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RESEARCH ARTICLE

Safety Evaluation of Nano Iron Zero Valente Green Synthesized: A Comparative Study

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ABSTRACT

Objective(s): Nowadays, examining the toxicity of nanoparticles including the synthesized and functionalized iron nanoparticles using methods like green synthesis is highly considered, due to their increasing usage in various fields of medicine, biology, industrial, and pollution removal. Hence, in this study, the toxicity of the nano Fe0 particles functionalized the Myrtus communis (MC-ZVINP) was investigated

Methods: Cell line of human skin (normal fibroblast) was used to examine cellular toxicity using the MTT method. Also, biochemical factors such as liver enzymes level, and factors such as the number of white and red globules, lymphocytes, platelets, amount of blood hemoglobin, and histopathological test of liver tissue in laboratory small rats were examined after intraperitoneal injections of the MC-ZVINP with different concentrations daily and a duration of 3-month, with the groups receiving trivalent iron, the extract of plant-case, and normal saline.

Results: Cytotoxicity concentration of iron-case nanoparticles was obtained for 50% of HFF cells (CC50=149.23±4.45µg/mL). The results obtained from the blood factors examination showed a decreased the serum level of liver enzymes as well as an increase in the number of red and white globules and hemoglobin rate in mice receiving iron nanoparticles compared to the trivalent iron receiving group. Receiving the concentrations of 100 and 200 mg/kg/bw of iron nanoparticles have caused the incidence of mild and moderate inflammation in the liver of mice.

Conclusions: Generally, it can be concluded that, the MC-ZVINP have shown no significant toxicity on the levels of blood cells, enzymes, and liver tissue.

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INTRODUCTION

The worldwide spread of nanotechnology and the increasing usage of the products of this technology including Nano-sized metals to apply them in the health sector and in the field of human health are highly considered [1-3]. Iron is an essential nutrient for many cellular processes including oxygen transfer, energy generation, and DNA production [4, 5]. The conversion of iron from the oxidation form to the reduction form and vice versa has enabled it to play an effective role in the activity of

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many important enzymes involved in biochemical interactions [6]. Dysfunction of iron-regulating mechanisms in the body and/or receiving iron out of the body's physiological range lead to its deficiency or overloading in the body, which can be effective in causing some illnesses such as diabetes, coronary arteries disease of heart, and creation or recurrence of some types of cancer [4, 7, 8]. Furthermore, excessive receive of iron may cause free and reactive oxygen radicals [9]. One of the most important destructive effects of free radicals is the onset of lipids peroxidation, which can cause damages to the cell membrane. These radicals can lead to produce lipid peroxidase by alkylation protein groups and other cellular macromolecules as well as attacking to unsaturated fatty acids [10, 11]. Accordingly, this matter can lead to hepatic necrosis, which leads to elevated serum levels of alkaline phosphatase and aminotransferases enzymes. Increasing the levels of these enzymes is conventionally indicator of hepatic damages [12]. Iron nanoparticles are one of the first nanoparticles used in the purification process. So that, zero-valente iron nanoparticles, due to their high capacity in oxidation and reduction reaction, have been considered for water and soil purification and also for the removal of a wide range of contaminants such as hydrocarbon compounds, chlorinated compounds, nitro-benzenes, phenol chloride, biphenyl poly-chloride, and heavy metals and ions [13]. These nanoparticles have also highly reactive surfaces and a high potential for functionalization, so that they can be used by targeting the functionalization of these nanoparticles in various medical and biological fields such as magnetic drug-delivery systems, biotechnology, magnetic resonance imaging, hyperthermia, biodegradation, biosensors, and pollution removal [14]. Recently, the synthesis of iron nanoparticles has been considered by researchers using polyphenolic compounds derived from plant extracts [15-17]. There are relatively few reports on the toxicity of zero-valente iron nanoparticles [18, 19]. Clarifying the toxicity of zero-valente iron nanoparticles is particularly important, due to the increasing usage of these nanoparticles in water purification, soil remediation, and medicine. The phenyl groups and the hydroxyl groups present in these compounds give them the characteristics of a good coating agent to stabilize the active surfaces of the nanoparticles and also to reduce the bio-toxicity of the produced nanoparticles [20]. The plant-case has various phenolic compounds [21], which can be used to synthesize the iron nanoparticles in a green method and can also reduce the toxicity of iron nanoparticles in regard to its therapeutic properties [22-24]. It is important to note that, liver diseases and the incidence of significant damages to the liver cells will be a threat for life. For this reason, efforts are increasing to discover and use the effective compounds with no detrimental effects on the liver. With regard to the numerous usages of iron nanoparticles [25], up to now, few studies have investigated the toxicity of zero-valente iron nanoparticles, and these studies have been mainly conducted on bacteria, as well as the therapeutic uses of the *Myrtus communis* plant. Also, no studies have been conducted to examine the toxicity of green synthesis of iron nanoparticles using the plant extract; the aim of this study was to evaluate the toxicity of zero valente nano-iron particles that were synthesized using the green method (MC-ZVINP) [26] at the cellular level and in the animal model.

MATERIALS AND METHODS

Bio-synthesis of zero valente iron nanoparticles

The MC-ZVINP were prepared by reducing Fe (III) to Fe (0) using *Myrtus communis* leaf methanolic extract (MCLE) and ascorbic acid without air evacuation. 12 g L^{-1} of solution prepared from the extract in 50 ml of 0.1 ml NaOH was poured in a burette and added drop by drop into 0.1 M iron chloride and 0.1 M ascorbic acid solution with strenuous shaking. The immediate appearance of a black color suggested the reduction of iron ions. After 30 minutes stirring the reaction ends. The obtained MC-ZVIN were centrifuged at 20 000 rpm for 20 minutes, then washed using deionised water and ethanol to eliminate any unreacted biomolecules. The resultant nanoparticles were redispersed in normal saline for further studies. The bio-synthesized MC-ZVIN are biphasic in structure and amorphous in nature (Based on the XRD pattern), spherical with a diameter ranging 40-60 nm (Based on the SEM image). The TEM image exhibits a transparent organic layer coating around the nanoparticles, which was due to the surrounding phytochemicals that serve as a capping agent to prevent agglomeration (Fig. 1). The zeta potential of the MC-ZVIN (−22.4 mV), [26].

