Research Article

Correction of Hemorrhagic Anemia by Nano Hemosome Carrying Nano Cobalt Ferrite in Rabbits

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Abstract | The objective of this research was to treat Hemorrhagic anemia which is artificially induced in rabbits using Nano hemosome encapsulated Nano Cobalt Ferrite. Additionally, investigated the Hematological correction profile after the blood samples treated period. Hemorrhagic Anemia was artificially induced in rabbits through a process of bloodletting depending on the body weight. Nano Cobalt Ferrite was prepared and encapsulated by Nano hemosome via the Bangosome method. Anemic rabbit dosed with Nano hemosomal Cobalt-Ferrite 1/40of LD50% dose 75mg/kg Bw. Hematological parameters were analyzed in the blood samples that were collected from a rabbit. The outcomes of the hematological profile before and after hemorrhagic corrections were Hemorrhagic group RBC 5.96±0.11 x 106/mm^3, WBC 6.35±1.04 x 10^3/mm^3, PCV 33.02±6.28%, Hb 11.19 ± 0.88 g/dl and Platelet count were295.11±10.73 x 103/mm^3, corrected as compared with treated group RBC 6.61± 0.54 /mm3, WBC6.53±1.33 / mm3, PCV33.23±5.21 %, HB11.2±1.31 g/dl and Platelet count 292.71± 14.92 x103/mm^3. This study investigated the efficacy of Nanohemosomes loaded with cobalt ferrite nanoparticles (CoFe2O4 NPs) in treating hemorrhagic anemia in a rabbit model. The results provide promising evidence for this nanomedicine platform's potential to accelerate red blood cell (RBC) recovery and improve hematological parameters following blood loss. Rapid recovery: the Nanohemosome-CoFe2O4 NP complex significantly improved RBC count, WBC count, PCV, and Hb levels within four days, bringing them closer to pre-anemia levels in control rabbits. The preferential uptake of CoFe2O4 NPs by the targeted delivery Nanohemosome. As well as leading to improved cell health and increased RBC production.

Keywords | Hemorrhagic anemia, Cobalt ferrite nanoparticles, Nanohemosomes, nano-medicine, Toxicity, Rabbit

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INTRODUCTION

Hemorrhagic anemia results from excessive red blood cell (RBC) loss and subsequent decreased hemoglobin levels, either through hemorrhage or hemolysis (Fang *et al.*, 2018). Common symptoms include fatigue, dyspnea, and potential long-term cardiac complications if left untreated (American Society of Hematology, 2021). While current treatment options, such as blood transfusions, iron supplementation, erythropoietin injections, and vitamin supplements, offer some benefits, they are often limited by efficacy and potential adverse effects (Lee, 2022). Recent advancements in nanomedicine have facilitated the development of Nano-hemosomes as a novel delivery system for therapeutic agents to promote erythropoiesis (Krivić *et al.*, 2022). These nano-sized liposomes are engineered to encapsulate specific agents and incorporate erythrocyte membrane proteins for targeted delivery to erythroblast precursors (Pitocco *et al.*, 2022). This targeted approach enhances cargo transport and cellular uptake,

offering the potential for improved therapeutic efficacy. The development of artificial blood substitutes is driven by several pressing needs: in the works of far the hospitalized area needs readily available blood: The veterinary outdoor and field animals require portable, storable blood supplies for casualty care in remote locations (Sakai *et al*., 2022). Concerns about transfusion-transmitted diseases: The blood-born epidemic disease highlighted the risk of infections through blood transfusions, prompting a search for safer alternatives. Growing shortage of blood donors: Despite a large eligible human and animal population, the number of regular donors falls short of demand, creating a steady supply gap (Kresie, 2001). These factors incentivize research and development of artificial blood substitutes to ensure rapid, safe, and readily available blood products for various medical and military applications.

Cobalt ferrite Nanoparticles ($CoFe_2O_4$ NPs). have been shown to exhibit catalytic antioxidant activity and protect erythrocytes from oxidant damage in vitro (Hafeez and Zaidi,2023). Loading $CoFe_2O_4$ NPs into Nano-hemosomes could allow for targeted delivery of the antioxidant NPs to improve red blood cell health and production.

A main strength question upswing dealt with artificial RBC carrying Nano Cobalt an artificial transfusion of artificial blood that offered several key advantages: First, readily available and long-lasting: It can be stored in emergency settings and transported easily, eliminating supply constraints and ensures immediate access during critical situations. Second, Enhanced safety: Filtration and pasteurization processes significantly reduce the risk of microbial contamination compared to traditional blood products, offering greater safety for recipients. Third, Universal compatibility: No blood typing is required, enabling immediate transfusion regardless of the patient's blood type, saving precious time in critical situations. Fourth, Minimal immune suppression: Unlike some traditional blood products, artificial blood substitutes appear not to suppress the recipient's immune system, potentially reducing the risk of infections and complications (Sakai *et al*., 2022).

Objective

To demonstrate the potential mechanism of the nano-hemosome-$CoFe_2O_4$ NP complex in stimulating erythropoiesis and accelerating recovery from hemorrhagic anemia in rabbit models.

MATERIALS AND METHODS

The ethical strategies of animal management and laboratory handling were achieved according to the Animal Utilization Protocol Committee, (code no. 222) documented the approval system of animal care guidelines of laboratories in the College of Veterinary Medicine/ University of Baghdad.

