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Comparative analysis of biochemical abnormalities induced by iron oxide nanoparticles in some organs of albino rat and domestic pigeon and the role of deferoxamine as iron chelator

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Abstract: Recently, the use of nanomaterials in different biological and biochemical fields took an increased trend especially Iron oxide nanoparticles (IONPs) because of its physical, chemical and magnetic properties. Despite wide usage of IONPs, it has lethal side effects. This study was constructed to evaluate the harmful effects of IONPs on different organs of mammalian and avian models (Rattus norvegicus domestica) and (Columba livia domestica) respectively, and to assess the role of deferoxamine as an iron chelator for alleviation of IONPs-induced side effects. Both animal models were divided into six groups served as control, IONPs-treated for 1 hour or 1 week, deferoxamine (DFO)-treated for 1 week, IONPs/DFO-co-treated for 1 hour or 1 week. Atomic absorption spectrophotometric (AAS) analysis and biochemical analysis for hepatic and renal functions were performed after animal sacrifice. The AAS analysis showed that the iron levels almost increased significantly in the blood, liver, kidney and spleen after injection of the IONPs alone for 1 week compared to control. While the co-injection of IONPs and DFO for 1 week caused a significant decrease in iron levels compared to the IONPs alone. Concomitantly, the levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase and (AST) and levels of creatinine and uric acid showed a significant increase in the IONPs-treated animals compared to control groups. Whereas a significant decrease of ALT, AST, creatinine and uric acid in the animals treated with IONPs and DFO together compared to animals treated with IONPs alone.
Keywords: Iron oxide nanoparticles, Comparative toxicity, Deferoxamine, Biochemical analysis, Mammals, Aves

1. Introduction

In the last few decades magnetic nanoparticles got a great interest in many research areas specially the biomedical and biological fields [1-3]. Duo to their unique chemical and physical properties, iron oxide nanoparticles (IONPs) were found to be a great solution in the biological and biomedical fields, so that they were used in magnetic resonance imaging (MRI) [4-6], magnetic hyperthermia [4, 7, 8], and drug delivery [9, 10]. However, the great obstacle facing the expansion of using the magnetic nanomaterials is their side effects [3].

It was proposed that the toxicity of magnetic nanoparticles is due to its size, large surface and the penetration ability [11]. The intravenous (IV) injection of ultrasmall Fe3O4, SiO2 and Au nanoparticles with dose of 100 mg/kg in albino mice caused an increase in the concentration of the reactive oxygen species (ROS) in the serum, heart, spleen and liver, as well as it led to an elevation in the levels of the alanine amino transferase (ALT) and aspartate amino transferase (AST) enzymes indicating a liver toxicity [12]. Additionally, it was reported that the dose of 214 mg/L of IONPs on a neuronal cell line (Rat pheochromocytoma-PC12 cells) in vitro caused neurotoxicity [13]. Furthermore, it was found that the liver is one of the most organs that the nanoparticles accumulate in because of the mixing of the blood that comes from the portal vein and hepatic artery in its hepatic sinusoids [14]. Therefore, a pervious study informed that the concentrations of 25, 50, 75 and 100 mg/L of IONPs caused a reduction in cell viability and oxidative damage for human hepatoma cells (HepG2) [15]. Furthermore, a dose of 10, 25, 50, 75, and100 mg/L caused a moderate toxicity in human peripheral lymphocytes in vitro [16]. Additionally, the lower doses as 10, 25, 50 and 100 mg/mL in the fertilized eggs of the domestic chick (Gallus gallus domestica) resulted in neurotoxicity and the higher dose of 200 µg /ml led to a total mortality [17]. On the same trend, in the Balb/c mouse the liver enzymes ALT, AST as well as alkaline phosphatase (ALP) showed a significant increase after oral administration of 150 and 300 µg/gr of IONPs for three days; however the lower doses as 25, 50, and 75 µg/gr showed no significant increase in the same enzymes [18]. Similarly, it was reported that exposure to 150 µg/kg body weight of IONPs for 15 days leads to a significant increase in AST, ALT and ALP levels in albino rats [19]. In the other hand, the toxic effect of ferric oxide (Fe2O3) and zinc oxide (ZnO) nanoparticles was assessed in male Wister rats after the nanoparticles inhalation with a dose of 8.5 mg/kg for three days and resulted in an increase in the Fe2O3 and ZnO content in the lung and liver tissues, however the liver enzymes as AST, ALT and ALP as well as the total protein revealed a significant decrease compared to the control group [20]. Additionally, the IV injection of IONPs was found to cause pathological conditions due their deposition in the rat's spleen, as vacuolar cytoplasmic degeneration, lysosomal changes and

