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The Effect of Short-term Intraperitoneal Injection of Fe2O4Zn Nanoparticle on Liver Enzymes and Tissue in Male Wistar Laboratory Rats

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ABSTRACT

The present study sought to examine the short-term effect of Fe2O4Zn nanoparticle on histological and enzymatic changes of liver in male Wistar laboratory rats. 24 male Wistar laboratory rats were divided into three groups. The control group included rats which received 0.5 ml of physiological saline. The second and third groups received 0.5 ml of Fe2O4Zn Nano fluid at concentration levels of 100 and 200 ppm for 7 consecutive days. The rats were phlebotomized on days 2, 7, and 14 following the treatment. Serum concentration levels of liver enzymes were measured. The livers were removed from the bodies on day 14. Serum glutamic oxaloacetic transaminase (SGOT), serume glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) levels saw a significant increase in both treatment groups compared to the control group on days 2, 7, and 14. Lactate dehydrogenase (LDH) levels, however, showed a reduction in the treatment groups compared to the control group which was not statistically significant. The histopathological analysis of liver tissues in treatment groups pointed to intrahepatocellular lipid accumulation, mild portal inflammation, hepatocyte inflammation, hyperemia, centrilobular, intravascular and portal red blood cell (RBC) aggregation, and centrilobular necrosis. Results from the second treatment group were more drastic than the first treatment group. Results demonstrate the histopathological effects of Fe2O4Zn on liver tissues and enzyme levels in the treatment groups compared to the control group

Keywords: Fe2O4Zn, liver enzymes, histopathology

INTRODUCTION

Numerous applications in medical and biological sciences can be considered for nanoparticles containing iron, nickel, and cobalt due to their unique magnetic properties and various potentials [1, 2, 3]. The introduction of nanoparticles to body bia the respiratory and digestive systems as well as their rapid uptake by bloodstream has been vastly reported, as they can easily pass through physiological barriers [4]. Numerous studies have been conducted on the toxicity of zinc oxide nanoparticles despite their wide application in the paint and coating industry, and in medical, biological, and cosmetic fields. It has been demonstrated that zinc oxide nanoparticles lead to intracellular reactive oxygen species (ROS) increase and accumulation which contributes significantly to nano-induced apoptosis [5]. These toxic effects are often accompanied by oxidative stress through diminished catalase and superoxide dismutase activities [5]. The toxicity of zinc oxide nanoparticles in bacterial systems has been corroborated [6, 7]. Nanoparticle studies reveal that despite their wide application in the gene transfer system to tissues and cells, magnetic resonance imaging (MRI), celltherapy, labeling macromolecules, safety survey, tumor therapy, and cancer cell thermotherapy, they can directly interact with the DNA structure via introduction to the nucleus [8]. This can lead to several consequences such as ROS creation, apoptosis, genotoxicity, and DNA damage.

Altered and elevated liver enzyme levels are associated with the specified points, leakage of cell contents, and liver cell membrane damages [9]. Environmental contact with zinc oxide nanoparticles leads to their aggregation in the stratum corneum and hair follicles. Similarly, bones, kidneys, and the pancreas are among the target organs for zinc

oxide nanoparticles [10, 11]. Contact with low concentrations of zinc oxide nanoparticles unlocks their genotoxic potential, realized through lipid peroxidation and oxidative stress [12].

Numerous studies point to their destructive effects on epidermal, cancer, and liver cells [12, 13]. Liver is one of the most important body organs assigned with the task of detoxification [14, 15]. The highest concentration level of nanoparticle uptake belongs to the liver. Therefore, it can be a target for the introduction of various effects of nanoparticles in in-vivo environments. Liver enzymes are significant markers of necrosis and damaged liver cells. Liver enzyme levels increase in several liver diseases. Considering the central role of liver in removing nanoparticles from the bloodstream and that their biosafety is still a subject of controversy, the effects of Fe2O4Zn nanoparticle on liver enzymes and histopathological changes of the liver tissue were examined in this study [16]. Studies have shown nanoparticles to be rapidly absorbed by liver cells. Liver is an organ of the reticuloendothelial system that is highly sensitive to oxidative stress due to its high blood flow [17].

