mail: asalsadoon@uowasit.edu.iq

Submission: 24-Oct-2024 **Accepted:** 08-Jun-2025 **Published:** 23-Jul-2025

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How to cite this article: ALsadoon AK, Al-Maamori JAI, Rheima AM. The physio-histological improvement of CeO_2NPs and calcitriol treatment on inflamed chronically kidneys in male laboratory rats. Med J Babylon 2025;22:S109-14.

pharmacokinetics, controlled release, fewer toxic side effects, and the capacity to increase dosage. Many effective therapeutic medicines are water-insoluble, restricting their clinical use.[7] Hydrophobic drug-loaded nanoparticles increase solubility, bioavailability, stability, pharmacokinetics, pharmacodynamics, and reduce drug toxicity and improve therapeutic effectiveness, triggered release, and targeted delivery.[8] The objective of this research is to use CeO_2NPs, calcitriol, and CeO_2NPs loaded calcitriol therapy to enhance renal functions and tissues, as well as to mitigate adverse effects arising from kidney inflammation.

Materials and Methods

Basil leaves were used to make therapeutic CeO_2NPs utilizing green approaches. Calcitriol was then added to CeO_2NPs. Chemical experiments assessed the effectiveness of these nanoparticles for treatment. Thirty healthy male albino mice weighing 180–220 g were studied. The controlled investigation began after 14 days of acclimatization. Adenine (100 mg/kg) from Sigma-Aldrich Company in Germany was orally given to 24 male rats to cause chronic kidney disease (CKD) for 60 days. The experimental groups were then separated into a control group): male rats got distilled water for 60 and 28 days. (Group 1): male rats with CKD and untreated. (Group 2): male rats with CKD received solely oral calcitriol (RHAWN Company, China) at 0.16 mg/kg based on a study.[9] CeO_2NPs (200 mg/kg) were given to CKD-afflicted male rats in (Group 3) and, (Group 4): male rats with CKD got CeO_2NP-loaded calcitriol. After 28 days of therapy, blood urea, creatinine, albumin, and total protein were determined, and the kidneys from each rat were removed and weighed immediately. The kidneys in each group were kept at 10% formalin until the histological study.

Statistical analysis

It was conducted using Microsoft Excel and IBM SPSS V26, specifically employing one-way analysis of variance. The data is provided as the mean with the standard error. In addition, the mean differences of the tried groups were analyzed using the least significant difference (LSD) method.[10]

Ethical approval

All procedures and protocols adhered to the ethical guidelines set by the Ethical Committee of Wasit University–College of Dentistry, with the project being assigned No. 2024An.1 on March 10, 2024.

Results

Effect of (CeO_2NPs, calcitriol, and CeO_2NPs loaded calcitriol) on serum urea and creatinine levels

Table 1 showed a significant decrease ($P \leq 0.05$) of serum urea and creatinine in all treatment groups (G2, G3, and G4) when compared to the CKD. The data indicated a significant increase ($P \leq 0.05$) in the CKD group than control rats.

Effect of (CeO_2NPs, calcitriol, and CeO_2NPs loaded calcitriol) on serum Albumin and total protein levels

Table 2 indicates an important statistical decrease ($P \leq 0.05$) in the level of serum albumin for G1 when associated with the control group. While, high significant increase in G2, and non-significant decrease in (G3, and G4) when compared to the CKD group. Also, the data showed a significant increase in serum total protein stages in the CKD group compared with the healthy group, so, a high decrease in the G2 group, and a non-significant difference in (G3, and G4), when compared to male rats in the CKD group.

Effect of (CeO_2NPs, calcitriol, and CeO_2NPs loaded calcitriol) on histopathology of kidney tissue in CKD-treated rats

Through a histopathological study of the renal tissue of male rats, Eosin and Hematoxylin stains were used to indicate the severity of kidney inflammation resulting from the use of adenine. Histopathology study of rat kidney tissue showed abnormalities in the glomeruli, degeneration of tubular cells associated with areas of atrophy of the renal tubules, a multifocal area of collagen fiber deposition (fibrosis) around the tubules, and mild swelling of the glomeruli as shown in Figure 1A and B compared with the control group in Figure 2A and B.