mitochondrial abnormalities in kidney [21]. In chicks, the supplementation of IONPs with different doses (20-80 mg/kg body. weight.) displayed a significant increase in AST and creatine kinase compared to control group. Neither the ALT, ALP, total protein nor the creatinine, urea and uric acid showed an significant difference compared to the control animals [22]. Besides, a research group informed that a dose of 0.8 mg/kg of IONPs intravenously injected in a male Wister rat reached a few number of body organs and caused toxicity in the liver, lung and kidney [23]. Furthermore, the intraperitonially injection of IONPs with doses of 50, 100 and 150 mg/kg body weight caused insignificant differences in the renal function parameters as urea, uric acid and creatinine compared to control group in Wister rats as well as no mortalities were recorded during the study [24]. In a previous studies, Awaad et al. reported that the intratesticular installation of IONPs with high doses caused oxidative reactions which led to release of reactive oxygen species (ROS) which promotes cell death [25]. However, another study revealed that ultrasmall IONPs with size less than 5 nm can trigger the cells to release the ROS while the nanoparticles with size larger than 5 nm hadn't shown the same behaviour [12]. In other studies on albino rat as mammalian model, an exposure to Cu-NPs (40 and 60 nm diameter) resulted in the proliferation of endothelial cells of brain capillaries when they were injected with low concentrations (about 1.5μg/mL)[26]. Another study informed that the accumulation of IONPs significantly altered the levels of other metals as Cu, Mn, and Zn in brain, heart, lung, liver and spleen and increased the level of the Lactate Dehydrogenase (LDH) enzyme in the blood as well as the secretion of a pro inflammatory cytokine (IL-6) and a chemokine (IL-8)[27].

Deferoxamine (DFO) is a natural product isolated from the bacterium Streptomyces pilosus. DFO was the first known iron chelator over more than 50 years ago which helped in reducing the mortality rate of thalassaemia major [28, 29]. DFO was found to be an effective drug that was used for both acute iron intoxication and iron-overload anaemia such as thalassemia [30]. It is degraded into smaller metabolites which have an iron chelating ability that plays a role also in releasing iron out of the body [31, 32]. Many researchers reported that the main iron excretion route is the urine, and the minor one is the faeces; however, a higher concentration of DFO was used to get rid of cardiac iron which may cause cardiac failure in some patients [33, 34].

Therefore, in this study the abnormalities induced by IONPs in different organs of two animal representatives such as albino rat (Rattus norvigicus domestica) and domestic pigeon (Columba livia domestica) will be analysed comparatively using biochemical analysis. Animals were injected intravenously with IONPs alone in order to evaluate the iron oxide toxicity in the blood and some body organs at different time points. Additionally, some animals were injected with IONPs with DFO (subcutaneous injection) to evaluate the ameliorative role of deferoxamine as iron chelator against IONPs-induced toxicities in both animal models using atomic absorption spectrophotometric analysis (AASA) and biochemical parameters. The obtained data from this study will provide some information about the toxicities produced by IONPs in different organs of two animals models and the role of DFO as iron chelator to reduce these abnormalities.