Previous studies on nanoparticles, in particular on iron oxide nanoparticles, have yielded conflicting results regarding their lack or low levels of toxicity, induction of inflammatory responses and apoptosis [18, 19]. Previous studies have demonstrated that iron oxide nanoparticles are able to stop the cell cycle during the G1 phase [20] and increase endothelial cell permeability [21]. Elevated SGOT, SGPT, ALP, and LDH serum levels are indicators of a damaged liver tissue. Iron oxide nanoparticles are used as contrast agents in MRIs. They are also used to mark and track stem cells. On account of their physiochemical properties, they are used as drug carriers in treating cancer cells in in-vivo environments. However, their impacts on human health have not yet been thoroughly identified [22]. Consequently, the effects of hybrid iron-zinc oxide nanoparticles on histopathological characteristics of liver and its enzymes were examined in the present study.

MATERIALS AND METHODS

5 gr of Fe2O4Zn was bought from Yas Aria Teb Company, which itself was commercially purchased from Sigma-Aldrich. The specifications are presented in Table 1.

Table 1.the specifications of Fe2O4Zn nanoparticle

Specifications	
Particle size (APS)	<100nm
Trace metal basis	>99%
Linear formula	Fe_2O_4Zn
Form	Nano powder
CAS number	12063-19-3
Molecular weight	241/08

Two stock solutions were prepared to determine the concentration of Fe2O4Zn:
1) 100Nmol (stock solution 1): 100 mg of Fe2O4Zn was dissolved in 10 ml of distilled water (100 mg/ 10 ml). A 100 nm concentration level was thus obtained. The amount of required nanoparticle for injection to a 150-g rat at the concentration of 100 mg/ 1 kg was calculated from the stock solution as follows:

Table 2.The injection amount to a 150-g rat at the concentration of 100 mg/ 1 kg equals 1.5 ml

100 mg	1000g
150g	X=1.5 ml

2) 200 Nmol (stock solution 2): 200 mg of Fe2O4Zn was dissolved in 20 ml of distilled water (200 mg/ 20 ml). A 200 nm concentration level was thus obtained. The amount of required nanoparticle for injection to a 150-g rat at the concentration of 100 mg/ 1 kg was calculated from the stock solution as follows:

Table 3.The injection amount to a 150-g rat at the concentration of 200 mg/ 1 kg equals 3 ml:

1000g	200 mg
150g	X=3 ml

The required amounts for injection were thus calculated and injected intraperitoneally by insulin syringes.

Grouping and treatment
24 male Wistar laboratory rats with a mean weight of 220 +/- 33 g were bought from Kermanshah University of Medical Sciences and kept in the animal nest of the Islamic Azad University of Sanandaj for two weeks for preparation purposes. The rats were kept at proper laboratory temperature (22 +/- 2 °C) and condition under

sufficient room light (12 hours of light and 12 hours of darkness). They were randomly divided into three groups of eight. The control group received 0.5 ml of physiological saline for 7 days. The second and third groups received Fe2O4Zn at 100 and 200 mg/ 1000 g concentrations of body weight (dissolved in 0.5 ml of distilled water) via intraperitoneal injection for 7 days [23].

Chemical analysis of blood

On days 2, 7, and 14 following the treatment, all rates were phlebotomized. Bloods were taken from the corner of the eyelid using capillary tubes. The specimens were then centrifuged at 3000 rpm for 15 minutes for their serum to be extracted. They were kept at -20°C until the measurement of enzyme concentrations. The concentration levels of SGOT, SGPT, ALP, and LDH were measured using the enzyme-linked immunosorbent assay (ELISA) method [24].

Histological analysis

After phlebotomy, the liver parts were removed under deep sedation. Small liver bits were fixed in 10% neutral buffered formalin on day 14. After preparing molds using molten paraffin, the specimens were dissected, put on microscope slides and stained by hematoxylin and eosin [25].

Statistical analysis

Data were analyzed using SPSS v.16. ANOVA was used to determine whether a significant difference existed between treatments. Dunnett's T3 test was utilized to compare the control group with experimental ones. Tukey's range test was employed to compare experimental groups. Results were reported in terms of mean scores +/- standard deviations at the significance level of < 0.05 (P < 0.05).