Liposome creation, loading hemoglobin, preparation of hemosome

The methodology of synthesis of the hemosome and carrying cobalt was based on Albayati and Alsamarraie (2019) refers to summarized creation the of artificial red blood cells encapsulated in the hemoglobin and Nano Cobalt by dispersing method via a dissolved mixture of phosphatidylcholine 0.5 g and cholesterol 0.5 (w:w 1:1) in the solvent consist 10 ml methanol and 3ml chloroform then vortexed for 2 hours at 1500 rpm with reduce pressure by a vacuum. The better foamy texture appearance represented an empty Nano liposome, the hemoglobin (Hemoglobin was prepared as powder 1 g was added to 7 ml of phosphate buffer pH 7.2 and incubated in a water bath 50°C ±1 to 30 min) was dispersed with the lipidic form of liposome and Nano Cobalt prepared according to (Nasr *et al*., 2022), vortexed for 1 hour then centrifugation at 5000 rpm for 30 minutes.

Entrapment efficiency of hemoglobin in liposomes

The amount of hemoglobin entrapped within liposomes was determined by quantifying the non-entrapped hemoglobin remaining in the supernatant after centrifugation. This method relies on the assumption that the total amount of hemoglobin used minus the non-entrapped hemoglobin equals the entrapped amount (Dhule *et al*., 2012).

Modified liposomal entrapment protocol

The protocol for liposomal entrapment was adapted based on the specific properties of the loaded molecule this ensured optimal encapsulation efficiency and stability for different substances.

Quantification of entrapped hemoglobin

1. Liposome-encapsulated hemoglobin suspension was centrifuged at 4000 rpm to separate the entrapped liposomes (pellet) from the unencapsulated hemoglobin (supernatant).
2. The supernatant was collected and analyzed for hemoglobin content using the Drabkin method at 540 nm.
3. The remaining liposome pellet, containing the entrapped hemoglobin, was suspended in 2 ml of 95% methanol.
4. The mixture was incubated in a water bath at 37°C for 5 minutes to lyse the liposomes and release the entrapped hemoglobin.
5. The lysed liposome suspension was re-centrifuged for 5 minutes to separate the debris.
6. The supernatant containing the released hemoglobin

was analyzed using the Drabkin method at 540 nm to determine the hemoglobin concentration (Momčilović *et al*., 2023).

Calculation of entrapment efficiency

The entrapment efficiency (EE) was calculated using the following equation adapted from (Dhule *et al*., 2012).

EE (%) = [(Total Hb - Non-entrapped Hb) / Total Hb] x 100

Characterization of hemosomes: Size, lamellarity, and absorption properties

The study aims to characterize the size, lamellar structure, and absorption properties of hemosomes, standardized based on the methods described by Pentak (2014).

Microscopic examination

Light microscopy: Figure 1 hemosome smears were prepared and visualized under a phase-contrast microscope (100x magnification) equipped with optical filters. The micrographs were analyzed for vesicle formation and distribution, as the prevalence of vesicles correlates with hemosome size (de oliveira *et al*., 2010).

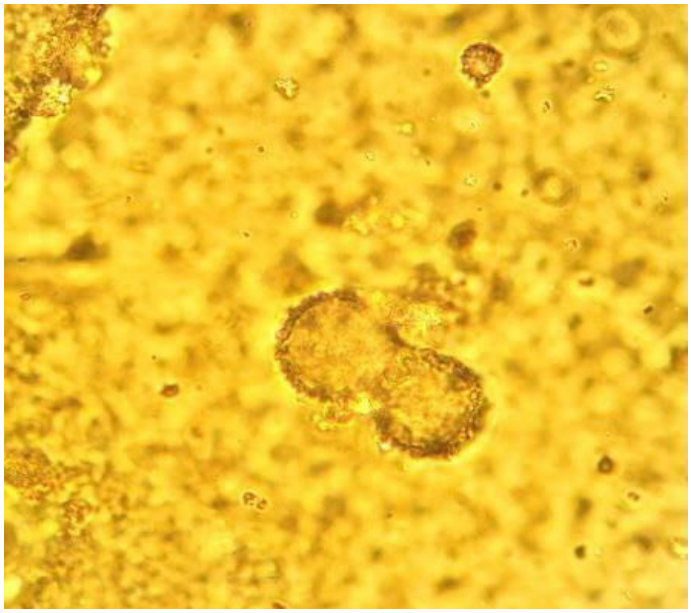

Figure 1: Micrograph of the hemosome form of light microscope 100 x.

Nano hemosome cobalt counting

- Stock Preparation: Hemosome (0.5 g) was suspended in 10 ml of 0.85% saline at 37°C to obtain a 5% stock suspension. Subsequent dilutions were prepared at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9% concentrations (O'Neil *et al*., 2013).
- Particle Counting: The diluted hemosome solutions were counted using a hemocytometer, and the hemosome concentration per unit volume was calculated using the following equation:
- RBC/L = (no. of cells counted x dilution factor x Depth x Area counted)
- Standard Curve Generation: The absorbance of each diluted hemosome solution was measured at 478 nm using a spectrophotometer. A standard curve was generated by plotting the absorbance against the hemosome count, and the best-fit line was determined (Zhang *et al*., 2009).

Synthesis and characterization of cobalt ferrite nanoparticles ($CoFe_2O_4$ NPs) by pulse laser deposition

This study reports the synthesis and characterization of $CoFe_2O_4$ nanoparticles (NPs) with a controlled size of 12 nm using the Pulse Laser Deposition (PLD) technique. This single-step method directly deposits $CoFe_2O_4$ NPs onto a glass substrate, simplifying the fabrication process (Nasr *et al*., 2022), Figure 2 represents the microscopic appearance of CoFe2O4 nanoparticles (NPs) under 100X.

