MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 6449-6455
DOI: 10.12659/MSM.909591

Received: 2018.02.22
Accepted: 2018.03.19
Published: 2018.09.14

One-Pot Facile Fabrication of Bioavailable Iron Nanoparticles with Good Biocompatibility for Anemia Therapy

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: This study was supported by the Chongqing National Health and Family Planning Commission Medical Research Project (2015MSXM179) and the Chongqing Qijiang District Science and Technology Project (QJ2015090)

Background: Iron deficiency anemia (IDA) has been a major public health problem all over the world. Developing new iron (Fe) fortificants with both high bioavailability and negligible food sensory changes for IDA is in urgent demand.

Material/Methods: The Fe nanoparticles were fabricated through a one-pot reduction process under the protection of bovine serum albumin (BSA). The BSA-Fe nanoparticles were characterized systematically. The comparisons between BSA-Fe nanoparticles and $FeSO_4$ in bioavailability were carried out through hemoglobin (Hb) repletion method. The biocompatibility of BSA-Fe nanoparticles was also investigated through *in vitro* and *in vivo* assays.

Results: BSA-Fe nanoparticles have super-small size and good water solubility as well as water stability. The Hb repletion assay demonstrated that BSA-Fe nanoparticles have comparative bioavailability with $FeSO_4$. The *in vitro* cell viability assay, *in vivo* histological analysis, and biochemical measurements proved the remarkable biocompatibility of BSA-Fe nanoparticles.

Conclusions: The BSA-Fe nanoparticles fabricated through a one-pot facile method have good water solubility, comparative bioavailability with $FeSO_4$, and acceptable biocompatibility, exhibiting good potential for further clinical translations.

MeSH Keywords: Anemia • Materials Testing • Nanoparticles

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909591

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Background

Iron deficiency anemia (IDA) is a major public health problem all over the world [1]. Iron (Fe) fortification through diet can be an effective strategy for cure and prevention of IDA [2,3], but food fortification with Fe is challenging. Water-soluble Fe fortificants with high bioavailability, such as ferrous sulfate ($FeSO_4$) and sodium iron ethylene diamine tetra acetic acid (EDTA), often cause adverse sensory changes in foods [4–6]. Poorly soluble Fe fortificants such as ferrous fumarate and ferric pyrophosphate tend to have fewer sensory changes but have low bioavailability or aggregate in beverages [7,8]. Therefore, developing new Fe fortificants with both high bioavailability and negligible food sensory changes for IDA is urgent.

Recently, iron nanoparticles have shown exciting potential for novel iron fortificants with high bioavailability and low reactivity compared with current sodium iron EDTA and $FeSO_4$ [9,10]. However, iron nanoparticles with limited colloidal stability can be easily oxidized and aggregated rapidly in the solution, causing concern regarding biosafety [9,11–13]. As a result, facile fabrication of iron nanoparticles with good solubility, high bioavailability, and acceptable biocompatibility remains challenging.

Protein-mediated fabrication of functional nanomaterials with desirable properties has attracted research attention due to its simple procedure, mild synthesis conditions, and good reproducibility [14]. Various nanostructures with fascinating features have been fabricated by using proteins as stabilized agents via mimicking biomineralization processes [15,16]. Albumin is often chosen as a biocompatible template for the synthesis of water-soluble nanoparticles due to its abundant hydrophilic functional groups, such as thiol, amine, and carboxyl groups [17]. Albumin has been approved by the U.S. Food and Drug Administration (FDA) for clinical use, showing reliable biosafety. Therefore, it is very attractive to develop albumin-directed Fe nanoparticles with good solubility and biocompatibility for Fe fortification through the facile biomineralization-mimicking method.

Here, we present novel albumin-protected Fe nanoparticles with good solubility and inherent biocompatibility for IDA treatment. We also compared the bioavailability of Fe nanoparticles and $FeSO_4$. Systematic results demonstrated that the proposed albumin-protected Fe nanoparticles have the same high bioavailability as $FeSO_4$, showing good potential for clinical translation.