2. Materials and methods:

2.1. Materials

The required kits to measure the levels of AST, ALT, total protein, bilirubin, urea, uric acid and creatinine in the blood serum were purchased from Spectrum company (21, Misr El Tameer Bldgs. Zone one Masaken El Sheraton 11361, Cairo, Egypt). Nitric acid, hydrogen peroxide, hydrazine monohydrate and iron III acetylacetonate were purchased from sigma Aldrich (Ontario, Canada). Deferoxamine were purchased from Novartis pharmaceutical company (Basel, Switzerland).

2.2. Methods:

2.2.1. Preparation of iron oxide nanoparticles (IONPs)

Iron III acetylacetonate was used to prepare the IONPs according to previous method brief modification. In this method, a solution of iron III acetylacetonate was prepared by dissolving 618 mg of iron III acetylacetonate in 25 ml ethyl alcohol. Another mixture solution of 1.26 ml distilled water with 620 µl hydrazine monohydrate was prepared. The two mixtures were mixed and incubated under steering in dry oven at 80 °C for overnight. The resulted precipitate was collected, washed by distilled water, and centrifuged for 3 times and stored at 4 °C for further investigations. To ensure the homogeneity and dispersity of the prepared IONPs the solution was sonicated for 1 hour before injection.

2.2.2. Animal experimental design

Thirty adult males of albino rats (Rattus norvegicus domestica) and thirty adult males of domestic pigeons (Columba livia domestica) were used to achieve the goals of this study. The animals were kept in separated metal cages with free access to food and water. The animals of each species were randomly divided into main six groups (5 members/each group). For each animal (albino rats, domestic pigeons), the first group was considered as control group and administrated by phosphate buffer saline (PBS) for 1 week. The second group was subcutaneously injected with 30 mg/kg (1 dose/day) of DFO 1 week. The third group was intravenously injected with a single dose of 5mg/kg IONPs only 1 hour before dissection. The fourth group was subcutaneously injected with 30 mg/kg DFO for 1 week and at the same time intravenously injected with 5mg/kg IONPs for 1 hour just before dissection. The fifth group was intravenously injected with 5mg/kg IONPs only for 1 week. While the sixth group was subcutaneously injected with 30 mg/kg DFO and intravenously injected with 5mg/kg IONPs for 1 week. All experiments were carried out under the guidelines of animal care committee in Faculty of Science at Sohag University with approval number CSRE-8-23.

2.2.3. Animal dissection and organ collection

The animals were sacrificed at each specific time point by an overdose of diethyl ether and dissected in a sterilized area. The blood and blood serum, liver, spleen and kidney of each animal were collected and stored at appropriate temperature for further investigation at -20 °c.

2.2.4. Blood and blood serum tests

The collected blood was divided used to evaluate the levels of IONPs contents, blood parameters abnormalities and liver and kidney function.

2.2.5. Blood and tissue digestion

A known blood volume and wights from each tissue was digested in a mixture of nitric acid and hydrogen peroxide with ratio of 70% nitric acid: 30% hydrogen peroxide. The samples were then put in clean dried 10 ml glass vial and heated at 180 C for 10 min, after solution dried the same process was repeated again until the digestion of the tissues was completed perfectly. A well know volume of deionized water was used to dilute the samples after digestion according to the method of [35, 36]. Using blank samples, the iron concentration in each gram of tissue will be calculated using atomic absorption spectrophotometer.

2.2.6. Blood parameters measurement

Blood was collected in blood collecting tubes containing EDTA in order to evaluate the blood parameters abnormalities in each group. The targeted parameter in this experiment includes red blood corpuscles (RBCs) and platelets numbers (PLT), haemoglobin (HB) and haematocrit (HCT).

2.2.7. Liver and kidney functions evaluation

To evaluate liver and kidney functions, blood was centrifuged at 4000 RPM for 20 minutes. Then blood serum was collected and placed in a labelled dry clean Eppendorf tube in – 20 C. The kidney and liver function tests were carried out by: T80 UV spectrophotometer (PG Instruments company, UK), according to [37].