RESULTS

The effect of Fe2O4Zn on SGOT serum levels at 100 and 200 ppm concentrations

The effects of Fe2O4Zn on SGOT at 100 and 200 ppm concentrations on days 2, 7, and 14 are as follows :

According to the results, on day 2 following the treatment, a statistically significant difference was seen between the treatment groups and control group in terms of SGOT serum concentration levels in that in both groups the enzyme levels were elevated (P< 0.05). On day 7, enzyme levels in the two treatment groups (100 and 200 ppm) saw a significant increase compared to the control group (P< 0.05 and P<0.01, respectively). On day 14, SGOT blood serum levels saw a significant increase compared to the control group (P<0.01).

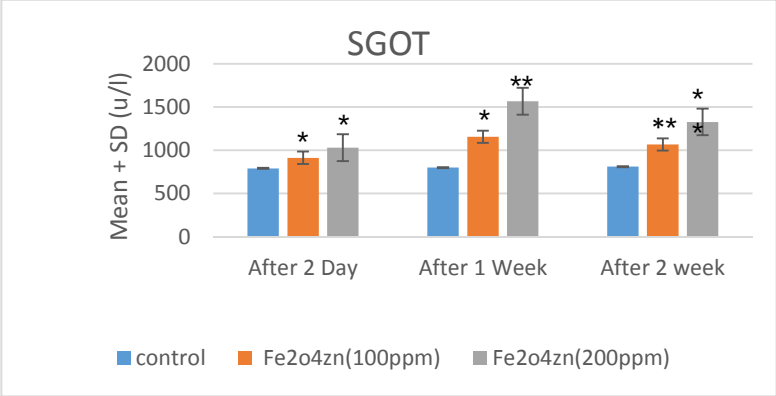

Figure 1. SGOT levels in 100- and 200-ppm treatment groups on days 2, 7, and 14

Indicates a significant difference between treatment groups and the control group (P < 0.05(* **Indicates a significant difference between 100- and 200-ppm treatment groups (P < 0.01)*

The effect of Fe2O4Zn on SGPT serum levels at 100 and 200 ppm concentrations

The effects of Fe2O4Zn on SGOPT at 100 and 200 ppm concentrations on days 2, 7, and 14 are as follows:

According to the results, on day 2 following the treatment, a statistically significant difference was seen between the treatment groups and control group in terms of SGPT serum concentration levels which was highly significant (P < 0.05*). On day 7, enzyme levels in the two treatment groups (100 and 200 ppm) saw a significant increase compared to the control group (P< 0.05 and P<0.01, respectively). On day 14, SGPT blood serum levels saw a significant increase compared to the control group, which was statistically significant (P<0.01***).

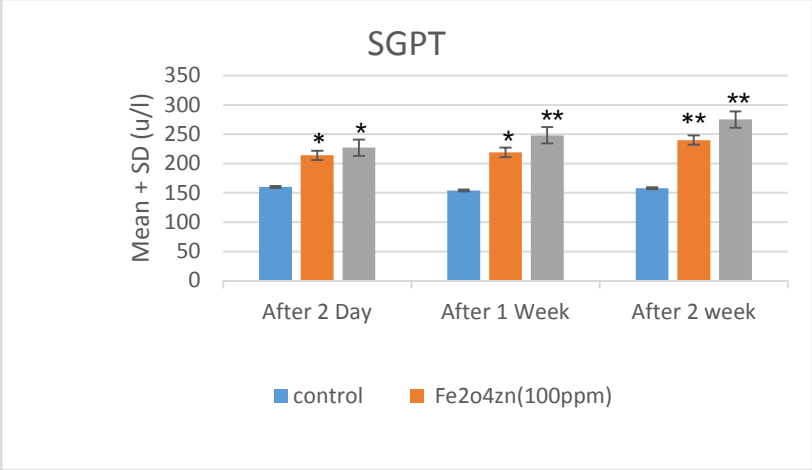

Figure 2.the effect of different amounts of Fe2O4Znon SGPT plasma concentrations on days 2, 7, and 14
**Indicates a significant difference between treatment groups and the control group (P < 0.05*)*
Indicates a significant difference between 100- and 200-ppm treatment groups (P < 0.01)*

The effect of Fe2O4Zn on ALP serum levels at 100 and 200 ppm concentrations
The effects of Fe2O4Zn on ALP at 100 and 200 ppm concentrations on days 2, 7, and 14 are as follows:
According to the serum levels, on day 2 following the treatment, a statistically significant difference was seen between the treatment groups and control group in terms of ALP serum concentration levels which was not significant (P> 0.05). On day 7, enzyme levels in the two treatment groups (100 and 200 ppm) saw a significant increase compared to the control group (P< 0.01 and P<0.001, respectively). On day 14, ALP blood serum levels saw a highly significant increase for the two treatment groups compared to the control group (P<0.05* and P< 0.01**).