Material and Methods

Materials and reagents

Double-distilled water was used throughout this work. Bovine serum albumin (BSA) and methyl thiazolyl tetrazolium (MTT) were bought from Aladdin Reagent Co., Ltd. (Shanghai, China). $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot H_2O$, and $NaBH_4$ were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Synthesis of BSA-Fe nanoparticles

BSA-Fe nanoparticles were fabricated by *in situ* chemical reduction of $FeCl_3 \cdot 6H_2O$ in BSA [10]. Briefly, 250 mg of BSA was mixed with 0.1 M $FeCl_3 \cdot 6H_2O$ in 10 mL of water. Fe(III) ions bound to the functional groups of BSA were then chemically reduced to Fe by adding $NaBH_4$ (0.2 M). After reacting for 30 min, the final product was obtained through dialysis and freeze-drying. The lyophilized powder of BSA-Fe nanoparticles was stored at –4°C until further use.

Characterizations

The morphology and size of BSA-Fe nanoparticles were characterized via high-resolution transmission electron microscopy (HRTEM, Philips, Holland). Fourier trans-form infrared (FT-IR) spectra of BSA and BSA-Fe were recorded with a spectrometer (Nicolet, USA) ranging from 650 to 4000 cm^{-1}. The hydrodynamic size of the BSA and BSA-Fe nanoparticles was measured on a Malvern Zetasizer instrument (Nano series ZS, UK). The Fe content in BSA-Fe was determined through atomic absorption spectroscopy (AAS).

In vitro solubility

The *in vitro* solubility of BSA-Fe and $FeSO_4$ was tested as follows: A compound containing 20 mg Fe was dissolved in 250 mL of hydrochloric acid (0.1 M, pH 1). The mixture was then placed on a shaker at 200 rpm at 37°C. The percentage of dissolved Fe element was measured after 5, 15, 30, 45, and 60 min. The Fe content of the supernatant solution after centrifugation was measured by AAS.

Cell viability assay

4T1 cells (a mouse-derived breast cancer cell line) and 3T3 cells (a mouse embryonic fibroblast cell line) were regularly cultured in RPMI-1640 (Hyclone, USA) supplemented with 10% FBS (Gibco, USA) and 1% penicillin-streptomycin (Hyclone, USA) in an atmosphere of 5% CO_2 at 37°C. Cell viability of BSA-Fe was measured through MTT assay in 4T1 cells and 3T3 cells. 4T1 cells and 3T3 cells were seeded in a 96-well plate at a density of 8×10^3 cells/well in 200 µL of fresh culture medium

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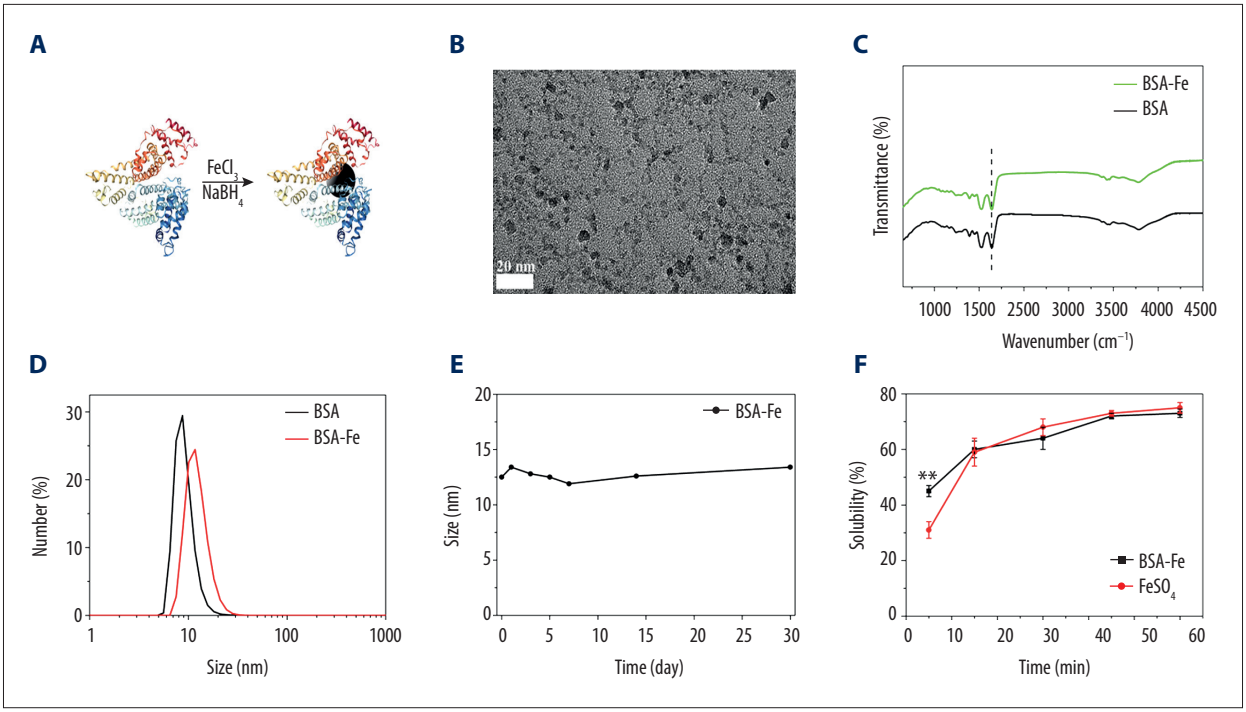