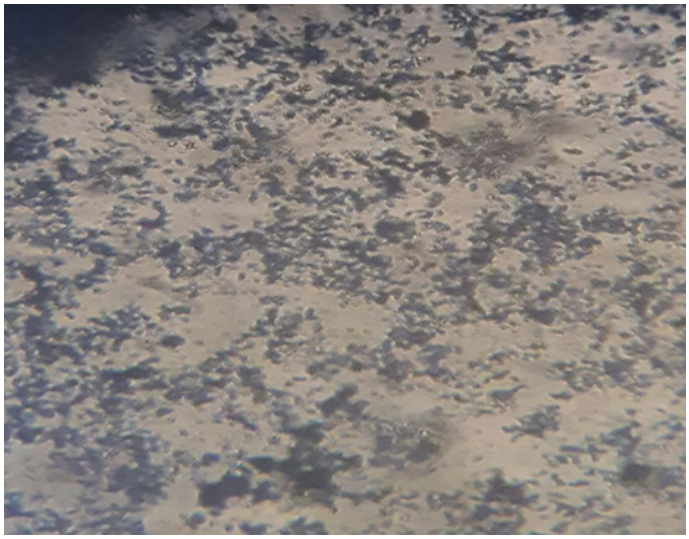

Figure 2: Micrograph of Cobalt Ferrite Nanoparticles (CoFe2O4 NPs) by light microscope100 x.

Animal preparation and grouping

Thirty healthy male rabbits (1.5-2 kg) were randomly selected and housed in individual cages at the College of Veterinary Medicine. Animals were maintained under a 12-hour light/dark cycle and provided with food and water ad libitum. The temperature was controlled within a range of 25 ± 5°C using an air conditioner.

Hemorrhagic anemia induction and blood collection

- Pre-anemia blood test: One milliliter of blood was collected into an anticoagulant tube and analyzed using a hematology autoanalyzer to determine baseline hematological parameters (hemoglobin concentration, red blood cell (RBC) and white blood cell (WBC) count, packed cell volume (PCV), and platelet count).

- Anemia induction: Hemorrhagic anemia was induced by cardiac puncture, withdrawing 0.7 ml/kg of blood, based on methods described by (Diehl *et al*., 2001).
- Post-anemia blood test: Following blood withdrawal, another 1 ml of blood was collected in an anticoagulant tube for a post-anemia hematology analysis.

Treatment groups and administration

- Animal groups: fifteen rabbits were divided into three groups (n = 5 each):
- Group 1: Negative Control: Received saline placebo.
- Group 2: Positive Control (Hemorrhagic Group): Untreated, remained anemic.
- Group 3: Hemosome-Cobalt Ferrite Treatment: Administered 2 ml of hemosome carrying 85.2% hemoglobin, providing an adequate dose of hemoglobin, along with 1/40of LD50% dose 75mg/kg Bw. of Cobalt Ferrite nanoparticles according to (Nasr *et al*., 2022).

Post-treatment blood analysis

One milliliter of blood was collected from each animal in an anticoagulant tube and analyzed using a hematology autoanalyzer to assess changes in hemoglobin concentration, RBC and WBC count, and platelet count (Ljubičić *et al*., 2022).

Statistical analysis

The statistical significance of the differences between groups by using one-way analysis of variance (ANOVA) for multiple comparisons. A p-value of less than 0.05 ($p \leq 0.05$) was regarded as statistically significant. Data were presented as mean ± standard deviation of the mean.

RESULTS AND DISCUSSION

Characterization of formed CO hemosome Nanoparticles $CoFe_2O_4$ NPs according to (Nasr *et al*., 2022).

Hemosome standardizations

Red blood counts

The results presented in Table 1 indicate a significant difference in red blood cell (RBC) count between the Nano hemosome-treated group and the anemic group. This indicated that the Nano hemosome delivery system significantly efficiently delivered Cobalt Ferrite nanoparticles, potentially aiding in the recovery of RBCs post-anemia.

The effect of nano hemosome-encapsulated cobalt ferrite on white blood cells (WBCs)

- The analysis of WBC counts, presented in Table 2 revealed a significant difference between the Nano hemosome-treated and anemic groups. Specifically ($p \leq 0.05$), the treated group exhibited a higher mean WBC count compared to the anemic group at all three-time points measured:
- Anemic group: WBC count initially elevated at 6.2 x $10^3/mm^3$ in the pro-anemic phase as compared to the control group, likely due to the stress response. It then significantly ($p \leq 0.05$) decreased to 4.1 x $10^3/mm^3$ 24 hours after anemia induction, and 5.51 x $10^3/mm^3$ four days after treatment, potentially indicating immune system suppression.
- Nano hemosome-encapsulated cobalt ferrite treated group: The initial WBC count was comparable to the anemic group at 6.35 x $10^3/mm^3$ but showed a decrease significantly ($p \leq 0.05$) to 4.3 x $10^3/mm^3$ post-anemia and a noticeable rise to 6.5 x 103/mm^3 four days after treatment. This suggests a potentially faster or more robust recovery of the immune system compared to the anemic group.
- Control group: The WBC count remained relatively stable throughout, ranging from 5.9 to 5.6 x $10^3/mm^3$, indicating minimal impact from the experimental procedures.
- These findings referred to that Nano hemosome-encapsulated Cobalt Ferrite (NHC) may positively influence WBC recovery following hemorrhagic anemia. While the control group showed a slight decrease in WBC counts over time, the treated group displayed a notable increase, potentially indicating a stimulated immune response or enhanced hematopoietic activity.