Culture and Preparation of Human normal Foreskin Fibroblast Cells

Cell line of human skin (normal fibroblast) was provided by Pasteur Institute (Amol, Iran). The fibroblasts were implanted in culture medium

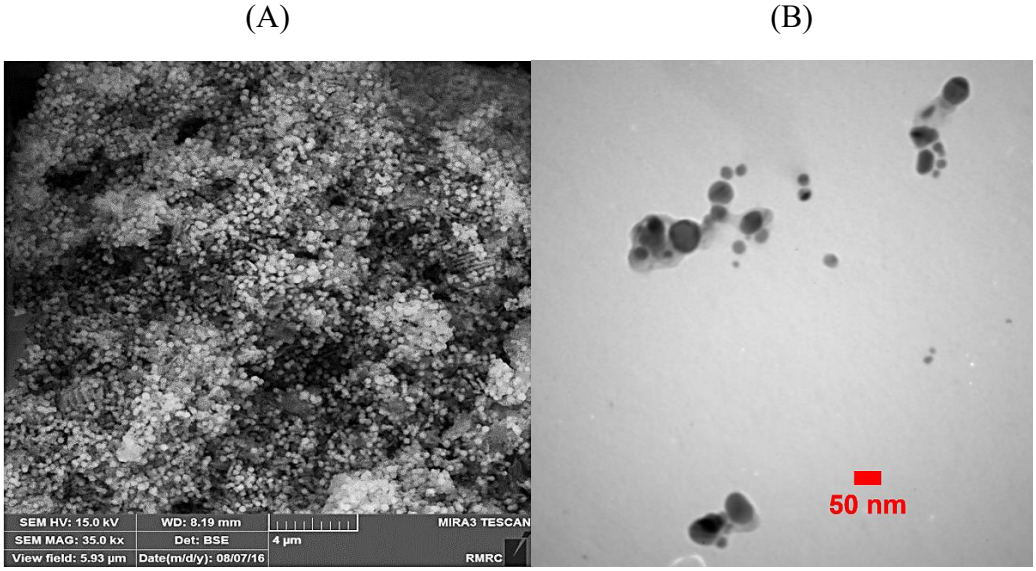

Fig. 1. (A) SEM image, (B) TEM image of zero valente iron nanoparticles and the bio-organic components of *Myrtus communis* leaf extract.

of DMEM/F12 containing serum of fetal bovine (10%) as supplementation, streptomycin (100 µg mL^{-1}) and penicillin (10,000 IU mL^{-1}). The cells were stored in an incubator containing CO_2 gas (5%) at the same temperature as the human body. Treatment was performed by replacing the fresh medium containing different concentrations of the desired compounds by passing 24h from cells culture on 96-home plates.

cytotoxicity examination (MTT)

To determine the effects of biocompatible plant nanoparticles containing zero valente iron on cell survival; the cells were exposed at concentrations (0.78125–200 µg/mL) per ml of the MC-ZVINP after one night and day of their implantation on the plates. After a 48-hour incubation, the exposure time was terminated by removal of the culture medium and the cells were cleaned with saline phosphate buffer (PBS). MTT dye in the amount of 100µL, added to the well containing the cells so that at the end of the concentration reached 5.0 mg ml^{-1}, after 4 hours incubation with derived formazan crystals, it was dissolved in DMSO. Also, blue-violet light absorption at wavelength 570 nm was read to determine cell survival. Inverted metallurgical microscopy was used to investigate the morphology of the cells. Equation $(A_S / A_C) \times 100$ was considered to find the survival rate and cell proliferation (A is optical absorption for the sample and the control). The concentration of MC-ZVINP was determined to estimate the inhibitory effects of MC-ZVINP on the growth of (HFF) cells, which cause cytotoxicity incidence in 50% of the cells (CC_{50}). In all the treatments, CC_{50} values were expressed using nonlinear regression compared to evaluation control and as (Mean ± S.D).

animal study

This study was performed on laboratory small mice in the weight range of 20 ± 5 g. Animals were placed at the animal-house of Pharmacy Faculty of Medical Sciences University of Mazandaran under the temperature conditions 25 ± 1 °C, 5 ± 60% humidity, 12h light, and 12h in darkness, and after one week of animal habituation to the cage environment, mice were categorized into 6 groups at random so that each group had 6 mice.

The first experimental group was with no treatment, receiving normal saline and was considered as the control group. The second group was the receiver of 200 mg/kg/bw concentration of *Myrtus communis* extract, the third group received only 200 mg/kg/bw concentration of trivalent iron. The fourth group received a concentration of 50 mg/kg/bw of nano Fe^0 particles synthesized with the plant extract daily and through intraperitoneal injection and for three months, the fifth group and

the sixth group received 100 and 200 mg/kg/bw concentration of these nanoparticles in the same way. Then, blood collection from mice and separating the liver tissues were done, to perform subsequent tests by observing ethical principles and under complete anesthesia conditions with ether.

Blood parameters Examination

Examining biochemical factors such as the levels of AST, ALT and ALP hepatic enzymes was done by Pars test kits using colorimetric method and BT3000 automatics assessment system. Factors such as the numbers of white and red globules, lymphocytes, platelets, and blood hemoglobin amount were also measured.

Histopathological examination

At the end of the 3-month period, all mice were placed in an anesthetic closed container containing ether-impregnated cotton, and abdominal area was cut after anesthesia, and after removing the liver tissue (cuts of different lobes were provided in all groups) and washing it with physiological serum, it was placed in a fixator (10% formalin) (the fixator was replaced for three consecutive days). After 72 hours, tissue preparation stages were performed and microscopic sections with 5 µm thickness were prepared for examining the tissue damages. The slides were stained through hematoxylin and eosin method, and were then prepared for being studied with optimal microscopy. Ten slides were provided from each specimen, and different parts of each slide were randomly examined in 10 fields. The specimens were blindly observed and confirmed by the pathologist.