Figure 1. Synthesis and characterizations of BSA-Fe nanoparticles. (**A**) Schematic illustration of the fabrication for BSA-Fe nanoparticles. (**B**) HRTEM of BSA-Fe nanoparticles. (**C**) FT-IR spectra of BSA and BSA-Fe nanoparticles. (**D**) Hydrodynamic sizes of BSA and BSA-Fe nanoparticles. (**E**) Hydrodynamic size monitoring of BSA-Fe nanoparticles. (**F**) Solubility of BSA-Fe nanoparticles and $FeSO_4$ at pH 1.

at 37°C and 5% CO_2. After 24-h incubation, the stale medium in each well was replaced with 200 μL of fresh medium containing different concentrations of BSA-Fe nanoparticles. After another 24-h incubation, the cells were incubated with fresh medium containing 10 μL of MTT (5 mg mL^{-1}). Four hours later, the supernatant in each well was replaced with 150 μL of DMSO. Finally, the cell viabilities were calculated depending on the absorbance values at 490 nm of each well.

The bioavailability and biocompatibility *in vivo*

All the animal experiments throughout this work were approved by the Animal Care and Use Committee of our institution. The bioavailability of BSA-Fe nanoparticles was investigated by the hemoglobin (Hb) repletion method. Sprague-Dawley (SD) rats (n=50, 21±2 days old) were housed and fed individually in stainless steel cages. The rats were kept with a 12-h light/dark cycle at 24°C. The anemic rat model was built according to a previous report by an Fe-deficient (Fe-def) diet (2.5 mg Fe/kg) for 14 d. After the depletion period, rats were randomly assigned to 5 groups. The rats in each group consumed the same Fe-def diet with different fortificants at 2 levels ($FeSO_4$, 10 or 20 mg/Kg diet; BSA-Fe, 10 or 20 mg/Kg diet), or no added Fe (Fe-def, 2.5 mg Fe/kg diet) for 10 d. In the meantime, 10 rats (35 days old) fed normal diets were used as the normal control group. During the 10-d period, these rats were weighed every 2 days. At the tenth day, the blood samples of these rats were collected for assessment of Hb, red blood cells count (RBC), and other biochemical indicators (e.g., alanine aminotransferase, ALT; aspartate aminotransferase, AST; creatinine CREA; urea). The vital organs (heart, liver, spleen, lung, and kidney) of these rats were extracted for comparisons in the histological assessment.

Statistical analysis

All quantitative data are presented as means ±SEM. Significance differences between groups were determined by the 2-tailed *t* test. All statistical analyses were calculated with SPSS version 15. $P<0.05$ was considered significant.