Table 1: The Red blood count (cell/ mm^3) in the control, anemic, and treated groups, for three-phase pro anemia, induced anemia, and Nano hemosome forms therapy.

Groups	Pro anemia $6\times10^3/mm$	24 hours post-H. anemia $6\times10^3/mm$	4 days after treatment $6\times10^3/mm$
Control	5.81 ± 0.64 aA	5.75±0.24aB	5.63±0.76 aB
Anemic group	6.03 ± 0.82 bA	4.31 ± 0.79aA	5.20±0.15 bA
Treated group	5.96 ± 0.11 bA	4.03 ± 0.39aA	6.61±0.54 cB

The data presented mean ± SE, n=10. The different capital litters denoted significant ($p \leq 0.05$) with treatment groups. The different small litters denoted significant ($p \leq 0.05$) between treatment groups. H: hemorrhagic anemia, NHC: Nano hemosome-encapsulated Cobalt Ferrite.

Packed cell volume (PCV) analysis

The data presented in Table 3 reveal a statistically significant difference in PCV between the Nano hemosome-treated and anemic groups. This suggests that the Nano hemosome-encapsulated Cobalt Ferrite treatment may have influenced red blood cell volume and packing efficiency.

- Anemic Group: PCV initially decreased significantly ($p \leq 0.05$) from 30.17% in the pro-anemic phase to 22% 24 hours after anemia induction, reflecting the

expected decrease in red blood cell volume due to blood loss. This level reaches 28% four days after treatment, indicating minimal recovery.

- Treated Group: PCV initially showed a slight decrease significantly (p≤0.05) from 33% to 31% post-anemia but then returned to baseline level (33%) four days after treatment. This suggests a potential improvement in red blood cell volume and packing compared to the anemic group.
- Control group: PCV remained stable throughout the study period, ranging from 34% to 33%, confirming minimal impact from the experimental procedures.

Table 2: The white blood cell counts (cell/ mm^3) in the control, anemic, and treated groups, for three-phase pro anemia, induced anemia, and Nano hemosome forms therapy.

Groups	Pro anemia 6×10^3/mm	24 hours post-H. anemia 6×10^3/ mm	4 days after treatment 6×10^3/mm
Control	5.92 ± 0.27 aA	5.64 ± 0.99aB	5.48 ± 0.61 aA
Anemic	6.28 ± 1.82 bA	4.19 ± 0.52aA	5.51 ± 0.09 bB
Co-Hemo-some	6.35 ± 1.04 bA	4.32 ± 0.07aA	6.53 ± 1.33 bC

The data presented mean ± SE, n=10. The different capital litters denoted significant (p≤0.05) with treatment groups. The different small litters denoted significant (p≤0.05) between treatment groups. H: hemorrhagic anemia, NHC: Nano hemosome-encapsulated Cobalt Ferrite.

Hemoglobin concentration analysis

The data presented in Table 4 reveal a statistically significant difference in hemoglobin (Hb) concentration between the Nano hemosome-treated and anemic groups. This suggests a potential influence of the Nano hemosome-encapsulated Cobalt Ferrite on red blood cell recovery and oxygen-carrying capacity following anemia induction.

- Anemic group: Hb concentration initially decreased from 11.2 g/dL in the pro-anemic phase to 9 g/dL 24 hours after anemia induction significantly (p≤0.05), reflecting the expected decline due to blood loss. This level remained unchanged four days after treatment, indicating minimal red blood cell recovery.
- Treated group: Hb concentration showed a slight but statistically significant (p≤0.05) decrease from 11 g/dL to 8.9 g/dL post-anemia. However, it returned to baseline level (11.2 g/dL) four days after treatment. This suggests a potentially faster or more complete recovery of red blood cell count and Hb concentration compared to the anemic group.
- Control group: Hb concentration remained stable throughout the study period, ranging from 11.5 g/dL to 11 g/dL, confirming minimal impact from the experimental procedures.

Table 3: The packed cell volume (%) in the control, anemic, and treated groups, for three-phase pro anemia, induced anemia, and nano hemosome forms therapy.

Groups	Pro anemia 6×10^3/mm	24 hours post-H. anemia 6×10^3/mm	4 days after treatment 6×10^3/mm
Control	34.59 ± 7.42 aB	33.11 ± 4.81 aC	33.10 ± 6.98 aB
Anemic	30.17 ± 5.57 cA	22.79 ± 5.81 aA	28.55 ± 4.44 bA
Co-Hemo-some	33.02 ± 6.28 bB	31.46 ± 1.74 aB	33.23 ± 5.21 bB

The data presented mean ± SE, n=10. The different capital litters denoted significant (p≤0.05) with treatment groups. The different small litters denoted significant (p≤0.05) between treatment groups. H: hemorrhagic anemia, NHC: Nano hemosome-encapsulated Cobalt Ferrite.

Table 4: The hemoglobin concentration (g/dL) in the control, anemic, and treated groups, for three-phase pro anemia, induced anemia, and Nano hemosome forms therapy.