2.2.8. Statistical analysis

The data resulted from the biochemical tests and atomic absorption spectrophotometric analysis were analysed by IBM SPSS statistics version 25. The One-Way ANOVA and test (Tukey's test) were performed to compare the differences between groups. All data were demonstrated in the figures as means ± standard deviation of the mean (SD). Statistical significance was considered at a level of $P < 0.05$.

3. Results:

3.1. Biodistribution profiles of IONPs:

3.1.1. Biodistribution profiles of IONPs in the blood

The AASA analysis showed that, there were different variations in the biodistribution profiles of the iron (representing IONPs) in the blood in both species. The blood of rat and pigeon (Fig. 1A and 1B, respectively) showed a significant increase in the iron levels after 1 week of IONPs intravenous injection alone compared to that in the control group. Quantitatively, iron levels in the blood of rat and pigeon increased by 30% and 69.8% respectively. While the iron levels in the blood of both species showed a significant decrease after 1 week of IONPs intravenous injection along with oral administration of DFO. Compared to iron levels in the blood after 1 week of IONP injection, blood rat and pigeon showed 29.2% and 69.3% decrease respectively compared to iron levels after injection of IONPs alone. There was no significant difference in iron levels between control serum and after IONPs and DFO simultaneous injection. Injection of IONPs alone for 1 hour showed no significant increase in iron levels compared to the injection of IONPs and DFO simultaneous injection in the serum of both species.

3.1.2. Biodistribution profiles of IONPs in the liver

In the case of iron levels in the liver of rat and pigeon as shown in (Fig. 1C and 1D, respectively) the iron levels showed a slight increase after 1 week of IONPs injection alone compared to the control (16.5% and 17.6% for rat and pigeon respectively).

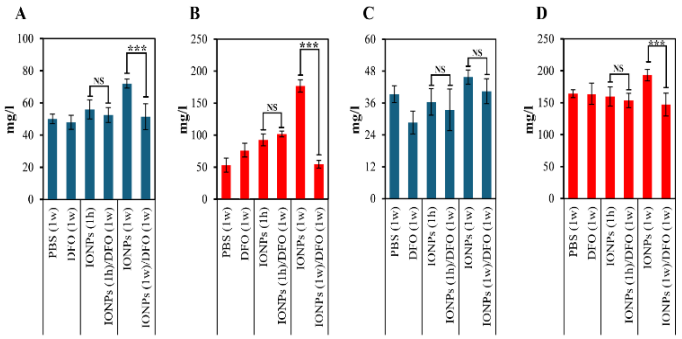

Figure 1: AASA of iron levels (representing IONPs) in the blood and liver of albino rat (A and C, respectively) and in domestic pigeon (B and D, respectively) after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The iron levels in the blood of both rat and pigeon showed a significant increase after 1 week injection of IONPs, while iron levels significantly decreased after 1 week of IONPs injection along with DFO administration for 1 week. The levels of IONPs showed a significant increase in pigeon's liver but not in rat's liver after 1 week of injection, and significantly decreased after 1 week injection of IONPs and DFO. There were no significant changes of the iron levels in blood and liver after 1 hour of IONPs or IONPs and DFO injection into rat or pigeon. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

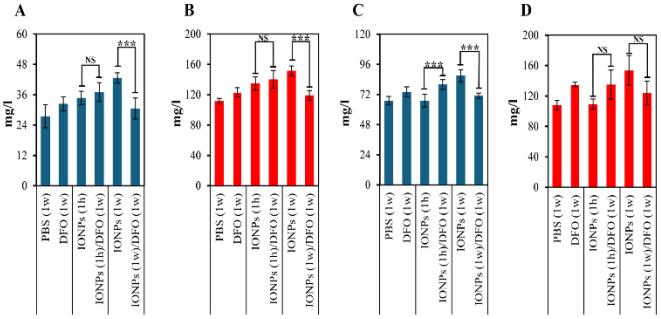

Figure 2: AASA of iron levels (representing IONPs) in the kidney and spleen of albino rat (A and C, respectively) and in domestic pigeon (B and D, respectively) after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The iron levels showed a significant increase in the kidney of rat and pigeon after 1week of IONPs injection compared to that after 1week of IONPs and DFO simultaneous injection. While the iron levels showed a significant increase in spleen of rat after 1week of IONPs injection compared with that after 1week of IONPs and DFO injection. The iron levels significantly increased in the rat spleen after 1hour of IONPs and DFO injection compared to that after 1hour injection of IONPs alone. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