Statistical analysis method

In order to analyze the data, the mean and standard deviation were calculated and entered into SPSS software, and judgment was made among the groups with the obtained P-value using ANOVA and tukey tests. Here, the groups receiving iron nanoparticles were separately compared separately with the groups receiving trivalent iron and control.

RESULTS AND DISCUSSION

Cytotoxicity evaluation

At First, the effects of iron-case nanoparticles on cells viability were investigated using MTT method for 48 hours. The results of Fig. 2 show that, MC-ZVINP at the concentration of 149.23 µg/mL has caused toxicity in 50% of growth HFF cells.

In the present study, the cytotoxicity of iron-case nanoparticles was concentration-dependent, so that by increasing the concentration, cell viability rate decreased.

Since the toxicity of nanoparticles on different cell lines is varied depending on the property of the nanoparticles and the cell type under examination; various researches have been conducted to investigate the subsequent effect of iron nanoparticles on different cells, and their results expressed the effect of nanoparticle concentration as well as cell line applied on cell death. So that, by increasing the concentration of nanoparticles, their cytotoxicity also increases, and of course this toxicity effect cannot observed in all cells [27].

The survival of human bronchial mucosal tissue cells was investigated in the presence of zero-valente iron nanoparticles synthesized by chemically resolved in physiological serum and divalent iron at concentrations of 100 and 200 µM resolved in physiological serum. These cells have had a higher survival rate in confronting zero-valente iron nanoparticles compared to divalent iron [28], which is consistent with our study showing that, the iron-case nanoparticles have less toxicity than trivalent Iron.

Studies show that, chemical zero-valente iron nanoparticles can cause toxic effects on the microglia of rodent neurons [29].

The most important mechanism that can be considered for existence of the toxicity effect for these nanoparticles, is the production of oxygen free radical, which when its concentration exceeds the specified and controlled amount inside the cell, will cause the incidence of negative effects on the function of intracellular organelles such as mitochondria, proteins, and even DNA, which eventually leads to cell death [30-32].

Other studies have also shown that, the most important mechanism of toxicity after exposure to zero-valente nanoparticles, is oxidative stress [33].

Modification of zero-valente iron nanoparticles; for example, coating zero-valente iron nanoparticles with Asparaginate through reducing the direct contact of nanoparticles with cells has resulted in toxicity reduction [32].

Biochemical study

Assessment of AST, ALT and ALP enzymes in terms of serum levels

The results of biochemical parameters assay are shown in Fig. 3, and indicated that the serum

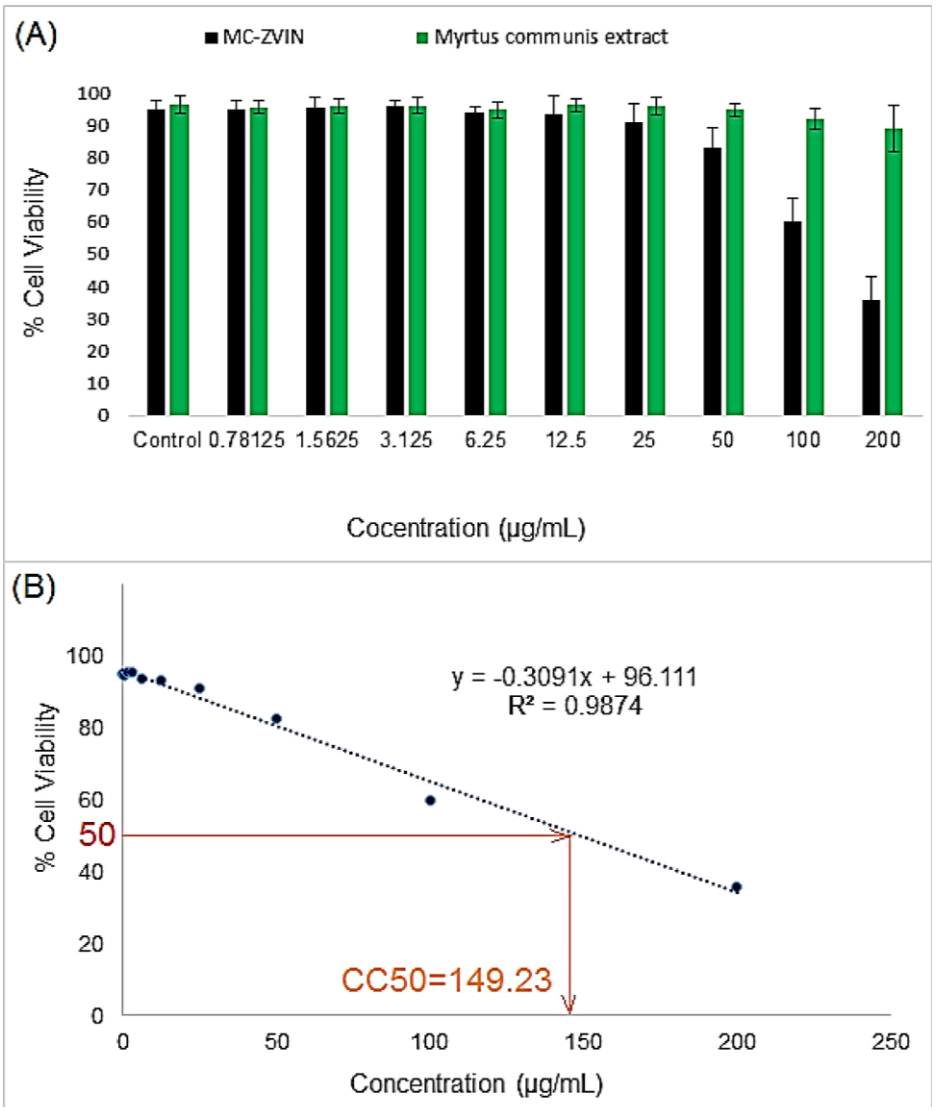

Fig. 2. Cytotoxicity of green synthesized MC-ZVINP (A) on cell line of human skin (normal fibroblast) and CC_{50} calculation method (B).

activity of ALT and ALP enzymes in mice receiving trivalent iron, has significantly increased compared to the group receiving the normal saline ($P < 0.001$) while, in the mice that received iron nanoparticles in different doses, serum concentrations of AST, ALT, and ALP enzymes have significantly decreased compared to the group receiving the trivalent iron ($P < 0.05$).