When histological examination of kidney sections from the G2 group was treated with calcitriol, it was observed that there was degeneration in the renal cortex. This degeneration was characterized by hydropic edema with cytoplasm eosinophilia. Additionally,

Table 1: Effect of (CeO_2NPs, calcitriol, and CeO_2NPs loaded calcitriol) on serum urea and Creatinine levels

Parameters / Groups	Mean ± SE	
	Urea (mg/dL)	Creatinine (mg/dL)
Control	37.33 ± 0 .92^{d}	0.42 ± 0.03 ^{d}
G1 (CKD without treatment)	132.33 ± 14.41^{a}	1.65 ± 0.17 ^{a}
G2 (calcitriol)	88.25 ± 2.08 ^{BC}	1.06 ± 0.24^{b}
G3 (CeO_2)	89.60 ± 7.29^{bc}	0.92 ± 0.14^{bc}
G4 (CeO_2 loaded calcitriol)	92.33 ± 1.78^{b}	0.83 ± 0.08^{bc}
P value	0.001*	0.013
LSD	21.45	0.45

Data = Mean ± S.E. (n = 6 rats in each group)

Different letters of data are denoted by significant differences ($P < 0.05$)

Similar letters of data are denoted to non-significant difference ($P > 0.05$)

*: Highly significant ($P \leq 0.01$)

there was a reduction in renal tubules in certain areas, while other areas showed normal tubules without any degenerative changes in the glomeruli [Figure 1A and B].

The histological analysis of G3, which was treated with CeO_2NPs, revealed a reduction in degenerative changes in the renal cortex. However, some areas still exhibited these changes. In contrast, G4, which was treated with CeO_2NPs loaded with calcitriol, showed degenerative changes in the renal cortex, characterized by hydropic swelling with increased eosinophilia of the cytoplasm observed in certain areas, while other areas showed normal renal tubules and glomeruli without any degenerative changes as shown in Figures 3A and B, 4A and B.

Table 2: Kidney function values (mg/dL) of control and experimental groups treated with calcitriol, CeO_2 alone once and loaded with calcitriol at another time for 28 days

Parameters Groups	Mean ± SE	
	Albumin (mg/dL)	Total protein (mg/dL)
Control	4.03 ± 0.19[a]	5.91 ± 0.27[b]
G1 (CKD without treatment)	3.16 ± 0.30[b]	7.65 ± 0.24[a]
G2 (calcitriol)	4.21 ± 0.17[a]	6.10 ± 0.24[b]
G3 (CeO_2)	2.72 ± 0.07[bc]	7.14 ± 0.11[a]
G4 (CeO_2 loaded calcitriol)	2.90 ± 0.13[bc]	7.23 ± 0.28[a]
P value	0.001*	0.004*
LSD	0.55	0.90

Data = Mean ± S.E. (n = 6 rats in each group)

Different letters of data are denoted by significant differences ($P < 0.05$)

Similar letters of data are denoted to non-significant difference ($P > 0.05$)

*: Highly significant ($P \leq 0.01$)

Discussion

The significant increase in urea, creatinine, and albumin in CKD groups, which induced CKD by adenine, is a widely used model of chronic renal failure. It mimics the changes in human CKD, including widening with granular form, apoptotic injuries, damage to 70%–80% of renal tissue with fibrosis, and elevated levels of urea and creatinine serum.[11]

Calcitriol has a significant impact on enhancing glomerular cells, decreasing hypertrophy and fibrosis, and preventing cell death, it hinders the synthesis of pro-inflammatory cytokines while motivating the manufacture of anti-inflammatory cytokines. This improvement in kidney structure leads to a decrease in serum urea, creatinine, and total protein and an elevation of albumin levels, This is in line with the findings of Yeter *et al*.[12]

The current training showed a significant decrease in urea, creatinine, and total protein levels in the treatment groups when compared with the CKD group, this matches the study,[13] which used cerium oxide as a treatment for kidney inflammation and showed a significant increase in urea and creatinine levels. As a result, histological changes and apoptosis decreased significantly. Cerium oxide was able to reduce urea and creatinine. It is known that cerium oxide works to reduce apoptosis by reducing the immunological activity of caspase-3 in apoptosis, alleviating oxidative stress.[14] Another study discovered that the levels of uremic toxins in the blood and urine were reduced when CeO_2NPs were administered. This suggests that CeO_2NPs can improve renal failure caused by adenine by safeguarding the kidneys against various harmful factors, including high levels of phosphates, accumulation of uremic toxins, and inflammation caused by oxidative stress.[15]