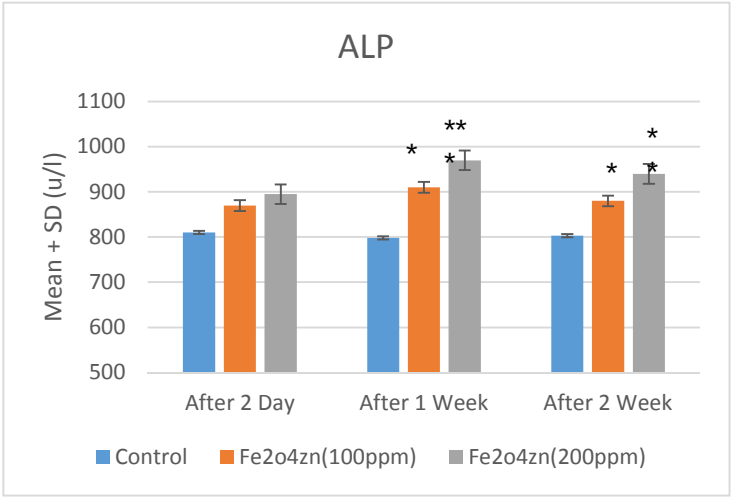

Figure 2. The effect of different amounts of Fe2O4Znon ALP plasma concentrations on days 2, 7, and 14.
Indicates a significant difference between treatment groups and the control group (P < 0.05(
***Indicates a significant difference between 100- and 200-ppm treatment groups and the control group (P < 0.01**)*
****indicates a significant difference between 100- and 200-ppm treatment groups and the control group (P < 0.001)*

The effect of Fe2O4Zn on LDH serum levels at 100 and 200 ppm concentrations
The effects of Fe2O4Zn on LDH at 100 and 200 ppm concentrations on days 2, 7, and 14 are as follows:
According to the results, on day 2 following the treatment, a reduction was seen in the treatment groups compared to the control group which was not statistically significant (P> 0.05). Similar results were obtained on days 7 and 14 following the injection (P> 0.05).

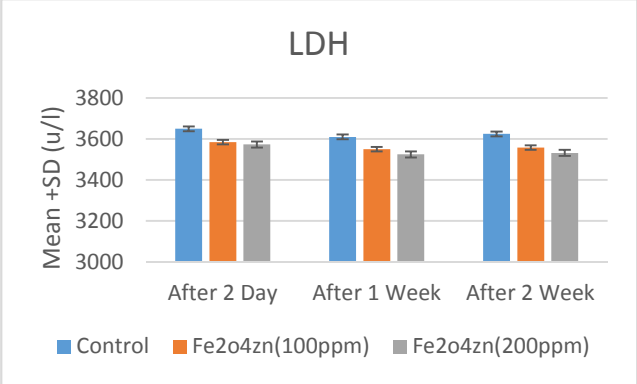

Figure 4. The effect of different amount of Fe2O4Zn on LDH concentration levels on days 2, 7, and 14
P < 0.05 is the significance level.

Histopathological results

Results from the H&E staining suggested that Fe2O4Zn influenced the histopathological characteristics of liver at 100- and 200 ppm concentrations. Hepatocytes were altered in both treatment groups. The effect of Fe2O4Zn nanoparticles on the 200 ppm treatment group was greater than that on the 100 ppm group. This could be explained by the greater tissue lesions in the 200ppm group with severe cellular inflammation and necrosis.