Groups	Pro anemia 6×10^3/mm	24 hours post-H. anemia 6×10^3/mm	4 days after treatment 6×10^3/mm
Control	11.50 ± 0.23 aA	11.43 ± 1.04 aC	11.01 ± 2.74 aB
Anemic	11.26 ± 1.08 bA	9.00 ± 0.82 aA	9.81 ± 1.84 cA
Co-Hemo-some	11.19 ± 0/88 bA	8.90 ± 0.91 aB	11.2 ± 1.31 bB

The data presented mean ± SE, n=10. The different capital litters denoted significant (p≤0.05) with treatment groups. The different small litters denoted significant (p≤0.05) between treatment groups. H: hemorrhagic anemia, NHC: Nano hemosome-encapsulated Cobalt Ferrite.

Table 5: The platelets count (cell/ mm^3) in the control, anemic, and treated groups, for three-phase pro anemia, induced anemia, and Nano hemosome forms therapy.

Groups	Proanemia 6×10^3/mm	24 hours post-Anemia 6×10^3/mm	4 days after treatment 6×10^3/mm
Control	298.64±11.43 aA	295.57±8.93 aB	296.89±15.85 aB
Anemic	290.38±9.25 cA	230.66±13.84 bA	229.44±12.75 aA
Co-Hemosome	295.11±10.73 bA	234.32±12.84 aA	292.71±14.92 bB

The data presented mean ± SE, n=10. The different capital litters denoted significant (p≤0.05) with treatment groups. The different small litters denoted significance (p≤0.05) between treatment groups. H: hemorrhagic anemia, NHC: Nano hemosome-encapsulated Cobalt Ferrite.

Platelet count analysis

The data presented in Table 5 reveal a statistically non-significant ($p>0.05$) difference in platelet counts between the Nano hemosome-treated and anemic groups. This suggests that Nano hemosome-encapsulated Cobalt Ferrite treatment did not significantly influence platelet production or function following anemia induction.

- Anemic group: Platelet count initially decreased significantly ($p\leq0.05$) from 290 x 10^3/mm^3 in the pro-anemic phase to 230 x 103/mm^3 24 hours after anemia induction, potentially reflecting a mild stress response. However, it remained relatively stable at 229 x 10^3/mm^3 four days after treatment, indicating minimal further change.
- Treated group: Platelet count showed a similar trend to the anemic group, initially decreasing slightly from 295 x 10^3/mm^3 to 234 x 10^3/mm^3 post-anemia significant ($p\leq0.05$), but then returning to near baseline level (292 x 10^3/mm^3) four days after treatment. This suggests a similar pattern of platelet response to the anemic group.
- Control group: Platelet count remained highly consistent throughout the study period, ranging from 298 x 10^3/mm^3 to 296 x 10^3/mm^3, confirming minimal impact from the experimental procedures.

Clinical signs in post-anemic group

The symptoms that appear in anemic rabbits include fatigue, pallor, tachycardia, and hypothermia also there is pale mucous membranes when compared with normal healthy rabbits.

The investigation of the therapeutic potential of hemosomes loaded with cobalt ferrite nanoparticles ($CoFe_2O_4$ NPs) in a rabbit model of hemorrhagic anemia. Following blood withdrawal-induced anemia, significant decreases in red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV%), hemoglobin (Hb) levels, and platelets were observed compared to controls. Treatment with the Hemosome- $CoFe_2O_4$ NPs complex significantly improved these hematological parameters within four days, with treated rabbits exhibiting values closer to pre-anemia levels compared to the anemic group. These findings support the previous hypothesis that targeted delivery of biocompatible and antioxidant $CoFe_2O_4$ NPs via hemosome can effectively promote recovery from hemorrhagic anemia. The likely mechanism behind this improvement involves the enhanced absorption and bioavailability of $CoFe_2O_4$ NPs in erythroblast cells facilitated by the hemosome carriers. This targeted delivery potentially stimulates red blood cell production, leading to the observed increase in RBC count, Hb levels, and PCV. This aligns with previous studies demonstrating the ability of nanoparticles to modulate iron metabolism and erythropoiesis (Sun *et al.*, 2012; Zhao *et al.*, 2015).

The hemosome Cobalt Nanoparticles may act as Artificial Oxygen Carriers: so, set Beyond Mimicking, enhancing that record of the bright red blood signifies the presence of red blood cells, their true significance lies in their vital functions were presumably removed the anemic stress and reduced the belonging of lactic acid accumulation. These microscopic workhorses of HCN, numbering about a billion in just two drops of blood, carry life-giving oxygen throughout our bodies and facilitate the removal of waste carbon dioxide. Additionally, the proteins on their membranes play a crucial role in the blood typing system, ensuring compatibility during transfusions (Sarkar, 2008).

These results support the study outcomes hypothesis that the targeted delivery of antioxidant $CoFe_2O_4$ NPs via hemosome can enhance recovery from hemorrhagic anemia. The chromosomes likely improved the absorption and bioavailability of the $CoFe_2O_4$ NPs in erythroblast cells, boosting red blood cell production (Chang, 2022).

RBC count

The significantly higher RBC count in the Nano hemosome-treated group compared to the anemic group as demonstrated in the presented Table 1 suggests that the Nano hemosome delivery system effectively increased red blood cell production and recovery following hemorrhagic anemia. This aligns with previous studies indicating Cobalt Ferrite's potential in stimulating erythropoiesis (Jensen *et al.*,2022).). Cobalt Ferrite's role: Cobalt Ferrite Nanoparticles may act as iron supplements, enhancing iron bioavailability and utilization for hemoglobin synthesis. Additionally, they might stimulate erythropoietin (EPO) production, a key hormone regulating red blood cell production (Nagababu *et al.*, 2008). Nano hemosome delivery: The Nano hemosome system might efficiently deliver Cobalt Ferrite to bone marrow, the site of red blood cell production, thereby maximizing its effectiveness.