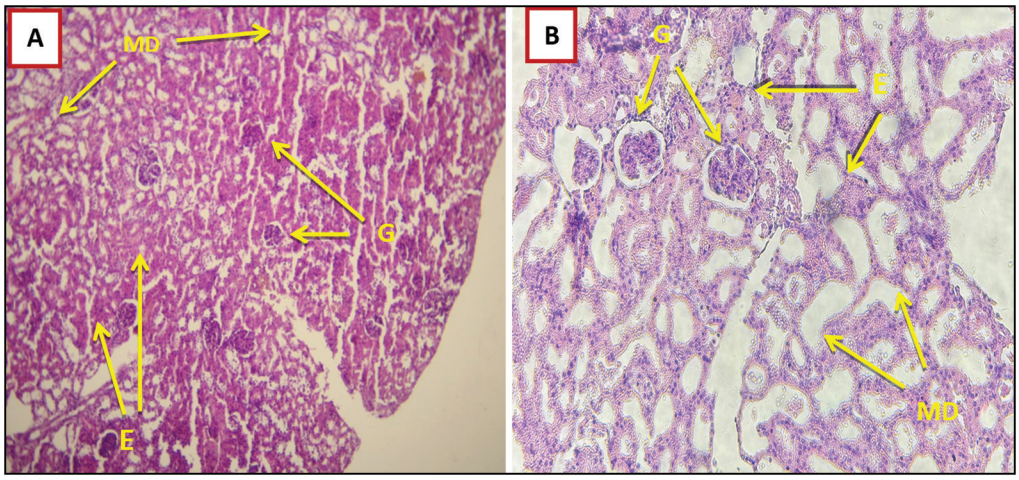

Figure 1: Photograph of renal tissue in G2 group (treated by calcitriol) after 28 days showed hydropic swelling with eosinophilia (E) of Cytoplasm, Mild dilated tubules (MD) in certain areas, whereas others exhibit typical tubules without degenerative alterations and normal glomeruli (G) (stain H&E). (A) ×100 and (B) ×400

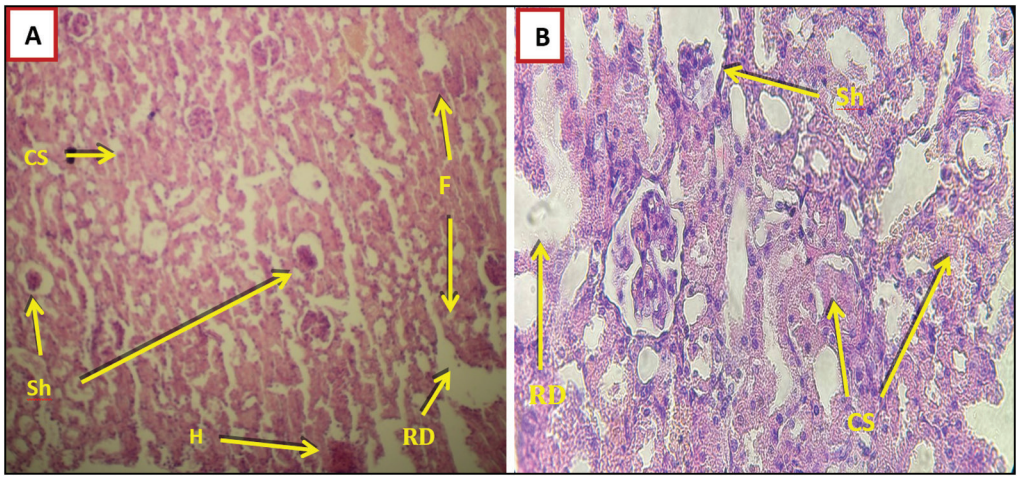

Figure 2: Photograph of renal tissue in control group showing normal histological criteria of Capsule (CA), Cortex (CO), Medulla (M), glomeruli (G), and renal convoluted tubules (T) (stain H&E). (A) ×200 and (B) ×400

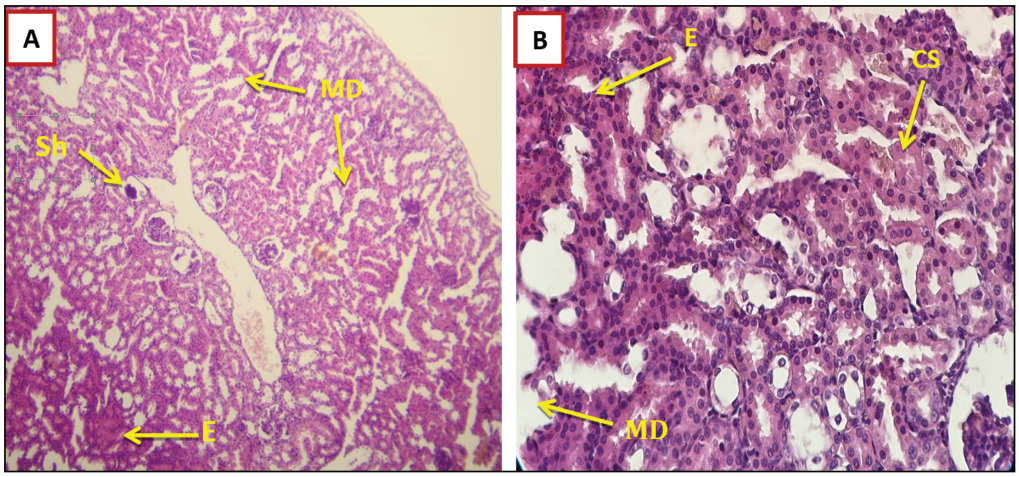

Figure 3: Photograph of renal tissue in G3 group (treated by CeO_2NPs) after 28 days. Showed mild dilated tubules (MD), cell watery swelling (CS) with increased eosinophils (E) of cytoplasm, and shrinkage of some renal glomeruli (Sh) (stain H&E). (A) ×100 and (B) ×400