Therefore, in the present study, it can be said that, unlike trivalent iron, detrimental and destructive effects on serum level of liver enzymes such as AST, ALT and ALP were not observed by injection of the MC-ZVINP. In other word, it does not only create any increase in the level of important hepatic enzymes, but also can significantly modify the destructive and additive effects of iron on these enzymes. Because the first step in the diagnosis of liver damage is to do a simple blood test showing the presence of sensitive and high-consumption liver enzymes such as AST, ALT, and ALP aminotransferases. Under normal conditions, these enzymes exist in the liver cells, but they enter the bloodstream when the liver is damaged [34].

However, other studies reported this matter that, serum levels of ALT and AST enzymes are directly affected by poisoning with the iron

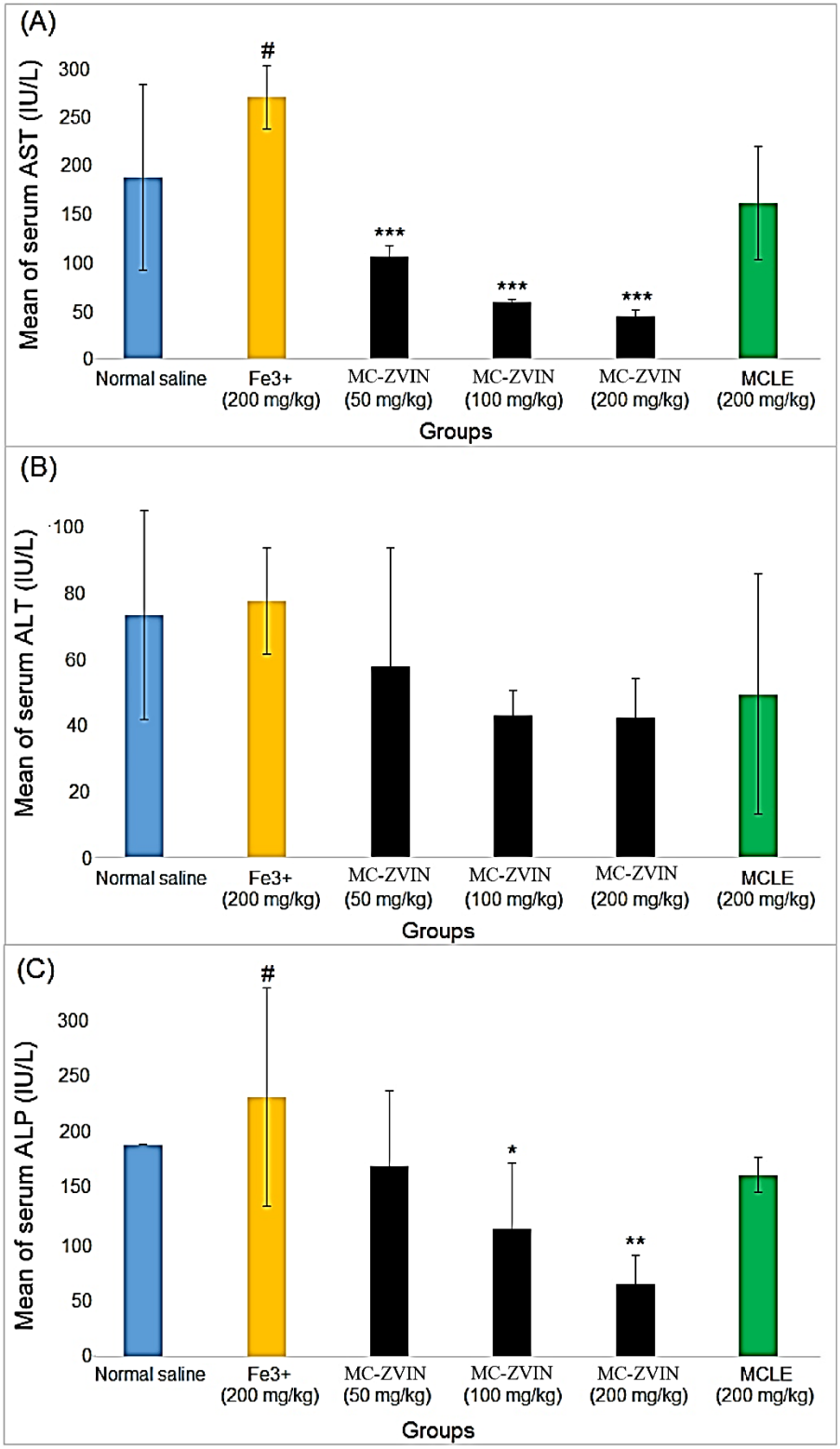

Fig. 3. Serum concentrations of liver enzymes in mice; (A) AST, (B) ALT and (C) ALP (n=6). Values expressed as mean ± standard deviation. $P < 0.01$ (*), $P < 0.001$ (**) and $P < 0.0001$ (***) compared with Fe^{3+} group.

nanoparticles [35].

Significant increase was observed in ALT, AST, and ALP enzymes levels in the groups receiving trivalent iron compared to the group receiving normal saline. Investigation of the safety and pharmacokinetics properties of iron particles indicated that, ferumoxtran-10 particles can also lead to alter liver enzymes [36].

