RESEARCH PAPER

Impact of Green Synthesized Hematite Nanoparticles Loaded Calcitriol on Anemia Associated with Chronic Kidney Disease in Male Rats

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ABSTRACT

Anemia is a common difficulty in chronic kidney disease (CKD) and promotes morbidity and mortality and lowers the quality of life. Most patients get oral or intravenous iron supplementation and erythropoiesis-stimulating medications. Therapy aims to improve renal function and red blood cell production. For chronic kidney disease anemia, erythropoiesis-stimulating medications and iron supplements are recommended. Oxides (hematite–α-Fe_2O3) nanoparticles were synthesized by an eco-friendly method using basil leaf extract. Calcitriol was loaded onto the surface of iron oxide nanoparticles by the physical adsorption process. Then, rats with anemia associated with chronic kidney disease caused by adenine orally (100 mg/kg) for 60 days, were treated by calcitriol (0.16 mg/kg). Fe_2O_3NPs (200 mg/kg) and Fe_2O_3 NPs loaded Calcitriol, for 28 days. The albino rats were divided into five groups: control, G1 infected with CKD without treatment, G2 treated with calcitriol only, G3 treated with Fe_2O_3NPS only, and G4 treated with calcitriol loaded on Fe_2O_3NPs. The results showed a significant increase in serum levels (Hb, folate, B12, TIBC, and UIBC), and no significant difference appeared in serum ferritin and iron levels. Iron oxide nanoparticles can effectively treat anemia, and loading calcitriol onto iron oxide increases its effectiveness.

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INTRODUCTION

Hemoglobin concentrations below 12.0 g/dL in men and 11.0 g/dL in females are typical signs of anemia. Anemia associated with chronic kidney disease (CKD) exhibits normocytic, normochromic, and hyperproliferative characteristics. The severity of anemia is increased when the glomerular filtration rate (GFR) surpasses 80 mL/min/1.73 m^2 [1].

Nanoparticles have a broad variety of chemical, physical, and biological properties, making them effective alternatives to traditional approaches, environmental sustainability, ubiquitous availability, biomedical uses, and biocompatibility make plant-based synthesis an ecologically acceptable method. Plant synthesis is of interest to researchers because of its various advantages. Plants include non-toxic compounds,

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and stabilizing agents, making them the ideal source of nanoparticles [2]. Delivery of therapy to targeted tissues and cells is as important as choosing the therapeutic material. Which can transported better using nanoparticles, and may readily penetrate through biological barriers and membranes to reach hard-to-reach anatomical areas due to their properties. While decreasing adverse effects, drug delivery systems provide the right dosage [3].

Darwish *et al*. show that iron-fortified meals reduce iron-deficient anemia. Many water-soluble drugs, such as ferrous sulfate ($FeSO_4$), sodium ferric dimethylamine, and ferrous glycinate chelate, may alter food taste, especially from oral nutritional supplements, and stay in the gut instead of being absorbed [4]. People are increasingly recommending iron oxide nanoparticles (Fe_2O_3 NPs) as an alternative to standard treatments for iron deficiency anemia [5]. Several clinical experiments have established that vitamin D impacts the hematopoietic system because bone marrow stromal and accessory cells have vitamin D receptors (VDRs). Vitamin D insufficiency, along with inflammation, iron deficiency, and EPO deficiency, may aggravate anemia, and hemoglobin levels are substantially associated with 25-hydroxyvitamin D levels, or (calcitriol). Several investigations found that chronic renal disease patients who took calcitriol or its derivatives had reduced EPO needs, less anemia, and higher blood hemoglobin [6].

This research aimed to assess the efficacy of Fe_2O_3NPS, produced using the environmentally friendly process of basil leaf-based green synthesis, both on its alone and when loaded with calcitriol, in improving markers of anemia in male laboratory rats with adenine-induced chronic kidney disease.