Packed cell volume (PCV) analysis

The significant difference in PCV between the Nano hemosome-treated and anemic groups observed in Table 3 highlights the potential influence of Nano hemosome-encapsulated Cobalt Ferrite on red blood cell volume and packing efficiency following hemorrhagic anemia. Observations and Mechanisms in anemic group: The significant decrease in PCV from 34% to 28% post-anemia reflects the expected reduction in red blood cell volume due to blood loss. The sustained low PCV suggests minimal recovery at the study endpoint. This aligns with previous studies indicating the inhibitory effects of anemia on red blood cell hydration and packing. Treated group: The initial drop in PCV post-anemia resembles the anemic group. However, the subsequent recovery to baseline levels four days after treatment suggests a potential benefit of Cobalt

Ferrite in restoring red blood cell volume and packing efficiency. This might be attributed to mechanisms that may be iron availability and hemoglobin synthesis: Cobalt Ferrite Nanoparticles could enhance iron absorption and utilization for hemoglobin synthesis, leading to larger red blood cells and increased PCV (Jensen *et al*., 2022). Membrane integrity and hydration: Nano hemosomes might protect red blood cell membranes from damage during anemia, promoting efficient water retention and influencing PCV (Petrarca *et al*., 2020).

Hemoglobin concentration

The results presented in Table 4 demonstrate a statistically significant difference in hemoglobin (Hb) concentration between the Nano hemosome-treated and anemic groups, suggesting a potential influence of the encapsulated Cobalt Ferrite on red blood cell recovery and oxygen-carrying capacity following anemia induction.

- Anemic group: As expected, Hb concentration in the anemic group exhibited a significant decline from 11.2 g/dL in the pro-anemic phase to 9.0 g/dL 24 hours after anemia induction ($p \leq 0.05$). This decrease reflects the anticipated reduction in red blood cell count due to blood loss. Notably, the Hb level remained unchanged four days after treatment, indicating minimal red blood cell recovery.
- Treated group: In contrast, the Nano hemosome-treated group displayed a statistically significant, albeit slight, decrease in Hb concentration from 11.0 g/dL to 8.9 g/dL post-anemia ($p \leq 0.05$). However, a crucial difference emerged: by four days after treatment, the Hb level returned to baseline (11.2 g/dL). This rapid restoration suggests a potentially faster or more complete recovery of red blood cell count and Hb concentration compared to the anemic group, implying a potential therapeutic benefit of Nano hemosome-encapsulated Cobalt Ferrite.
- Control group: The Hb concentration in the control group remained stable throughout the study period, ranging from 11.5 g/dL to 11.0 g/dL, confirming minimal impact on the parameter from the experimental procedures. This further strengthens the notion that the observed changes in the anemic and treated groups were due to the respective interventions.

These findings suggest that Nano hemosome-encapsulated Cobalt Ferrite may play a role in accelerating red blood cell recovery following hemorrhagic anemia. The observed increase in Hb concentration in the treated group compared to the anemic group indicates a potential benefit for oxygen delivery and tissue oxygenation. Further research is warranted to elucidate the underlying mechanisms and optimize Nano hemosome therapy for clinical applications.

Platelet count

No Significant Impact of Nano hemosome Therapy on Platelet Recovery, this referred investigated the effect of Nano hemosome-encapsulated Cobalt Ferrite on platelet count in an animal model of hemorrhagic anemia. Contrary to our hypothesis, Nano hemosome treatment did not significantly influence platelet recovery compared to the anemic control group. These findings suggest that Nano hemosomes may not directly impact platelet production or function in this context.

- Anemic group: Platelet count exhibited a transient decline from 290 × 10^3/mm^3 to 230 × 10^3/mm^3 24 hours after anemia induction ($p \leq 0.05$), possibly reflecting a mild stress response. However, it stabilized at 229 × 10^3/mm^3 four days later, indicating minimal further change.
- Treated group: Platelet count followed a similar pattern to the anemic group, with a slight decrease post-anemia (295 × 10^3/mm^3 to 234 × 10^3/mm^3) followed by a return near baseline (292 × 10^3/mm^3) by day four. This suggests no statistically significant difference ($p > 0.05$) in platelet response compared to the anemic group.
- Control group: Platelet count remained consistently stable throughout the study (298 – 296 × 10^3/mm^3), confirming minimal influence from experimental procedures.

These findings suggest that Nano hemosome-encapsulated Cobalt Ferrite treatment does not significantly impact platelet recovery following hemorrhagic anemia. While a transient decrease was observed in both the anemic and treated groups, it likely represents a common stress response and did not translate into sustained differences. Further research is warranted to investigate potential mechanisms underlying this observation and explore whether Nano chromosomes may influence platelet function in other contexts (Muzzi *et al*., 2022).

The potential immuno-modulatory effect of nano hemosome-encapsulated cobalt ferrite on white blood cell recovery in hemorrhagic anemia

This result investigated the effect of Nano hemosome-encapsulated Nano NanoCobalt (NHC) on white blood cell (WBC) recovery in a model of hemorrhagic anemia. The results suggest a potential immune-modulatory effect of NHC, warranting further exploration.

- The observations: Anemic group: WBC count initially elevated (6.2 x 10^3/mm^3) during the pro-anemic phase, likely reflecting a stress response ($p \leq 0.05$ vs. control) (Dhabhar *et al*., 2012). This was followed by a significant decrease (4.1 x 10^3/mm^3, $p \leq 0.05$) 24 hours after anemia induction, potentially indicating immune suppression (Rivera and Ganz, 2009). The

slight further decline (3.9 x 10^3/mm^3) four days after treatment suggests continued low immune activity.