Also, *Briley et al.* (2006) showed that, the high concentrations of iron oxide nanoparticles can have adverse effects on the liver function [37].

However, the study of *Eslami et al.* showed that, the plant-case extract prevented the increase of serum level of hepatic enzymes caused by iron accumulation in the liver of thalassemia model mice that have iron overload [26].

In this study, it was also found that, using the plant-case for green synthesis of iron nanoparticles have not led to an increase in liver enzymes and somehow have prevented the increasing of these enzymes through iron nanoparticles and have kept them in lower level.

On the other hand, it has been clarified that, the use of plant-case oily extract can significantly reduce the increased serum level of liver enzymes in the mice exposed to carbon tetrachloride [38]. Therefore, it can be deduced that, one of the reasons for keeping the liver enzymes in low level, is due to the use of the plant-*Myrtus communis* extract in the synthesis of zero-valente iron nanoparticles.

Evaluation of Hematological Parameters

In examining red and white globules count and blood hemoglobin rate (Fig. 4), the number of red globules and blood hemoglobin as well as blood platelets amount in the mice exposed to iron nanoparticles with concentrations of 50 and 100 mg/kg/bw significantly increased compared with the control group; however, number of the white globules showed an statistical decrease in all three doses ($P < 0.0001$). There was significant change in the number of lymphocytes in any of the iron nanoparticles concentrations prescribed for mice ($p < 0.01$).

Numerous studies have investigated the effects of various forms of iron nanoparticles, in the studies that were conducted by *Loeb et al.* (2008) on red blood cells, it was reported that, the iron oxide nanoparticles have toxic effects, which depend on how these nanoparticles enter in body as well as a particle size and its chemical form [39]. In our study, in general, no significant change was observed in the number of blood platelets. In addition, an increase was observed in the numbers of red globules as well as the rate of hemoglobin at some doses ($P < 0.01$). Accordingly, this difference can be due to the reduction of cell lysis and the protective and coating role of the plant-*Myrtus communis* [40], as in other studies, the protective role of different coatings such as magnetic nanoparticle coating with citric acid coating and magnetic nanoparticle coated with silica has been reported, which created no change in the percentage of blood cells by passing one month after the treatment [41].

Histopathologic examination of the liver

Histopathologic results of mice liver tissue (Fig. 5b) showed that, severe inflammation was observed in the trivalent iron receiving group with the presence of hemosiderin pigments around the port space compared to the control group. However, mild and moderate inflammations were observed in the liver of the mice receiving concentrations 100 and 200 mg/kg/bw iron nanoparticles compared to the control group, Fig. (5d, e).

In this study, increase in the concentrations of the synthesized iron nanoparticles has led to more inflammation around the port space. The concentration increase of hemosiderin, which indicated an increase in iron deposition in the cell, was also observed as dose-dependent. While, in the prescription of trivalent iron, severe inflammation of the liver was seen, especially in the areas around the port.

Stability and integrity of hepatocyte membrane are critical for liver functions, and chemical iron oxide nanoparticles disrupt this stability and cause the incidence of liver dysfunction with regard to their physicochemical properties. It has been shown that, accumulation of the nanoparticles in the liver and lungs was observed with one time intravenous injection of iron oxide nanoparticles into mice, which indicated the extensive absorption of these two organs [42]. On the other hand, some researchers have examined liver tissue damages caused by the iron oxide nanoparticles [43]. Studies have shown that, MC-ZVINP cause a decrease in serum iron concentration, which ultimately lead to a decrease in tissue accumulation and subsequently decrease the liver tissue damage [26]. Increasing the limited hepatic inflammation observed in the present study, can be due to the permeability of the nanoparticles to the tissue, as confirmed by other studies. Among the reasons mentioned for this damage, is the