MATERIALS AND METHODS

The nanoparticles with therapeutic effectiveness (Fe_2O_3) were synthesized using basil leaves by green methods. Subsequently, Calcitriol was loaded onto Fe_2O_3NPs and given to rats with CKD anemia for 28 days. The efficiency of these nanoparticles was evaluated through relevant chemical tests to determine their suitability for administration with calcitriol therapy.

The present study comprised 30 healthy male albino rats weighing 180–220 gm. After 14 days of acclimatization, the research was done in a controlled setting. A 12-hour dark-light cycle and 22–24°C room temperature were maintained. Animals have limitless food and water. Adenine, procured from (Sigma-Aldrich, Germany), was administered orally to 24 male rats to induce anemia associated with chronic kidney disease (100 mg/kg) dissolved in DIW for 60 days. Then the experimental groups were constructed and divided into (Control): male rats get distilled water, for 60-day periods and an extra 28 days. (Group 1): male rats with CKD-induced anemia which stays without treatment. (Group 2): male rats with CKD-induced anemia received only calcitriol (RHAWN Company, China) with formula ($C_{27}H_{44}O_3$) at an oraliy dose of (0.16 mg/kg/day) according to a study [7]. (Group 3): male rats with CKD-induced anemia, were treated with Fe_2O_3NPs (200mg/kg), and (Group 4): male rats with CKD-induced anemia get Fe_2O_3NPs-loaded Calcitriol.

Later 28 days of treatment, the rats were anesthetized with chloroform, and blood samples were taken. Hemoglobin tests were performed on (1 ml) of blood samples in EDTA anticoagulant tubes. Additionally, 4 ml of blood samples were placed in anticoagulant-free tubes and coagulated at temperature of room for 30 minutes. The sera were centrifuged at 3000 RPM for 15 minutes at 25°C. After storage at -20°C, the samples were biochemically tested. Anemia, Iron Status, Folate, Vitamin B12, Ferritin, Iron, TIBC, and UIBC were measured. Cobas/Roche Instrument Germany supplied and measured.

Statistical analysis

Microsoft Excel and IBM SPSS V26 were used for statistical analysis (one-way analysis of variance). The data reported mean with standard error. As well as, the mean differences of tested groups done by at least significant difference (LSD) [8].

RESULTS AND DISCUSSION

X-ray diffraction (XRD) of α - Fe_2O_3 NPs

By XRD analysis, the planes (012), (104), (110), (113), (024), (116), (122), (214), (300), and (220) can be assigned to a series of diffraction peaks at 2θ of 23.8665, 32.9681, 35.4494, 40.8637, 49.3151, 53.8772, 57.2123, 62.5967, 63.9079, and 75.55, respectively. With a=b = 5.0380 Å and c = 13.7720 Å, the pure rhombohedral phase of α-Fe_2O_3 (JSPDS Card no. 24-0072) was easily identified by all of the diffraction peaks. There were no impurity peaks seen. Additionally, the products' exceptional crystallinity is confirmed by

the strong and distinct diffraction peaks. Fig. 1.

The average crystallite size of the synthesized Fe_2O_3 NPs was calculated by using the Debye-Scherrer equation [9]:

$$D = \frac{k\lambda}{\beta Cos\theta} \quad (1)$$

Where:

D is the crystallite size

λ is the wavelength of the X-ray source (0.1541 nm) used in XRD

β is the full width at half maximum of the diffraction peak

K is the Scherrer constant with a value from 0.9 to 1

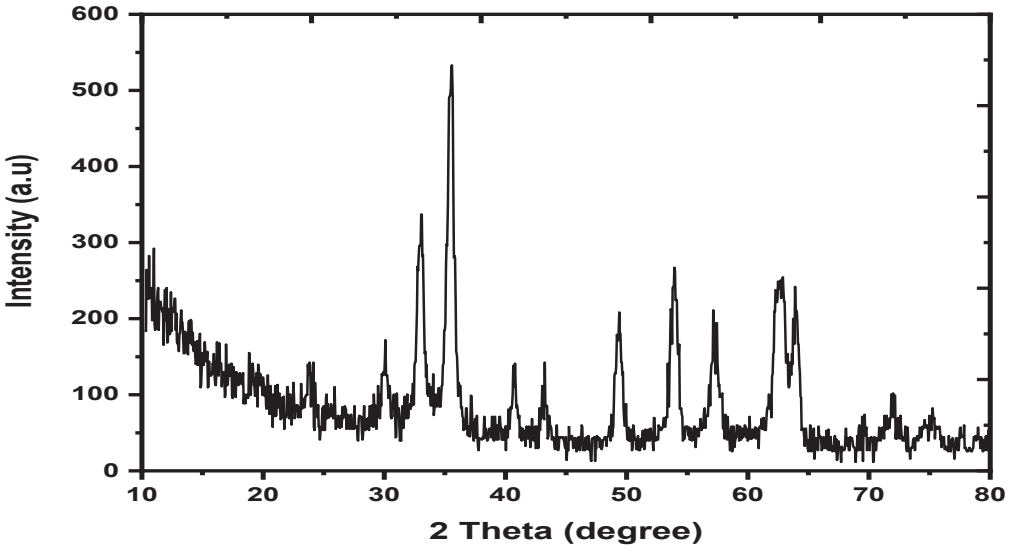

Fig. 1. XRD of α - Fe_2O_3 NPs.

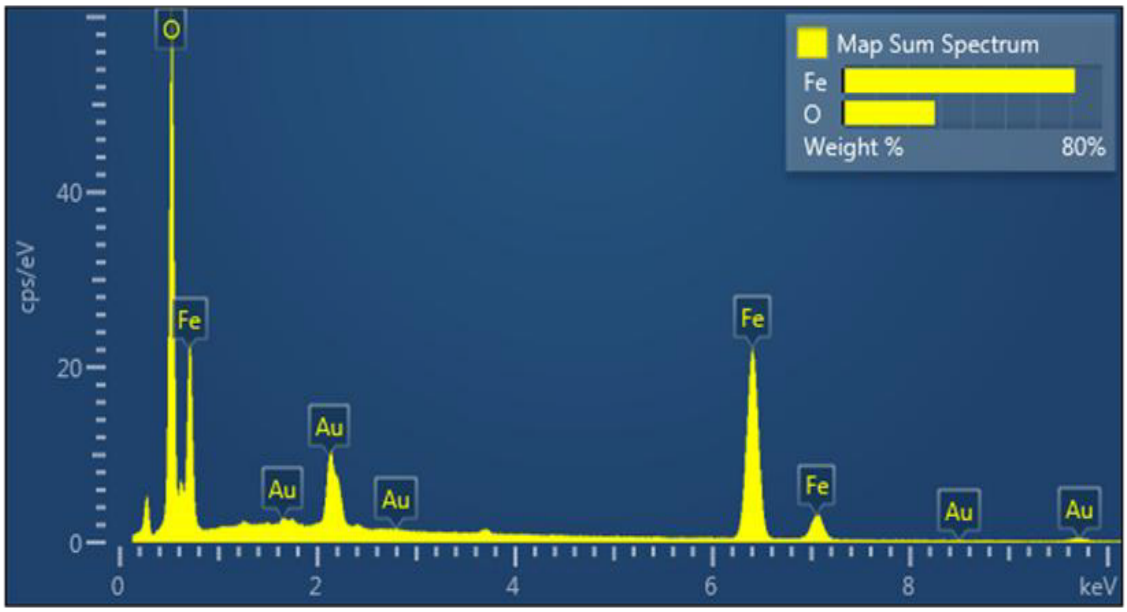

Fig. 2. EDX of α- Fe_2O_3 NPs

θ is the Bragg angle

Using the above equation, the average crystal size of α-Fe_2O_3 nanoparticles was 16 nm.

Energy-dispersive X-ray of α - Fe_2O_3 NPs

The EDX of Fe_2O_3NPs that were synthesized by a green method is shown in Fig. 2 which shows only Fe and O peaks, without other peaks referring to the presence of other elements in this sample.