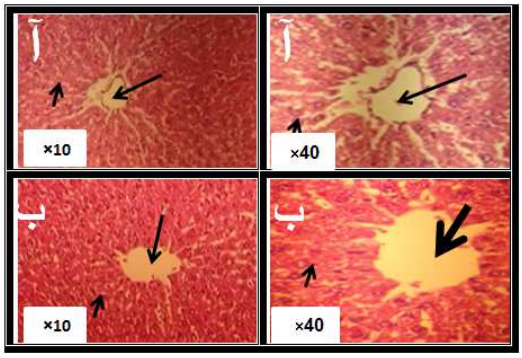

Figure 5.Dissection of H&E-stained liver tissues taken from the control group at the magnification level of (10× _ 40×)
Pictures A and B: central vein, hepatocytes, and hepatic portal (the arrow) in the control group. The hepatocytes are healthy, cytoplasm is red in color, cells have not been altered with no sign of inflammation.

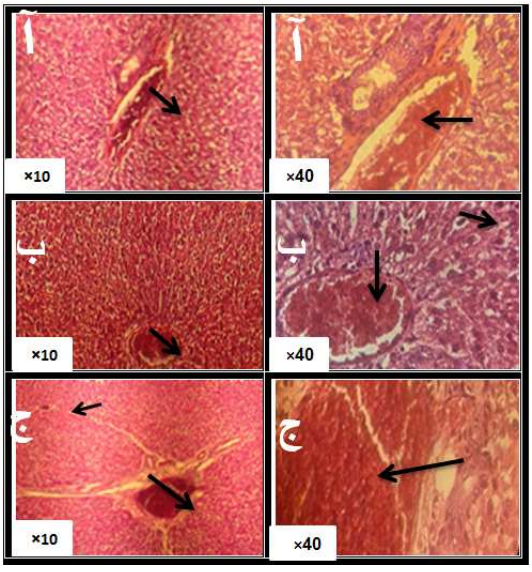

Figure 6.Dissection of H&E-stained liver tissues taken from the treatment group 1 at the magnification level of (10× _ 40×)
Picture A: intravascular hyperemia (the arrow); Picture B: Centrilobular hyperemia and lipid accumulation (the arrow); Picture C: Portal blood vessel and centrilobular(the arrow) hyperemia.

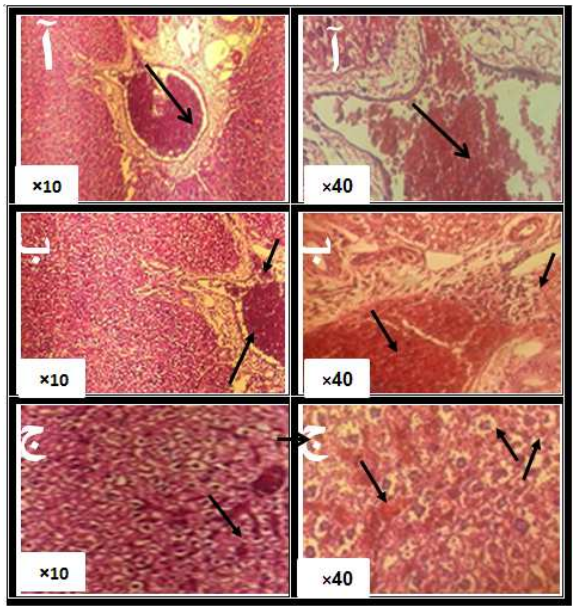

Figure 7.Dissection of H&E-stained liver tissues taken from the treatment group 2 at the magnification level of (10× _ 40×)
Picture A: Severe portal blood vessel hyperemia (the arrow); Picture B: More severe hyperemia, centrilobular necrosis, and pyknotic hepatocyte nuclei; a symptom of necrosis; Picture C: Lipid accumulation and sinusoidal congestion (the arrow).

DISCUSSION

Numerous studies have shown the toxic effects of nanoparticles; yet, few have attempted to reduce or neutralize their toxic effects. The toxic effects of Fe2O4Zn on liver histopathology and enzymes were examined in this study. The liver reduces bodily damages as it is an important organ in the metabolism and detoxification of foreign and toxic substances. This is done despite its vulnerability to foreign substances during metabolism in complex reactions which gives rise to adverse impacts on liver tissues. Elevated liver enzyme levels in blood are indicators of a damaged liver [26]. Damage to the liver membrane is the reason for the release of liver enzymes in blood. Previous studies have shown that lipid accumulation in the liver or vacuolization could be due to the complications of lipid peroxidation which are the result of a damaged or fragmented endoplasmic reticulum. These findings attest to an abnormal lipid metabolism [27].