Results

Synthesis and characterizations of BSA-Fe nanoparticles

Good water solubility is important for Fe nanoparticles to prevent strong colloidal aggregation when used in drinks and foods [18,19]. In this work, the Fe nanoparticles were fabricated through a one-pot facile reduction process under the protection of BSA (Figure 1A). When $FeCl_3 \cdot 6H_2O$ is mixed with BSA by stirring, Fe (III) ions can bind strongly onto BSA due to

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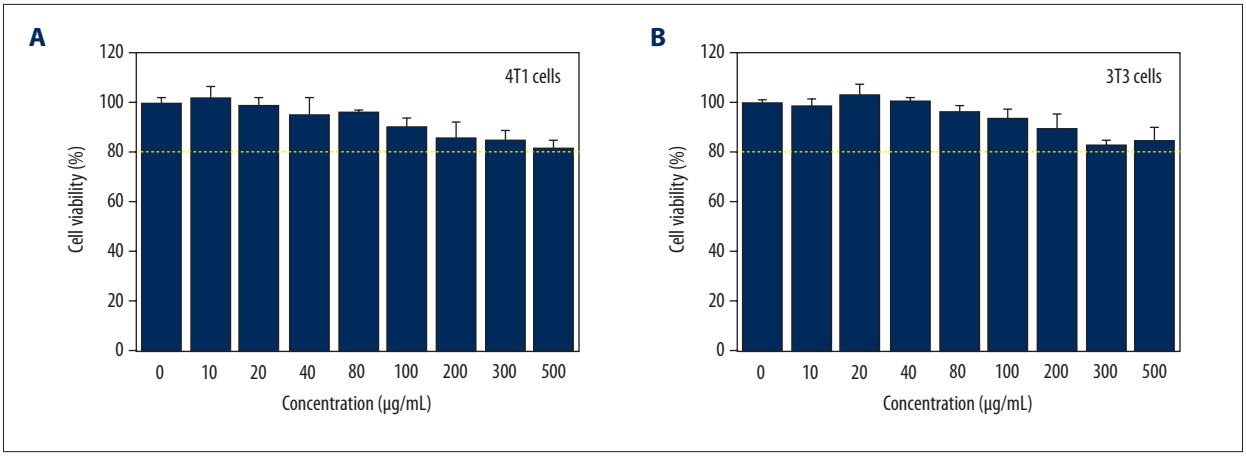

Figure 2. Cytotoxicity of BSA-Fe nanoparticles in 4T1 cells and 3T3 cells. (**A**) Cell viability of 4T1 cells when incubated with different concentrations of BSA-Fe nanoparticles. (**B**) Cell viability of 3T3 cells when incubated with different concentrations of BSA-Fe nanoparticles.