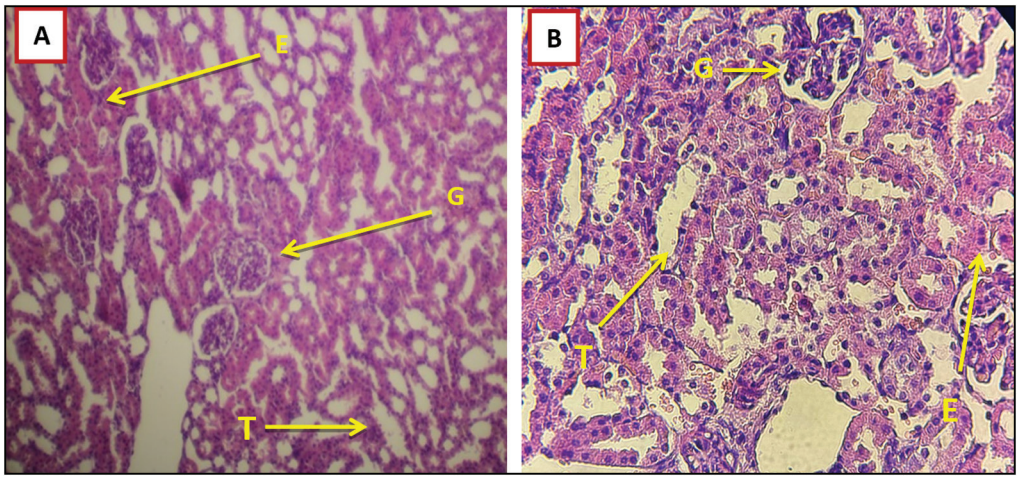

Figure 4: Photograph of renal tissue in G4 group (treated by CeO_2NPs loaded calcitriol) after 28 days showed disappear degenerative change about 60% and only40% still having the change in renal, watery swelling with increased eosinophils (E) of cytoplasm, the other area showing normal renal tubules (T), and normal glomeruli (G) (stain H&E). (A) ×200 and (B) ×400

Treatment with CeO_2NPs alone and loaded with calcitriol significantly reduced urea and creatinine concentrations in CKD rats. This demonstrates the ameliorative effects of nanoparticles with or without calcitriol on chronic kidney disease mice, possibly improving kidney function. If treatments are loaded onto nanoparticles, they will have a synergistic effect for two effective treatments, so the results will be better and more effective.[16]

On the other hand, the histological study supported the results of the existing study, as the histological analysis of the kidneys of rats in Figure 4A and B decrease in degeneration changes in the renal cortex of about 60%, and only 40% of tissue still having the changes in some areas, the other area showing normal renal tubules, and normal glomeruli without degenerative changes. Adenine-induced chronic kidney disease models have been used in several trainings.[17,18] The mechanism of adenine-induced chronic kidney disease includes the development and deposition of a fewer soluble adenine metabolite (i.e., 2, 8-dihydroxyadenine) in renal tubules, which is thought to cause renal failure.[19] In the earlier study using a rat model of CKD, treated by Ce_2O_3NPs which have positive effects on kidney function. Therefore, hypothesize that Ce2o3NPs improve kidney function.[20]

In this study, while the individual treatments of cerium oxide nanoparticles and calcitriol showed positive outcomes in treating kidney inflammation, the most favorable results were observed in group C5, where calcitriol was loaded onto cerium oxide nanoparticles. This result may be because the nanoparticles possess the capability to infiltrate inflammatory cells and exhibit anti-inflammatory properties. Hence, it may be regarded as a therapeutic approach to mitigating inflammation.[21] Additional studies have shown that NPs possess exceptional antioxidant characteristics and are capable of effectively eliminating free radicals.[22] The number of bonds between proteins and nanoparticles is directly proportional to the reduction in size of the particles, as a result of the increased surface range of the nanoparticles. Conversely, the minute dimensions of nanoparticles enable them to infiltrate almost all areas of the human body, including kidney cells.[23] The existing study is reliable with studies demonstrating the positive role of CeO_2NPs on renal tissue in rats.[24,25]

Conclusion

The current results prove that adenine-induced renal failure is diminished by administering (CeO_2NPs), which are considered biologically safe and have a protective physiological and histological effect on the kidneys by inflammatory biomarkers (urea, creatinine, albumin, and total protein), and improving the histological structure.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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