On the same way the injection of both IONPs and DFO simultaneously for 1 week showed a non-significant difference compared to iron levels in rat's serum after IONPs injection alone. Furthermore, the iron levels in the pigeon's serum revealed a significant decrease after 1 week of simultaneous injection of IONPs and DFO (23.8% decrease) compared to the IONPs alone. The injection of IONPs for 1 hour alone or along with DFO showed no significant variations in the liver of both species.

3.1.3. Biodistribution profiles of IONPs in the Kidney

Similar to the blood, the iron levels in the kidney in both albino rat and domestic pigeon (Fig. 2A and 2B, respectively) witnessed a significant increase in the iron levels after injection of IONPs alone for 1 week compared to the control group. The ratio of iron liver increase in the kidney for rat and pigeon increase was 35% and 26.5% respectively. However, the concurrent injection of IONPs and DFO for 1 week revealed a significant decrease in iron levels with 28.4% and 21.2% for albino rat and domestic pigeon respectively. The injection of IONPs alone or along with DFO after 1 hour showed insignificant differences in both species.

3.1.4. Biodistribution profiles of IONPs in the spleen

The albino rat's spleen (Fig. 2C) revealed a significant increase in the iron levels after injection of IONPs alone for 1 week in comparison to the control (23% increase), while the simultaneous injection of IONPs and DFO for 1 week led to significant decrease in the iron levels by 18.4% compared to the IONPs injection only. Interestingly, the iron levels in the rat's spleen showed a significant increase (8.9% increase) after simultaneous injection of IONPs for 1 hour and DFO compared to that of IONPs alone. On the contrary, the iron levels in the spleen of the domestic pigeon (Fig. 2D) showed no significant difference after injection the IONPs alone or accompanied with DFO compared to control group neither after 1 hour nor 1 week.

3.2. Biochemical changes of IONPs protective role of DFO:

3.2.1. Liver function:

3.2.1.1. ALT

As shown in figure 3A and 3B (rat and pigeon respectively), the single injection of IONPs in both albino rat and domestic pigeon for 1 week showed a significant increase in the ALT by 58% and 24% respectively. On the other hand, the concurrent administration of the IONPs and DFO for 1 week displayed a significant decrease of the ALT levels compared to that of IONPs alone injection for 1 week by 50% and 47.9% for albino rat and domestic pigeon respectively. The simultaneous injection of IONPs and DFO for 1 hour showed non-significant increase in comparison with the injection of IONPs alone or with the control in both species.

3.2.1.2. AST

AST displayed a remarkable change after the injection of IONPs and DFO for different times points in both species. In figure 3C and 3D (rat and pigeon respectively) the injection of IONPs alone in albino rat and domestic pigeon for 1 week revealed a significant increase in the AST enzyme levels by 70% and 51% respectively. While the simultaneous injection of IONPs and DFO for 1 week showed a significant decrease by 61% and 54% compared to IONPs injection alone in both albino rat and domestic pigeon respectively. In figure 3C, the AST levels in albino rat revealed a significant increase (42%) after the concurrent injection of IONPs and DFO after 1 hour compared to the IONPs after 1-hour single injection. However, similar simultaneous treatment of IONPs and DFO in domestic pigeon (Fig. 3D) caused a significant decrease (41.9%) in the AST enzyme levels compared to the IONPs after 1 hour of single injection.