Abnormal lipid accumulation in hepatocytes which is induced by most nanoparticles could possibly demonstrate the toxic and vulnerable effects of nanoparticles in the liver by causing lipolysis or lipomatosis [28]. These results are in line with previous histopathological findings. What is evident is that nanoparticles are rapidly absorbed by liver and Kupffer cells [16]. It has also been demonstrated that iron oxide nanoparticles are capable of entering the central nervous system, leading to oxidative stress and neuronal destruction [29]. Despite numerous studies on the toxicity of iron oxide nanoparticles conflicting results have been achieved [30, 31, 32].

Kim et al. reported no in vivo toxicity on the part of iron oxide nanoparticles in house mice [6, 25, 33]. However, Zhu et al. [2008] showed that iron oxide nanoparticles could cause lung damage, increased capillary permeability, and pulmonary airway epithelial cell destruction in house mice [6]. Factors influencing such conflicting results undoubtedly depend on physiochemical characteristics of nanoparticles, their size, shape, and solubility; experimentation methods, and exposure time to nanoparticles. Suggested mechanisms regarding induced in vivo toxicity by iron oxide nanoparticles include: 1- the role of ROS, and 2- hemochromatosis in tissues and organs [30]. Studies also point to the toxicity of zinc oxide nanoparticles through affecting ROS levels, causing damage to electron transport chain and the subsequent onset of apoptotic cascades [5]. Experimental data suggest that acute oral exposure to zinc Nano powder and its compounds could lead to gastrointestinal damages. Excessive consumption of zinc for several months results in anemia, pancreatic injury, reduced levels of high-density lipoprotein (HDL). Studies on the toxicity of zinc oxide nanoparticles on culturing mammalian nerve cells have demonstrated that these nanoparticles could lead to cellular abnormality in terms of size, cell contraction, and cell separation from culture dishes. They also significantly reduce LDH leakage [34]. Despite the numerous advantages of nanotechnology, studies show that most nanoparticles could bring about varying effects owing to their tiny size and unique characteristics. High toxic effects of nanoparticles disrupt the functioning of the respiratory and cardiovascular systems as well as the liver and kidneys [35].

In a study examining the effect of TiO2 nanoparticles on renal failure, it was revealed that these nanoparticles could significantly alter ALT and AST serum levels compared with the control group. Elevated liver enzyme levels are associated with cellular leakage and abnormal functioning on the part of liver cell membranes [Liu et al., 2010]. Nanoparticle-induced liver damages in histopathological studies have been reported to coexist with hepatocyte destruction [9]. It has also been reported that these nanoparticles cause disruptions in liver functioning and create inflammatory cascades. In addition, increased functioning of Kupffer cells in the liver and increased accumulation of liver macrophages have been reported frequently in the pathogenesis of nanoparticle-induced renal failures. When liver cells are damaged, the expression pattern of membrane receptors and such antigens as CD68 increases. In the pathogenesis of liver damages, active macrophages express such cytokines as TNF-α and IL-6 and chemokines that stimulate inflammatory responses. This contributes to the migration of microphages, T lymphocytes, and monocytes to the site of inflammation [35]. The increased activation of NF-κB transcription factor is a central molecular response to hepatocellular injury. With the import of such transcription factors to the nucleus, inflammation-associated gene transcriptions such as cytokines, chemokines, growth factors, cellular binding proteins, and cytokine receptors will increase [35]. Oxidative stress plays a key role in the activation of this transcription factor. Numerous studies show that nanoparticles of capable of migrating into the nucleus, causing damage to DNA chains and leading to several biological responses [35]. Prolonged severe oxidative stress and cell disruption are outcomes that reduce or disrupt mitochondrial membrane potential which could lead to cellular death [36].