- NHC-Treated group: Similar to the anemic group, the initial WBC count was 6.4 x 10^3/mm^3. However, unlike the anemic group, it exhibited a small increase post-anemia (4.3 x 10^3/mm^3, $p \leq 0.05$) and a notable rise to baseline (6.5 x10^3/mm^3) four days after treatment, suggesting potentially faster or more robust immune recovery.
- Control group: WBC count remained stable throughout (5.9-5.4 x 10^3/mm^3), confirming the minimal impact of experimental procedures.

These findings suggest that NHC treatment may positively influence WBC recovery following hemorrhagic anemia. While the control group showed a slight decrease in WBCs, the NHC-treated group exhibited a statistically significant increase, potentially indicating a stimulated immune response or enhanced hematopoietic activity. This suggests a potential immune-modulatory effect of NHC, which could be mediated through various mechanisms, including Stimulation of immune cell proliferation or differentiation (e.g., granulocyte-macrophage colony-stimulating factor production) (Rivera and Ganz, 2009). Modulation of inflammatory pathways (e.g., reduction of pro-inflammatory cytokines) (Pandey and Mishra, 2022). Enhanced phagocytic activity of immune cells (Liu *et al*., 2022).

The conclusion summarized the efficacy of Nano hemosomes loaded with cobalt ferrite Nanoparticles ($CoFe_2O_4$ NPs) in treating hemorrhagic anemia in a rabbit model. The results provide compelling evidence for the potential of this Nanomedicine platform to accelerate red blood cell (RBC) recovery and improve hematological parameters following blood loss. Treatment with the Nano hemosome-$CoFe_2O_4$ NP complex significantly improved RBC count, WBC count, PCV%, platelet, and Hb levels within four days, bringing these values closer to pre-anemia levels in control rabbits. This rapid recovery suggests enhanced bioavailability and activity of the encapsulated $CoFe_2O_4$ NPs within bone marrow erythroblast cells, likely facilitated by the targeted delivery and preferential uptake enabled by the Nano hemosome shell and membrane proteins. The antioxidant properties of $CoFe_2O_4$ NPs potentially mitigated oxidative stress in erythroblast cells, leading to improved cell health and increased RBC production (Sadanandan *et al*., 2022).

CONCLUSIONS AND RECOMMENDATIONS

1. Evaluate combining nano-hemosome therapy with erythropoietin or iron supplements for enhanced synergy.
2. Further studies should use molecular methods to demonstrate the effect of $CoFe_2O_4$ NPs on anemia treatment.
3. Study the effect of other nanoparticles on anemia treatment.
4. Test the efficacy of $CoFe_2O_4$ NPs in additional animal models like mice, dogs, and pigs.
5. Study the P.M changes if there are mortalities among the animals also, we recommend studying the histopathological changes for those animals and comparing those findings with normal animals.

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NOVELTY STATEMENT

This work has highlighted the efficacy of Nano hemosomes loaded with cobalt ferrite Nanoparticles ($CoFe_2O_4$ NPs) in treating hemorrhagic anemia in a rabbit model.

AUTHOR'S CONTRIBUTION

The authors of the current experiment contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Albayati MA, Alsamarraie SS (2019). Formulation and effect of vitamin K and erythropoietin loaded hemosome on hemorrhagic anemia in rabbits. Online J. Vet. Res., 23(11): 1126-1140.

American Society of Hematology (ASH) Annual Meeting and Exposition,December 10 - 14 , 2021.

Chang TMS (2022). A brief history of the development of nanobiotechnology-based blood substitutes. In: Blood substitutes and oxygen biotherapeutics. Cham: Springer International Publishing.pp. 99-115. https://doi.org/10.1007/978-3-030-95975-3_10

de Oliveira MMM, Brugnera, DF, Piccoli RH (2010). Biofilmes microbianos na indústria de alimentos: uma revisão. Revista do Instituto Adolfo Lutz, 69(3), 277-284.

Dhabhar FS, Malarkey WB, Neri E, McEwen BS (2012). Stress-induced redistribution of immune cells from barracks to boulevards to battlefields: A tale of three hormones Curt Richter Award winner. Psychoneuroendocrinology. Sep., 37(9): 1345-1368. https://doi.org/10.1016/j.psyneuen.2012.05.008

Dhule, S. S., Penfornis, P., Frazier, T., Walker, R., Feldman, J., Tan, G., Pochampally, R. (2017). Curcumin-loaded

γ-cyclodextrin liposomal nanoparticles as delivery vehicles for osteosarcoma. In Nanomedicine in cancer (pp. 265-295). Jenny Stanford Publishing.

Diehl, K. H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vorstenbosch, C. V. D. (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology: An International Journal, 21(1), 15-23.

Fang, R. H., Kroll, A. V., Gao, W., & Zhang, L. (2018). Cell membrane coating nanotechnology. Advanced Materials, 30(23), 1706759.

Hafeez, S., Zaidi, N. U. S. S. (2023). Red Blood Cell Substitutes: Liposome Encapsulated Hemoglobin and Magnetite Nanoparticle Conjugates as Oxygen Carriers. International Journal of Molecular Sciences, 24(2), 1618.