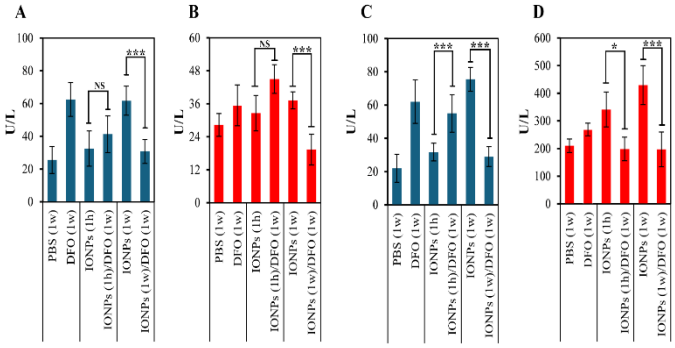

Figure 3: A graph showing the blood serum levels of ALT and AST in the albino rat (A and C, respectively) and ALT and AST in domestic pigeon (B and D, respectively), after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The liver enzymes (ALT and AST) showed a significant increase in albino rat after 1week of IONPs administration alone compared to those after 1week of IONPs and DFO simultaneous injection. While domestic pigeon showed a significant increase of AST only but not ALT after 1hour of IONPs administration alone in comparison to that after 1hour injection IONPs along with DFO.. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

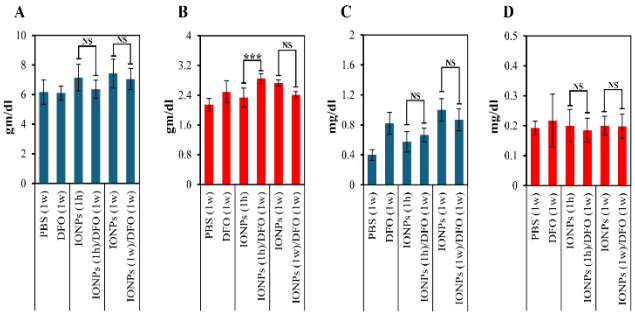

Figure 4: A graph showing the levels of total protein and total bilirubin in albino rat blood serum, (A and C, respectively) and in domestic pigeon (B and D, respectively), after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The domestic pigeon showed a significant increase in the total protein after 1 hour of IONPs and DFO injection in comparison to that after 1 hour of IONPs injection alone. While the albino rat showed no significant increase in the levels of total protein and bilirubin. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

3.2.1.3. Serum total protein

For the albino rat's total protein (Fig. 4A), revealed no significant abnormalities even after 1 hour or 1 week injection of IONPs alone or along with DFO compared to the control group. However, the total protein levels in domestic pigeon (Fig. 4B) showed no significant difference between injection of IONPs alone or IONPs along with DFO. While the injection of IONPs for 1 hour along with the DFO led to a significant increase (19.3%) in the serum total protein compared to that after single injection of IONPs.

3.2.1.4. Serum total bilirubin

In the case of total bilirubin, in albino rat (Fig. 4C) the injection IONPs alone or along with the DFO for 1 week showed a significant increase in the serum total bilirubin levels compared to the control by 60% and 53.4% respectively. However, the IONPs and DFO injection for 1 week showed no significant differences in comparison with the singular injection of IONPs for the same exposure time. The levels of serum total bilirubin in the domestic pigeon (Fig. 4D) revealed no significant differences after injection of the IONPs alone or along the DFO compared to the control.

3.2.2. Kidney function:

3.2.2.1. Serum creatinine

As shown in figure 5A and 5B the serum creatinine levels in albino rat and domestic pigeon respectively, showed no significant difference after the singular or simultaneous injection of IONPs and DFO for 1 week. However, the concurrent injection of IONPs for 1 hour and DFO for 1 week led to a significant increase (27%) in the creatinine levels in comparison to the single injection of IONPs for 1 hour.

3.2.2.2. Serum urea

The blood serum urea in albino rat and domestic pigeon (Fig. 5C and 5D respectively) showed no significant difference after the injection of IONPs alone or simultaneously with DFO for 1 week compared to the control group. However, the concurrent injection of IONPs for 1 hour and DFO for 1 week in the domestic pigeon (Fig. 5D) revealed a weak significant increase (25.5%) compared to the single injection of IONPs for 1 hour. On the other hand, the same treatment conditions in albino rat (Fig. 5C) showed no significant abnormalities.