According to Singh et al. [2015], since nanoparticles are initially reabsorbed by Kupffer cells, cell membranes will be damaged and toxic degradation products of iron oxide magnetic nanoparticles will be slowly imported to hepatocytes from macrophages [37]. SGOT, SGPT, and ALP levels in plasma undoubtedly determine the levels of liver diseases. SGOT and SGPT are specific liver enzymes used for the diagnosis of liver diseases [38, 39] . Previous studies [38, 40, 41]have shown that silver nanoparticles disrupt mitochondrial activity raising SGOT and SGPT plasma levels. This is a sensitive determining response in identifying cytoplasmic and mitochondrial membrane damage or destruction. In the study by Hussain et al. [2005] on the effects on silver nanoparticles in the induction of toxicity within 24 hours, it was revealed that an increased LDH plasmatic leakage as well as a significant toxicity level was created in the experimental groups compared to the control group. Similar results regarding LDH and liver enzymes were obtained using silver and AL ،MnO2 ، MoO3 and Fe3O4 nanoparticles, demonstrating that increased nanoparticles result in elevated levels of LDH in plasma [15].

The analysis of serum liver enzyme levels showed that serum SGOT level in plasma showed a significant increase in treatment groups 1 and 2 (100 ppm and 200 ppm) on days 2, 7, and 14 following the injection of nanoparticles. Results from the biochemical analysis showed that serum SGPT levels in plasma increased in both treatment groups on days 2, 7, and 14 compared to the control group. However, serum LDH levels in both treatment groups had hardly had any difference with the control group. LDH levels were indeed reduced, but it was not statistically significant. Increased serum ALP levels in both treatment groups were statistically more significant on the 7th day. Histopathological results of the liver showed that intrahepatic lipid accumulation, an early complication of liver cells, was more evident in the treatment groups compared to the control group (particularly upon increasing dosage). Mild portal inflammation (portal hepatitis) and mild cellular inflammation could be seen in the treatment group 1. Hyperemia was evident in hepatic centrilobular as well as in hepatic portal veins. In the treatment group 2 centrilobular cells were stricken with necrosis and apoptosis diagnosed by the presence of pyknotic hepatocyte nuclei. Severe hyperemia could also be seen in liver sinusoids. Treatment group 2 experienced more drastic changes than its 1st counterpart. In both treatment groups, however, hyperemia and RBC accumulation (in central and portal veins) can be found; which corresponds with the study of Khorsandi et al. [2015] on the effect of TiO2 on the liver tissue. The nanoparticles used in this study significantly raised serum or plasmatic levels of SGOT, SGPT, and ALP. Liver enzyme levels are significant indicators of damaged or destructed liver cells and tissue. The ALP enzyme can be found in many tissues such as the liver, bone marrow, intestines, and placenta and is a determining indicator of liver damages in bone diseases. It should be noted that bile duct obstruction increases ALP serum levels (ALP is localized to biliary ducts adjacent to hepatocytes)[16, 42].

In an unhealthy liver, biliary ducts are often blocked and filled with fluids. The enzyme is thus become concentrated and eventually enters the blood flow. These results are completely in line with those of Khorsandi et al. [2015]. ALP can be found in several tissues and is released abundantly from the liver, bones, intestines, and placenta. Bile duct obstruction increases ALP serum levels. Damaged liver cells release ALP into the blood circulatory system [42]. Increased concentration of liver enzymes could be also accounted for by their increased anabolism or decreased catabolism [43]. It seems that alterations in liver enzyme levels are due to their role in the liver metabolic activity. Stability and integrity of hepatocyte membranes is necessary for a vital hepatic functioning [42]. Given their physiochemical properties, nanoparticles will undoubtedly disrupt this stability and the proper hepatic functioning. In previous studies [Parivar et al., 2016] on the effect of iron oxide nanoparticles on plasmatic liver enzyme levels, AST, ALT, and ALP plasmatic levels (important markers of liver toxicity) were shown to have been increased,

which corresponds to the results of the present study. Results from the biochemical experiments suggest that reduced levels of LDH are probably due to intracellular toxic effects, in particular of Zn+2 particles. [2015]. Moreover, the most important reason for iron oxide nanoparticles not having any toxic impact on animals is opsonization followed by rapid removal of nanoparticles from the bloodstream by the reticuloendothelial system present in the spleen and lymph nodes, or liver cell tolerance enhancement that neutralizes its toxic effects [45].

CONCLUSION

Results from the present study show the histopathological effects of Fe2O4Zn on the liver tissue and alterations in liver enzyme levels in treatment groups compared to the control group.

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