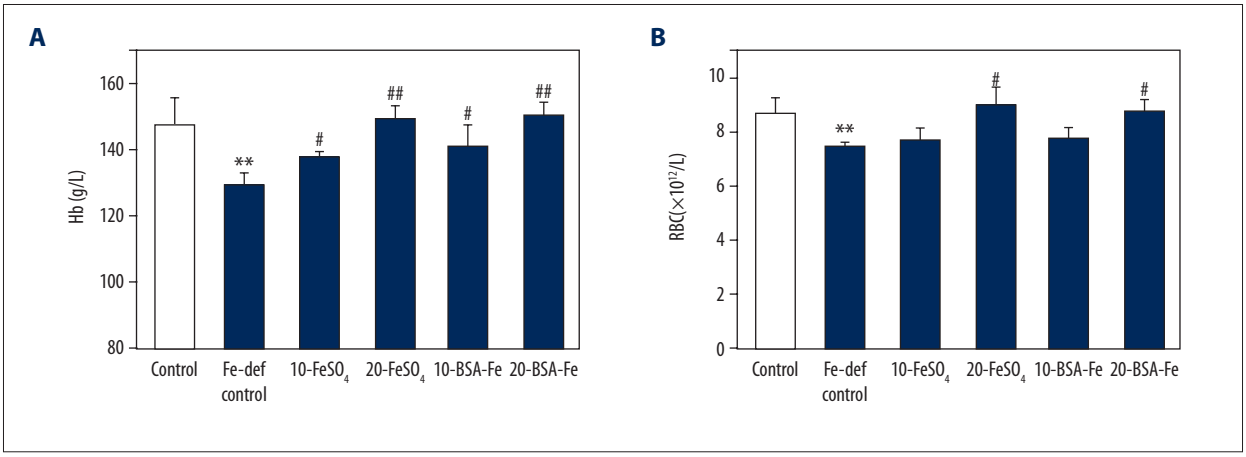

Figure 3. Bioavailability of BSA-Fe nanoparticles *in vivo*. (**A**) Serum Hb levels of rats treated with different fortifications. (**B**) RBC levels of rats treated with different fortifications. (** $p<0.01$ when compared with control group; # $p<0.05$ when compared with Fe-deficiency control group; ## $p<0.01$ when compared with Fe-deficiency control group).

the high affinity between Fe element and functional chemical groups (e.g., carboxy group, amino group, and sulfhydryl group) of BSA. After adding $NaBH_4$, iron ions are converted immediately into Fe nanoparticles by the strong reducing agent. Thanks to the protection of BSA as the reaction template, the size of Fe nanoparticles was under control. As shown in Figure 1B, the HRTEM image of BSA-Fe nanoparticles showed a round geometry with a super-tiny size of only <10 nm. FT-IR spectra showed the presence of BSA in obtained BSA-Fe nanoparticles (Figure 1C). BSA has perfect water solubility; therefore, BSA-protected Fe nanoparticles have a hydrodynamic size of only about 10 nm (Figure 1D). The hydrodynamic size of BSA-Fe nanoparticles had only negligible change during an observation period as long as 30 days (Figure 1E), exhibiting good water solubility and colloidal stability. To further mimic the actual gastric acid environment when the Fe fortificants are orally administrated, the solubility of BSA-Fe nanoparticles was investigated at pH 1. As shown in Figure 1F, BSA-Fe nanoparticles and $FeSO_4$ dissolved well at pH 1. Interestingly, the BSA-Fe nanoparticles were more soluble than $FeSO_4$ after 5 min, but there was no significant difference between the solubility of the BSA-Fe nanoparticles and $FeSO_4$ at the other time points. Our results show that the BSA-Fe nanoparticles fabricated through the facile one-pot reducing method have good water solubility, even better than that of $FeSO_4$.

The cytotoxicity of BSA-Fe nanoparticles

The cytotoxicity of the BSA-Fe nanoparticles was estimated through the cellular MTT assay with 4T1 cells and 3T3 cells. As shown in Figure 2, BSA-Fe nanoparticles with different concentrations ranging from 0 to 500 μg/mL resulted in negligible decrease in cell viability in 4T1 cells and 3T3 cells. Even at concentrations as high as 500 μg/mL, the cell viability was still

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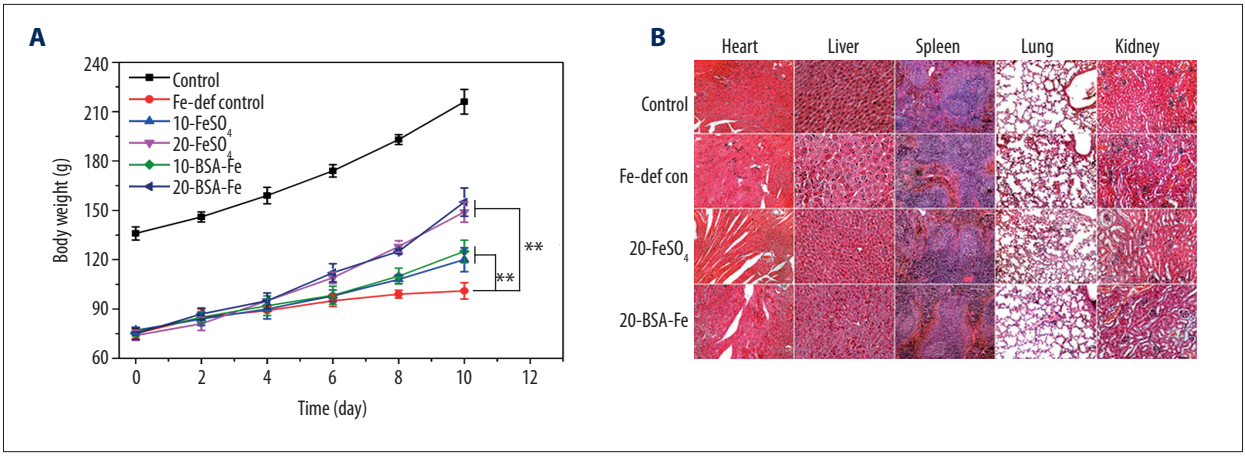