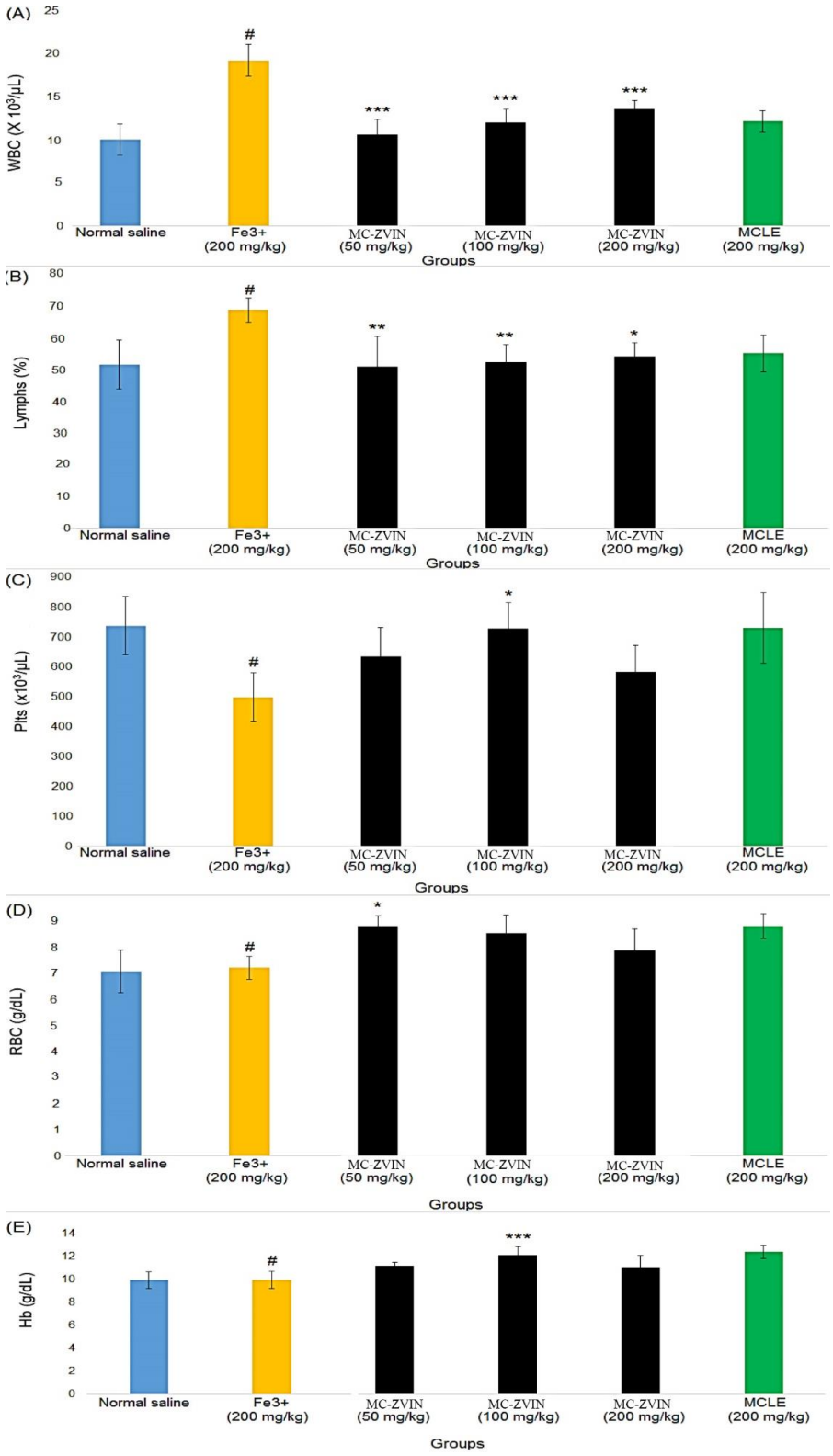

Fig. 4. The values of (A) WBC, (B) percentage lymphocytes (% Lymphs), (C) Platelets (Plts), (D) Red blood cell count (RBC) and (E) Hb in mice treated MC-ZVINP with different concentrations. $P < 0.01$ (*), $P < 0.001$ (**) and $P < 0.0001$ (***) compared with Fe^{3+} group.

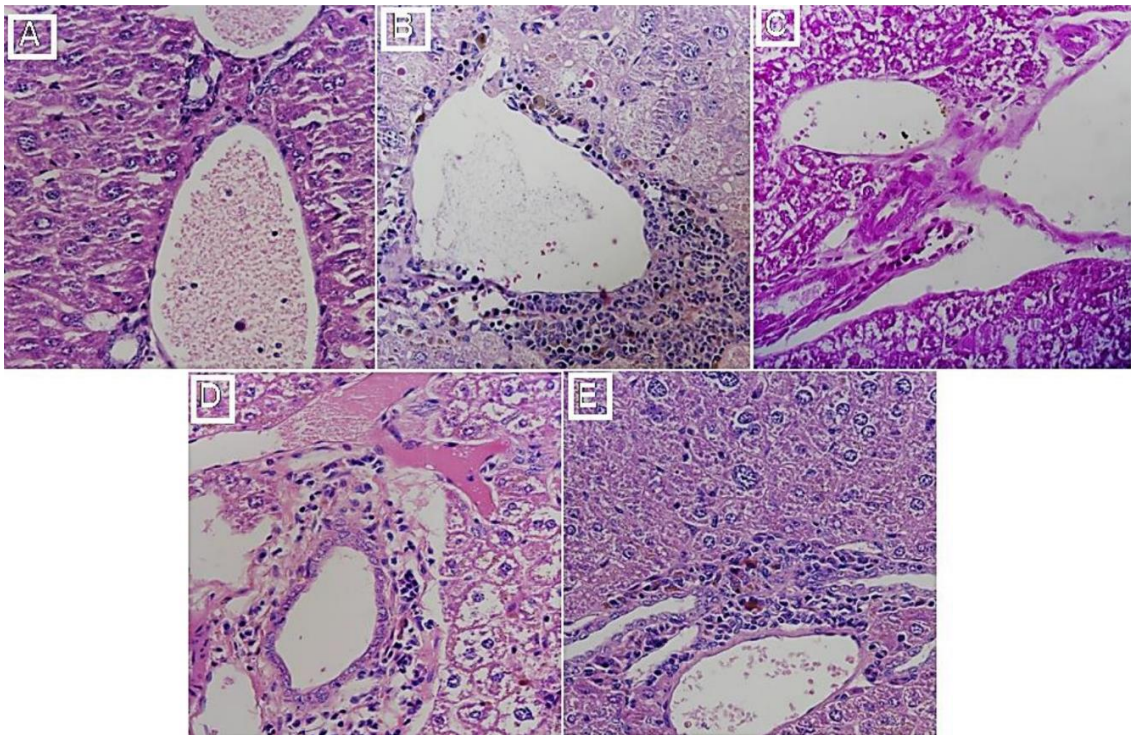

Fig. 5. Histopathologhy of liver sections from mice influenced by (A) Normal saline (control), (B) Fe^{3+} (200 mg/kg), (C) MC-ZVINP 50 mg/kg, (D) MC-ZVINP 100 mg/kg, (E) MC-ZVINP 200 mg/kg (Hematoxylin and Eosin staining, magnification ×400).

production of active free oxygen species in the presence of nanoparticles, which in its turn, can cause damage to tissues, and it was also clarified that, the rate of production of these free radicals is dose-dependent [43-45]. On the other hand, the plant-case has antioxidant properties caused by the existence of phenolic compounds in its extract [22]. Extract of the plant-*Myrtus communis* causes the increased superoxide dismutase and catalase enzymes, and on the other hand, by inhibiting free radicals and increasing the glutathione level can cause the inhibition of lipid peroxidation [22, 46] and reduce the damage caused by free radicals produced by zero-Valente iron nanoparticles [32]. With regard to the known antioxidant and anti-inflammatory properties of the plant [47], the observed decrease in inflammation can be expressed by the presence of biomolecules available in the extract of this plant in the structure of MC-ZVINP.