Transmission electron microscopy (TEM) of α - Fe_2O_3 NPs

TEM has investigated the shape and particle size of α- Fe_2O_3 NPs. As seen in Fig. 3, the mean particle size and distribution are randomly calculated from the samples' TEM images. The produced α-Fe_2O_3 NPs nanoparticles are spherical and free of aggregations, as demonstrated by TEM images. The production of clear nanoparticles was significantly aided by the extract's action as a surfactant. From the bottom up, synthesis mechanics were applied to it, producing the nanonucleus upon the addition of the surfactant. After that, growth continued until the needed particle was created. The primary cause of the tiny particles is this. The manufactured nanoparticles' diameters fell within the nanoscale (all measurements were less than 100 nm), indicating that they were nanoparticles

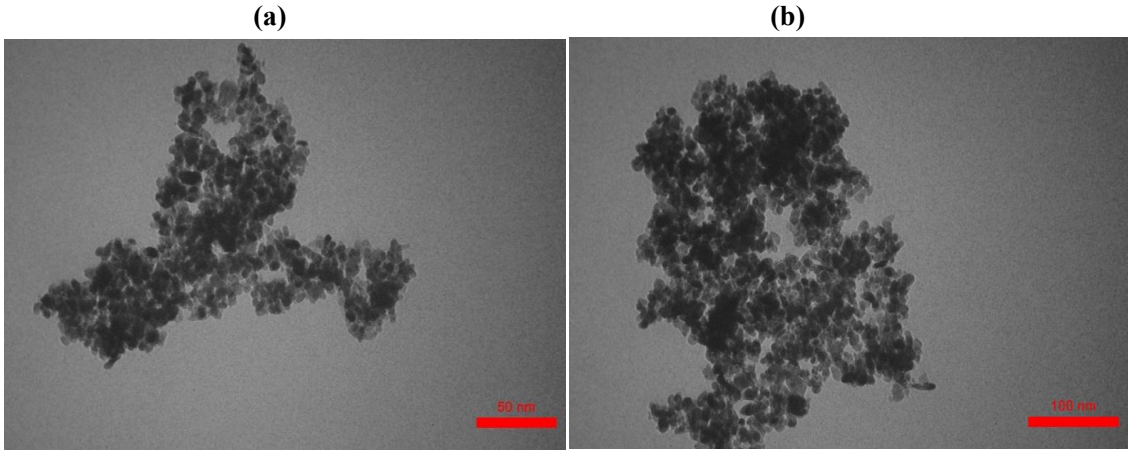

Fig. 3. TEM images of α- Fe_2O_3 NPs.

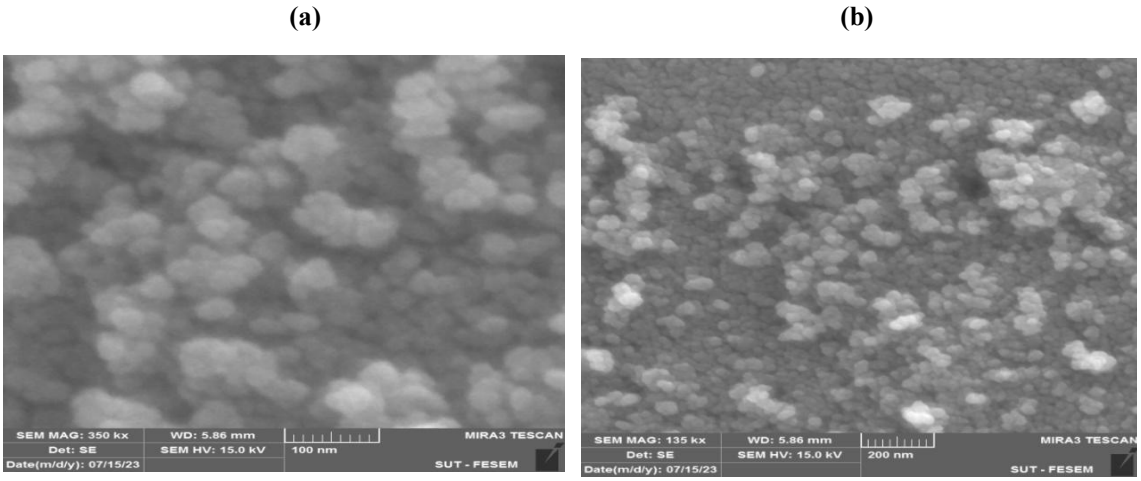

Fig. 4. FE-SEM images of α -Fe_2O_3NPs.