Figure 4. (**A**) Body weights of rats treated with different fortifications. (** $p<0.01$ when compared with Fe-deficiency control group). (**B**) Histopathological results of vital organs (heart, liver, spleen, lung and kidney) from rats treated with different fortifications.

above 80%. Low cytotoxicity is the essential requirement for BSA-Fe nanoparticles for further *in vivo* applications.

The bioavailability and biocompatibility of BSA-Fe nanoparticles *in vivo*

The bioavailability of BSA-Fe nanoparticles was investigated by the Hb repletion method [20]. As shown in Figure 3, the anemia rat model established through the Fe-deficiency diet showed obvious decrease in Hb and RBC levels compared with the normal control group. The Fe fortification of BSA-Fe nanoparticles and $FeSO_4$ can significantly elevate the Hb level of anemia rats, especially with higher Fe content (Figure 3A), and there was no significant difference between BSA-Fe nanoparticles and $FeSO_4$ at the same dosage of Fe. Even low Fe fortification (10 mg/Kg) made no significant contribution to the RBC enhancement; RBC levels of anemia rats obviously increased when given higher dosage of BSA-Fe nanoparticles and $FeSO_4$ and no significant difference was observed between them (Figure 3B). In addition, the body weights of anemia rats also remarkably increase during the treatments, especially with higher Fe fortification (Figure 4A). All these results demonstrate that the proposed BSA-Fe nanoparticles have bioavailability comparable to that of the most widely used Fe fortificants of $FeSO_4$.

Besides good bioavailability, biosafety is another important concern for further clinical translations. After Fe fortification, the vital organs of rats were extracted. The histopathological results showed no obvious histological changes in the susceptible organs (heart, liver, spleen, lung, and kidney) for 10 d after administrations of BSA-Fe nanoparticles and $FeSO_4$ when compared with the normal control group (Figure 4B). Blood biochemical analysis indicated that administration of BSA-Fe nanoparticles did not damage liver or kidney function (Figure 5). These results demonstrate the good biocompatibility of BSA-Fe nanoparticles for further clinical use.

Discussion

Iron deficiency is one of the highest risk factors for death and disability all over the world [1]. Targeted iron supplementation or food iron fortification is a reliable way to correct iron deficiency in populations [21]. Although $FeSO_4$ with high bioavailability is widely used as effective Fe fortificants, it is still criticized for causing adverse sensory changes in foods [22]. Recently, ferrous nano-compounds such as ferric phosphate ($FePO_4$) and Fe nanoparticles [9,10,20] have attracted increased attention due to their good potential as substitutes of $FeSO_4$.

For clinical translation of ferrous nano-compounds, several essential requirements must be met, such as simple production, comparative bioavailability, and good biocompatibility [23,24]. In the present work, BSA-Fe nanoparticles were fabricated through a one-pot facile reducing method within just 30 min. The whole procedure was conducted at room temperature without any other harsh conditions like starvation, high temperature, or high pressure. This simple, mild, and fast fabrication makes BSA-Fe nanoparticles suitable for further industrial production. Data show that BSA-Fe nanoparticles have good water solubility and stability, even in acidic environments. The comparative bioavailability with $FeSO_4$ can be also found in BSA-Fe nanoparticles. In addition, BSA-Fe nanoparticles showed remarkable biocompatibility both *in vitro* and *in vivo*. The BSA-Fe nanoparticles proposed in this work have a good potential for clinical translation.