CONCLUSION

In general, the results show that, iron nanoparticles synthesized by green method using plant-*Myrtus communis* extract have no significant detrimental effects on the liver and cause no increase in serum level of hepatic enzymes, as well as having no significant effects on blood parameters.

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CONFLICT OF INTEREST

No potential irreconcilable situations are proclaimed.

REFERENCES

1. Ayodele AT, Valizadeh A, Adabi M, Esnaashari SS, Madani F, Khosravani M, Adabi M. Biointerface research in applied chemistry. ultrasound, 2017;69:70.
2. Mujokoro B, Madani F, Esnaashari SS, Khosravani M, Ada-

bi M. Combination and Co-delivery of Methotrexate and Curcumin: Preparation and In Vitro Cytotoxic Investigation on Glioma Cells. Journal of Pharmaceutical Innovation. 2019.
3. Madani F, Esnaashari SS, Mujokoro B, Dorkoosh F, Khosravani M, Adabi M. Investigation of Effective Parameters on Size of Paclitaxel Loaded PLGA Nanoparticles. Advanced Pharmaceutical Bulletin. 2018;8(1):77-84.
4. Mascitelli L, Goldstein MR. Inhibition of Iron Absorption by Polyphenols as an Anticancer Mechanism. Polyphenols in Human Health and Disease: Elsevier; 2014. p. 1283-6.
5. Sornjai W, Nguyen Van Long F, Pion N, Pasquer A, Saurin J-C, Marcel V, et al. Iron and hepcidin mediate human colorectal cancer cell growth. Chemico-Biological Interactions. 2020;319:109021.
6. Kalinowski DS, Richardson DR. The Evolution of Iron Chelators for the Treatment of Iron Overload Disease and Cancer. Pharmacological Reviews. 2005;57(4):547-83.
7. Cherayil BJ. Iron and Immunity: Immunological Consequences of Iron Deficiency and Overload. Archivum Immunologiae et Therapiae Experimentalis. 2010;58(6):407-15.
8. Milner JA, Romagnolo DF, Connor J, Lee S. Bioactive compounds and cancer: Springer; 2010.
9. Basu T, Panja S, Shendge AK, Das A, Mandal N. A natural antioxidant, tannic acid mitigates iron-overload induced hepatotoxicity in Swiss albino mice through ROS regulation. Environmental Toxicology. 2018;33(5):603-18.
10. Toyokuni S. Mysterious link between iron overload and CDKN2A/2B. Journal of Clinical Biochemistry and Nutrition. 2010;48(1):46-9.
11. Niki E. Dual stressor effects of lipid oxidation and antioxidants. Oxidative Stress: Elsevier; 2020. p. 249-62.
12. Chaudhary S, Gupta RK, Gupta MK, Verma HC, Kumar H, Kumar A, Swain SR, El-Shorbagi A-N. Hepatoprotective response of Cordia sebestena L. fruit against simvastatin induced hepatotoxicity. Journal of Pharmacy & Pharmacognosy Research, 2020;8 (4):327-335.
13. Talley J. Bioremediation of recalcitrant compounds: CRC Press; 2016.
14. Eslami S, Ebrahimzadeh MA, Biparva P, Abedi Rad M. Zero Valent Iron-Based Nanoparticles: Synthesis, Characterization and their Application in Biology and Medicine. Journal of Mazandaran University of Medical Sciences, 2016;26 (142):285-310.
15. Desalegn B, Megharaj M, Chen Z, Naidu R. Green synthesis of zero valent iron nanoparticle using mango peel extract and surface characterization using XPS and GC-MS. Heliyon. 2019;5(5):e01750.
16. Njagi EC, Huang H, Stafford L, Genuino H, Galindo HM, Collins JB, et al. Biosynthesis of Iron and Silver Nanoparticles at Room Temperature Using Aqueous Sorghum Bran Extracts. Langmuir. 2011;27(1):264-71.
17. Shahwan T, Abu Sirriah S, Nairat M, Boyacı E, Eroğlu AE, Scott TB, et al. Green synthesis of iron nanoparticles and their application as a Fenton-like catalyst for the degradation of aqueous cationic and anionic dyes. Chemical Engineering Journal. 2011;172(1):258-66.
18. Møller P, Jacobsen NR, Folkmann JK, Danielsen PH, Mikkelsen L, Hemmingsen JG, et al. Role of oxidative damage in toxicity of particulates. Free Radical Research. 2009;44(1):1-46.
19. Nel A. Toxic Potential of Materials at the Nanolevel. Science. 2006;311(5761):622-7.
20. Nadagouda MN, Castle AB, Murdock RC, Hussain SM, Varma RS. In vitro biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols. Green Chem. 2010;12(1):114-22.
21. Barboni T, Cannac M, Massi L, Perez-Ramirez Y, Chiaramonti N. Variability of Polyphenol Compounds in Myrtus Communis L. (Myrtaceae) Berries from Corsica. Molecules. 2010;15(11):7849-60.
22. Aleksic V, Knezevic P. Antimicrobial and antioxidative activity of extracts and essential oils of Myrtus communis L. Microbiological Research. 2014;169(4):240-54.
23. Baharvand Ahmadi B, Bahmani M, Naghdi N, Saki K, Baharvand Ahmadi S, Rafieian Kopaei M. Review on phytochemistry, therapeutic and pharmacological effects of myrtus (Myrtus communis). Der Pharmacia Lettre, 2015;7 (11):160-165.
24. Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi FM, et al. Chemical composition and antioxidant activities of Myrtus communis L. berries extracts. Food Chemistry. 2010;123(4):1242-51.
25. Miraj S, Kiani S. A review study of therapeutic effects of Myrtus communis. Der Pharmacia Lettre, 2016;8 (9):281-285.
26. Eslami S, Ebrahimzadeh MA, Biparva P. Green synthesis of safe zero valent iron nanoparticles by Myrtus communis leaf extract as an effective agent for reducing excessive iron in iron-overloaded mice, a thalassemia model. RSC Advances. 2018;8(46):26144-55.
27. Liu N, Tang M. Toxic effects and involved molecular pathways of nanoparticles on cells and subcellular organelles. Journal of Applied Toxicology. 2019;40(1):16-36.
28. Keenan CR, Goth-Goldstein R, Lucas D, Sedlak DL. Oxidative Stress Induced by Zero-Valent Iron Nanoparticles and Fe(II) in Human Bronchial Epithelial Cells. Environmental Science & Technology. 2009;43(12):4555-60.
29. Sun Z, Yang L, Chen K-F, Chen G-W, Peng Y-P, Chen J-K, et al. Nano zerovalent iron particles induce pulmonary and cardiovascular toxicity in anin vitrohuman co-culture model. Nanotoxicology. 2016;10(7):881-90.
30. Chobot V, Hadacek F, Kubicova L. Effects of Selected Dietary Secondary Metabolites on Reactive Oxygen Species Production Caused by Iron(II) Autoxidation. Molecules. 2014;19(12):20023-33.
31. Huang G, Chen H, Dong Y, Luo X, Yu H, Moore Z, et al. Superparamagnetic Iron Oxide Nanoparticles: Amplifying ROS Stress to Improve Anticancer Drug Efficacy. Theranostics. 2013;3(2):116-26.
32. Yoon H, Pangging M, Jang M-H, Hwang YS, Chang Y-S. Impact of surface modification on the toxicity of zerovalent iron nanoparticles in aquatic and terrestrial organisms. Ecotoxicology and Environmental Safety. 2018;163:436-43.
33. Saccà ML, Fajardo C, Martinez-Gomariz M, Costa G, Nande M, Martin M. Molecular Stress Responses to Nano-Sized Zero-Valent Iron (nZVI) Particles in the Soil Bacterium Pseudomonas stutzeri. PLoS ONE. 2014;9(2):e89677.
34. Heibatollah S, Reza NM, Izadpanah G, Sohailla S. Hepatoprotective effect of Cichorium intybus on CCl4-induced liver damage in rats. Afr J Biochem Res, 2008;2 (6):141-144.
35. Karthikeyeni S, Siva Vijayakumar T, Vasanth S, Arul Ganesh MM, Subramanian P. Biosynthesis of Iron ox-