and had zero dimension (0D). The X-ray size determined from Scherrer's equation utilizing X-ray line broadening experiments and the nanoparticle size from TEM research agreed quite well. The average size of the particles was 17 nm.

Field emission scanning electron microscopy (FE-SEM) of α - Fe_2O_3 NPs.

The size and morphology delivery of α-Fe_2O_3 NPs nanoparticles were examined using a field emission scanning electron microscope (FE-SEM), as shown in Fig. 4.Nanoparticles have a smooth, well-crystallized surface. Based on the measurement images, we can see that the samples have a high porosity, which suggests that the samples have amorphous ratios. In the samples, the spherical shape predominates. Very small percentages of some clusters are found in the samples; this could be because the examiner did not properly prepare the sample for analysis. FE-SEM analysis indicates that spherical aggregates are produced as a roughly uniform dispersion of samples. Furthermore, the equal-sized produced nanoparticles' crystal nature is shown. The nano-sample's average particle size is 22 nm.

Effect of (Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol) on serum Hemoglobin levels

Male rats with chronic renal disease and adenine-induced anemia had significantly

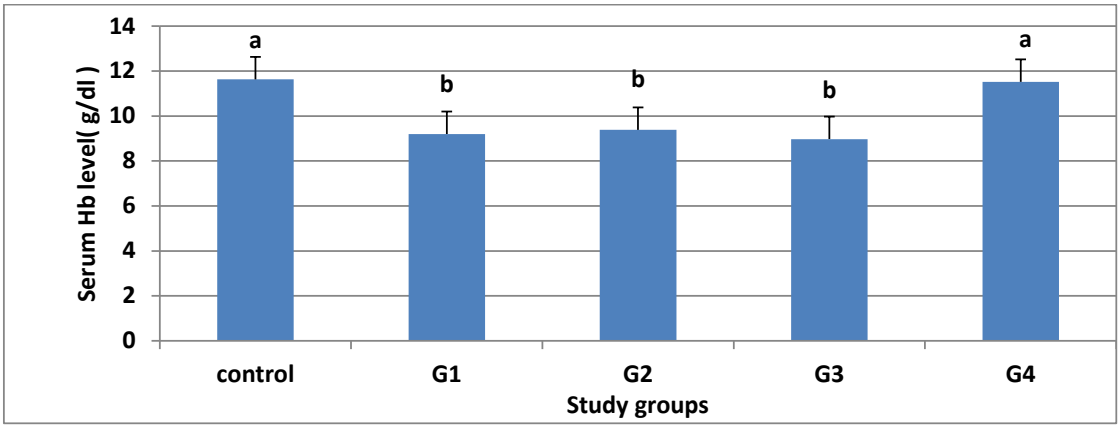

Fig. 5. Effect of (Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol) on serum Hemoglobin levels. (Data = Mean ± S.E. (n= 6 rats in each group). Different letters are denoted by significant differences ($p<0.05$). Similar letters denote non-significant difference ($p>0.05$)).