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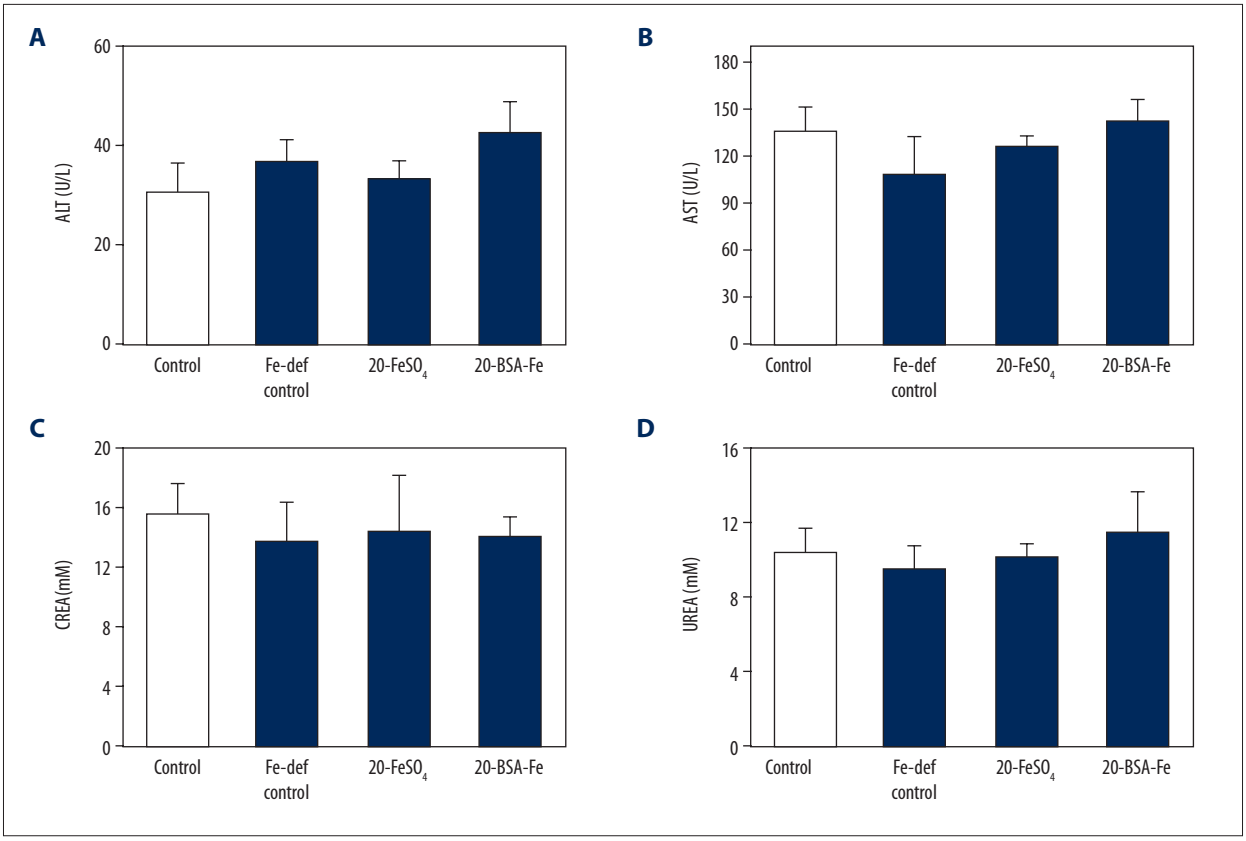

Figure 5. Biochemical indicators of ALT (**A**), AST (**B**), CREA (**C**), and UREA (**D**) in rats after different fortifications.

Conclusions

We fabricated the water-soluble BSA-Fe nanoparticles using a one-pot facile reducing method. It has bioavailability comparable to that of $FeSO_4$ and acceptable biocompatibility, showing good potential for further clinical translations.

Conflict of interest

None.