ide nanoparticles and its haematological effects on fresh water fish Oreochromis mossambicus. J Acad Indus Res, 2013;10:645-649.
36. Bourrinet P, Bengele HH, Bonnemain B, Dencausse A, Idee J-M, Jacobs PM, et al. Preclinical Safety and Pharmacokinetic Profile of Ferumoxtran-10, an Ultrasmall Superparamagnetic Iron Oxide Magnetic Resonance Contrast Agent. Investigative Radiology. 2006;41(3):313-24.
37. Briley-Saebo KC, Johansson LO, Hustvedt SO, Haldorsen AG, Bj??rnerud A, Fayad ZA, et al. Clearance of Iron Oxide Particles in Rat Liver. Investigative Radiology. 2006;41(7):560-71.
38. Ben Hsouna A, Dhibi S, Dhifi W, Mnif W, Ben Nasr h, Hfaiedh N. Chemical composition and hepatoprotective effect of essential oil from Myrtus communis L. flowers against CCL4-induced acute hepatotoxicity in rats. RSC Advances. 2019;9(7):3777-87.
39. Loeb SK, Asharani P, Valiyaveettil S. Investigating the toxicity of iron (III) oxide nanoparticles, zinc (II) oxide nanorods and multi-walled carbon nanotubes on red blood cells. National University of Singapore Available at: http://www nus edu sg/nurop/2010/Proceedings/FoS/Chemistry/Stephanie% 20Katharine% 20Loeb_NT081367E pdf, 2010.
40. Taee HM, Hajimoradloo A, Hoseinifar SH, Ahmadvand H. The effects of dietary Myrtle (Myrtus communis L.) supplementations on growth performance and some innate immune responses in rainbow trout (Oncorhynchus mykiss). International Journal of Aquatic Biology,2017;5(4):252-9.
41. Lourenço IM, Pieretti JC, Nascimento MHM, Lombello CB, Seabra AB. Eco-friendly synthesis of iron nanoparticles by green tea extract and cytotoxicity effects on tumoral and non-tumoral cell lines. Energy, Ecology and Environment. 2019;4(6):261-70.
42. Talesh S, Koohi M, Zayerzadeh E, Hasan J, Shabanian M. Acute toxicity investigation regarding clinical and pathological aspects following repeated oral administration of iron oxide nanoparticles in rats. Nanomedicine Research Journal, 2019;4(4):228-33.
43. Parivar K, Malekvand Fard F, Bayat M, Alavian SM, Motavaf M. Evaluation of Iron Oxide Nanoparticles Toxicity on Liver Cells of BALB/c Rats. Iranian Red Crescent Medical Journal. 2016;18(1).
44. Sadeghi L, Babadi VY, Espanani HR. Toxic effects of the Fe2O3 nanoparticles on the liver and lung tissue. Bratislava Medical Journal. 2015;116(06):373-8.
45. Sadeghi L, Tanwir F, Yousefi Babadi V. In vitro toxicity of iron oxide nanoparticle: Oxidative damages on Hep G2 cells. Experimental and Toxicologic Pathology. 2015;67(2):197-203.
46. Baz H, Gulaboglu M, Gozcu L, Demir M, Canayakin D, Suleyman H, Halici Z, Baygutalp K. Effects of aqueous extract of Myrtus communis L. leaves on streptozotocin-induced diabetic rats. J Res Med Dent Sci, 2016;4:214-218.
47. Minaiyan M, Khosropour P, Sajjadi S-E, Talebi A. Anti-inflammatory effect of Myrtus communis hydroalcoholic extract and essential oil on acetic acid–induced colitis in rats. Journal of Reports in Pharmaceutical Sciences. 2019;8(2):204.