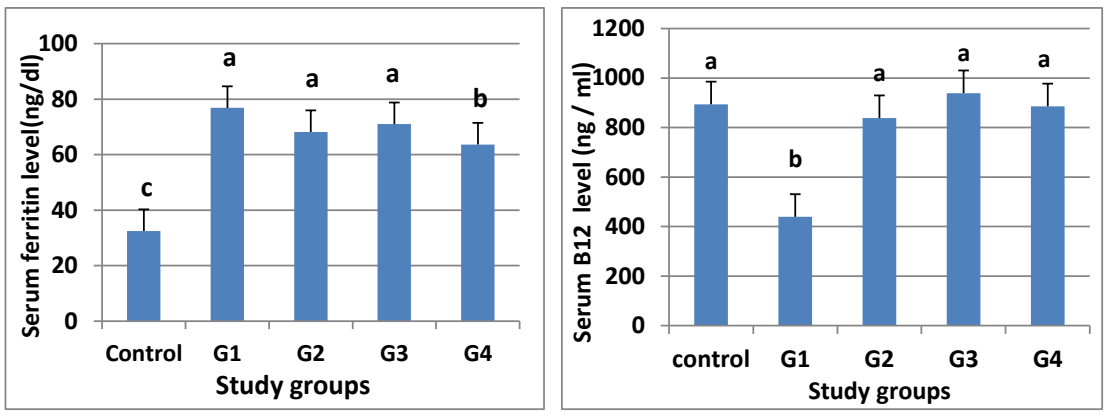

Fig. 6. Effect of (Calcitriol, Fe_2O_3 NPs, and Fe_2O_3 NPs loaded calcitriol) on serum Ferritin (a) and B12 (b) levels. (Data = Mean ± S.E. (n= 6 rats in each group). Different letters are denoted by significant differences ($p<0.05$). Similar letters denote to non-significant difference ($p>0.05$)).

lower(P≤0.05) serum hemoglobin levels (9.20±0.37 g/dL) compared to controls. Treatment groups G2 and G3 showed no significant changes (9.38±0.45, and 8.97±0.18 g/dL). Fig. 5 shows that the G4 group had a statistical increase (P≤0.01) of (11.52 ± 0.23 g/dL) compared to the CKD group.

Effect of (Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol) on serum ferritin and B12 levels

Male rats with chronic kidney disease associated with anemia had decreased significantly (P≤0.05) serum ferritin levels (76.83 ± 3.97 ng/ml) compared to controls (32.50 ± 3.33 ng/ml). while treatment groups showed no significant different in (G2: 68.16 ± 7.44 , and G3: 71.00 ± 4.91) , while significant increase in G4 (63.66 ± 3.98 ng/ml). In serum B12 levels, the result showed significantly decreased levels (P≤0.05) in the CKD group (439.33±28.81 pg/ml), compared to healthy rats(894.00±18.81 pg/ml), On the other hand, the result indicates that treated rats showed high significant in serum B12 levels (G2:838.40 ± 5.27, G3: 939.23 ± 68.32, and G4:886.00 ± 71.74 pg/ml) when compared with G1 (CKD group) Figs. 6a and b.

Effect of (Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol) on serum Folate and Iron levels

In the current study, the results showed no significant differences in the serum folate levels of male rats with CKD anemia group and all treatment groups (G2, G3, G4), when compared with the control group, while, the data showed a significant decrease (P≤0.05) in serum iron levels in male rats with CKD anemia (G1) compared with control group. and showed high significant increased (P≤0.05) in G2, G3, and G4 when , compared with the G1 group . Table 1.

Effect of (Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol) on serum UBIC and TBIC levels

Table 2 showed a decrease significantly (P≤0.05) in serum total iron binding capacity (TBIC) and, serum unsaturated iron binding capacity)

Table 1. Effect of (Calcitriol, Fe_2O_3 NPs and, Fe_2O_3 NPs loaded calcitriol) on serum Folate and Iron levels.

Parameters	Mean ±SE	
Groups	Folate (ng/ml)	Iron (µg/dl)
Control	13.16 ± 0.94	214.33 ±14.16 [a]
G1 (anemia associated with CKD)	12.00 ±0.85	124.16 ±10.54 [c]
G2 (Calcitriol)	11.83 ±0.74	184.40 ±14.23 [b]
G3(Fe_2O_3)	12.50 ± 0.76	245.00 ±46.74 [a]
G4(Fe_2O_3 loaded Calcitriol)	11.83 ± 0.60	222.33 ±8.37 [a]
LSD	2.30	41.54
P-value	0.720 *	0.001

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).
*: Non-significant.