References:

1. Camaschella C: Iron-deficiency anemia. N Engl J Med, 2015; 372: 1832–43
2. Miller JL: Iron deficiency anemia: A common and curable disease. Cold Spring Harb Perspect Med, 2013; 3: 114–19
3. Heeney MM, Finberg KE: Iron-refractory iron deficiency anemia (IRIDA). Hematol Oncol Clin North Am, 2014; 28: 637–52
4. Powers JM, Buchanan GR: Diagnosis and management of iron deficiency anemia. Hematol Oncol Clin North Am, 2014; 28: 729–45
5. Subramaniam G, Girish M: Iron deficiency anemia in children. Indian J Pediatr, 2015; 82: 558–64
6. Nielsen OH, Ainsworth M, Coskun M, Weiss G: Management of iron-deficiency anemia in inflammatory bowel disease: A systematic review. Medicine (Baltimore), 2015; 94: 963–68
7. Acosta E: Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Curr Opin Colloid In, 2009; 14: 3–15
8. Storey P, Lim RP, Chandarana H et al: MRI assessment of hepatic iron clearance rates after USPIO administration in healthy adults. Invest Radiol, 2012; 47: 717–24
9. Pereira DI, Bruggraber SF, Faria N et al: Nanoparticulate iron(III) oxo-hydroxide delivers safe iron that is well absorbed and utilised in humans. Nanomedicine, 2014; 10: 1877–86
10. Shen Y, Posavec L, Bolisetty S et al: Amyloid fibril systems reduce, stabilize and deliver bioavailable nanosized iron. Nat Nanotechnol, 2017; 12: 642–47
11. Hilty FM, Arnold M, Hilbe M et al: Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nat Nanotechnol, 2010; 5: 374–80
12. Rohner F, Ernst FO, Arnold M et al: Synthesis, characterization, and bioavailability in rats of ferric phosphate nanoparticles. J Nutr, 2007; 137: 614–19
13. Powell JJ, Bruggraber SF, Faria N et al: A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity. Nanomedicine, 2014; 10: 1529–38
14. Yao S, Jin B, Liu Z et al: Biomineralization: From material tactics to biological strategy. Adv Mater, 2017; 29: 110–25
15. Xie JP, Zheng YG, Ying JY: Protein-directed synthesis of highly fluorescent gold nanoclusters. J Am Chem Soc, 2009; 131: 888–89
16. Chen Q, Wang C, Zhan Z et al: Near-infrared dye bound albumin with separated imaging and therapy wavelength channels for imaging-guided photothermal therapy. Biomaterials, 2014; 35: 8206–14
17. Chen Q, Liang C, Wang X et al: An albumin-based theranostic nano-agent for dual-modal imaging guided photothermal therapy to inhibit lymphatic metastasis of cancer post surgery. Biomaterials, 2014; 35: 9355–62

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18. Huber DL: Synthesis, properties, and applications of iron nanoparticles. Small, 2005; 1: 482–501
19. Zimmermann MB, Hurrell RF: Nutritional iron deficiency. Lancet, 2007; 370: 511–20
20. Rohner F, Ernst FO, Arnold M et al: Synthesis, characterization, and bioavailability in rats of ferric phosphate nanoparticles. J Nutr, 2007; 137: 614–19
21. De Andrade Cairo RC, Rodrigues Silva L et al: Iron deficiency anemia in adolescents; A literature review. Nutr Hosp, 2014; 29: 1240–49
22. Kassebaum NJ, Jasrasaria R, Naghavi M et al: A systematic analysis of global anemia burden from 1990 to 2010. Blood, 2014; 123: 615–24
23. Aslam MF, Frazer DM, Faria N et al: Ferroportin mediates the intestinal absorption of iron from a nanoparticulate ferritin core mimetic in mice. Faseb J, 2014; 28: 3671–78
24. Latunde-Dada GO, Pereira DI, Tempest B et al: A nanoparticulate ferritin-core mimetic is well taken up by HuTu 80 duodenal cells and its absorption in mice is regulated by body iron. J Nutr, 2014; 144: 1896–902

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]