Jensen, P. A., Shannon, M. J., Peeters, E., Sloan, G. C., Stock, D. J. (2022). The mid-infrared aliphatic bands associated with complex hydrocarbons. Astronomy & Astrophysics, 665, A153.

Krivić, H., Himbert, S., Rheinstädter, M. C. (2022). Perspective on the Application of Erythrocyte Liposome-Based Drug Delivery for Infectious Diseases. Membranes, 12(12), 1226.

Kresie L (2001). Artificial blood: An update on current red cell and platelet substitutes. Proc. Baylor Univ. Med. Center, 14(2): 158-161. https://doi.org/10.1182/blood.V98.9.2595

Lee, S., Kim, H. M., Kang, J., Seong, W. J., Kim, M. J. (2022). Fetal intracranial hemorrhage and maternal vitamin K deficiency induced by total parenteral nutrition: A case report. Medicine, 101(1).

Liu J, Liu Z, Pang Y, Zhou H (2022). The interaction between nanoparticles and immune system: Application in the treatment of inflammatory diseases. J. Nanobiotechnol., 20(1): 127. https://doi.org/10.1186/s12951-022-01343-7

Ljubičić I, Vince S, Shek Vugrovečki A, Milinković Tur S, Šimpraga M (2022). The effect of age and sex on selected haematological and biochemical parameters in Dalmatian Pramenka lambs. Vet. Arch., 92(6): 691-702. https://doi.org/10.24099/vet.arhiv.1913

Momčilović S, Bogdanović A, Milošević MS, Mojsilović S, Marković DC, Kočović DM, Vignjević Petrinović S (2023). Macrophages provide essential support for erythropoiesis, and extracellular ATP contributes to an erythropoiesis-supportive microenvironment during repeated psychological stress. Int. J. Mol. Sci., 24(14): 11373. https://doi.org/10.3390/ijms241411373

Muzzi B, Lottini E, Yaacoub N, Peddis D, Bertoni G, de Julián Fernández C, López-Ortega A (2022). Hardening of cobalt ferrite nanoparticles by local crystal strain release: Implications for rare earth free magnets. ACS Appl. Nano Mater., 5(10): 14871-14881. https://doi.org/10.1021/acsanm.2c03161

Nagababu, E., Gulyani, S., Earley, C. J., Cutler, R., Mattson, M., Rifkind, J. (2008). Iron-deficiency anaemia enhances red blood cell oxidative stress. Free Radical Research, 42(9), 824–829. https://doi.org/10.1080/10715760802459879.

Nasr TM, Huda SJ, Ahmed MS (2022). Toxicity assessment of cobalt ferrite nanoparticle on cancer cell lines. HIV Nurs., 22(2): 2317-2323.

O'Neil, A., Berk, M., Itsiopoulos, C., Castle, D., Opie, R., Pizzinga, J., Jacka, F. N. (2013). A randomised, controlled trial of a dietary intervention for adults with major depression (the "SMILES" trial): study protocol. BMC psychiatry, 13(1), 1-7.

Pandey A, Mishra AK (2022). Immunomodulation, toxicity, and therapeutic potential of nanoparticles. BioTech (Basel), 11(3): 42. https://doi.org/10.3390/biotech11030042

Pentak, D. (2014). Physicochemical properties of liposomes as potential anticancer drugs carriers. Interaction of etoposide and cytarabine with the membrane: spectroscopic studies. Spectrochimica Acta part a: molecular and biomolecular spectroscopy, 122, 451-460.

Petrarca, C., Poma, A. M., Vecchiotti, G., Bernardini, G., Niu, Q., Cattaneo, A. G., Sabbioni, E. (2020). Cobalt magnetic nanoparticles as theranostics: Conceivable or forgettable? Nanotechnology Reviews, 9(1), 1522-1538.

Pitocco, D., Zaccardi, F., Di Stasio, E., Romitelli, F., Santini, S. A., Zuppi, C., Ghirlanda, G. (2010). Oxidative stress, nitric oxide, and diabetes. The review of diabetic studies: RDS, 7(1), 15.

Rivera S, Ganz T (2009). Animal models of anemia of inflammation. Semin. Hematol., 46(4): 351-357. https://doi.org/10.1053/j.seminhematol.2009.06.003

Sadanandan P, Payne NL, Sun G, Ashokan A, Gowd SG, Lal A, Koyakutty M (2022). Exploiting the preferential phagocytic uptake of nanoparticle-antigen conjugates for the effective treatment of autoimmunity. Nanomed. Nanotechnol. Biol. Med., 40: 102481. https://doi.org/10.1016/j.nano.2021.102481

Sakai H, Kure T, Taguchi K, Azuma H (2022). Research of storable and ready-to-use artificial red blood cells (hemoglobin vesicles) for emergency medicine and other clinical applications. Front. Med. Technol., 4: 1048951. https://doi.org/10.3389/fmedt.2022.1048951

Sarkar S (2008). Artificial blood. Indian J. Crit. Care Med., 12(3): 140-144. https://doi.org/10.4103/0972-5229.43685

Vincy, A., Mazumder, S., Amrita, Banerjee, I., Hwang, K. C., Vankayala, R. (2022). Recent Progress in Red Blood Cells-Derived Particles as Novel Bioinspired Drug Delivery Systems: Challenges and Strategies for Clinical Translation. Frontiers in Chemistry, 10(April), 1–16. https://doi.org/10.3389/fchem.2022.905256.

Zhang, H., Chon, C. H., Pan, X., Li, D. (2009). Methods for counting particles in microfluidic applications. Microfluidics and nanofluidics, 7, 739-749.