Table 2. Effect of Calcitriol, Fe_2O_3 NPs and Fe_2O_3 NPs loaded Calcitriol, on serum UBIC and TBIC levels.

Parameters	Mean ±SE	
Groups	TIBC (µg/d)	UIBC (µg/d)
Control	423.83 ± 23.16 [c]	261.00 ±6.76 [b]
G1(anemia associated with CKD)	371.16 ± 11.39 [d]	165.33 ±6.89 [c]
G2(Calcitriol)	438.20 ± 15.70 [b]	282.00 ±25.09 [a]
G3(Fe_2O_3)	480.63 ± 26.24 [b]	336.50 ±47.76 [a]
G4(Fe_2O_3 loaded Calcitriol)	532.66 ± 4.71 [a]	290.50 ±9.65 [a]
LSD	52.07	73.28
P-value	0.001	0.0001

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).

UBIC) levels in the CKD groups, compared to the control groups, and the treatment groups showed a significant increase($P \leq 0.05$) in serum TBIC and UBIC levels for all groups G2, G3, and G4 compared to G1 and the control group.

The observed decrease in some serum blood parameters in the current study (Hb, B12, folic acid, iron, TIBC, and UIBC) was consistent with [10], who found a significant decrease may be due to the use of adenine as a stimulant for kidney inflammation and the use of adenine as a stimulant in chronic kidney disease leads to a decrease in hemoglobin and is considered a direct cause of anemia and EPO insufficiency. The decrease in hemoglobin levels can be explained by the fact that it reduces the sensitivity of the oxygen detection system in erythropoietin-producing cells, This may explain why hypoxic kidneys in patients with chronic kidney disease produce less EPO, There is a complex interplay between infections chronic blood loss and kidney failure causing anemia in patients with chronic kidney disease [11].

Calcitriol had a role in increased serum iron, B12, TIBC, and UIBC. The data aligned with research conducted by [12] they demonstrated a significant increase in serum TIBC, UIBC, and B12. This means that calcitriol affects anemia associated with CKD, and improves iron status by increasing erythropoietin, suppressing PTH release, and increasing the rate of calcium absorption [13]. On the other hand, iron oxide nanoparticles (Fe_2O_3NPs) have a significant effect on serum folate, B12, iron, UIBC, and TIBC within normal values. Fe_2O_3NPs are commonly used in medicine and biotechnology,It's the most widely used because of its excellent biocompatibility, bioavailability, and easy synthesis properties for biomedical applications and the treatment of various diseases, they are promising materials for the effective absorption of drugs. They cause a significant increase in the number of red blood cells, hemoglobin concentration, and packed cell volume when treating anemia [14].

According to the results of the current study the calcitriol loaded on Fe_2O_3NPs showed a significant increase in Hb, B12, iron, UBIC, and TBIC compared with the anemia group associated with CKD,As it is known, NPs possess the property of selective, effective, and safe drug delivery, and for this reason they have been widely used to deliver different types of drugs, as NPs have developed the pharmacological properties and enhanced the targeting potential and bioavailability of drugs ,It was reported that therapeutics loaded with NPs contributed to the improvement of kidney inflammation caused by adenine in rats [15]. NPs loaded with drugs showed an effective more conveniently, it was concluded that these NPs might prevent chronic kidney disease by targeting affected kidney cells and attenuating inflammation, which indicated better therapeutic effects and fewer side effects than traditional ones [16].

CONCLUSION

The fast development of Nanomedicine has led to the improvement of medications that specifically target the kidneys, increasing drug concentrations and effectiveness while reducing toxicity and unwanted side effects. This research investigated the effectiveness of Fe_2O_3NPs, calcitriol, and Fe_2O_3NPs loaded calcitriol for treating anemia caused by CKD. The investigation detected significant elevations in serum Hb, iron, B12, TIBC, and UIBC. The results demonstrate the therapy's efficacy and the loading technique's capacity to deliver the drug to the intended destination.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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