3.2.2.3. Serum uric acid

In the albino rat, serum uric acid (Fig. 5E) showed a significant increase after singular injection of IONPs for 1 week (41.7%) compared to the control. Contradictory, the injection of IONPs along with DFO for 1 week caused a significant decrease (35.4%) compared to IONPs single injection. However, the concurrent injection of IONPs for 1 hour and DFO for 1 week led to significant increase (28.5%) in the serum uric acid levels in comparison with IONPs 1 hour injection. The serum uric acid levels in the domestic pigeon (Fig. 5F) displayed no significant abnormalities compared to that in the control at different treatment conditions.

3.2.3. Blood parameters:

3.2.3.1. HB and HCT value

The HB and HCT levels in albino rat (Fig. 6A, and 6C, respectively) and domestic pigeon (Fig. 6B, and 6D, respectively), the injection of IONPs for 1 hour and 1 week alone or along with DFO revealed a negligible non-significant abnormality among the treated groups and different treatment conditions.

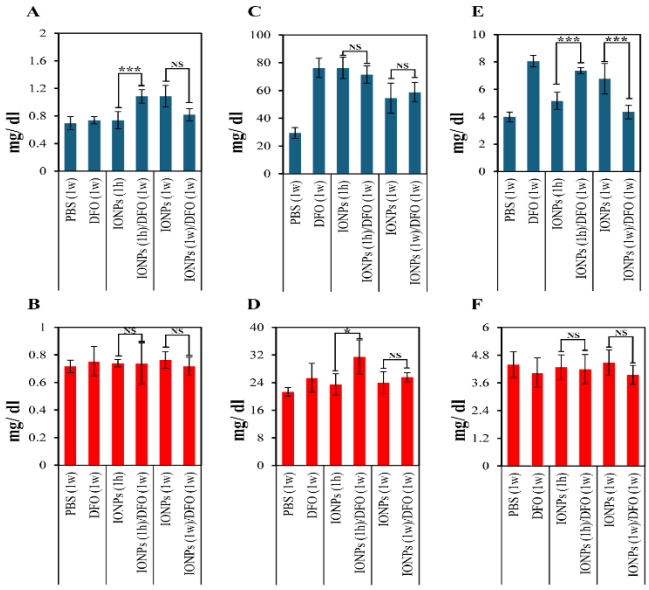

Figure 5: A graph showing the levels of kidney function parameters in serum creatinine (A and B), urea (C and D) and uric acid (E and F) in albino rat and domestic pigeon respectively, after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The creatinine and uric acid levels in albino rat and urea in domestic pigeon showed a significant increase after 1 hour of IONPs and DFO simultaneous injection compared to IONPs alone. Only the uric acid levels in albino rat showed a significant increase after 1 week of IONPs injection alone in comparison with simultaneous injection of IONPs and DFO. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

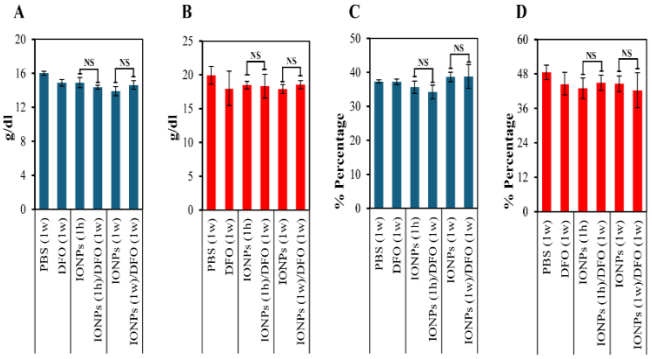

Figure 6: A graph showing HB and HCT levels in blood serum for albino rat (A and C, respectively) and for domestic pigeon (B and D, respectively), after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The HB and HCT showed no significant abnormalities in either rat or pigeon after injection of IONPs or DFO. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

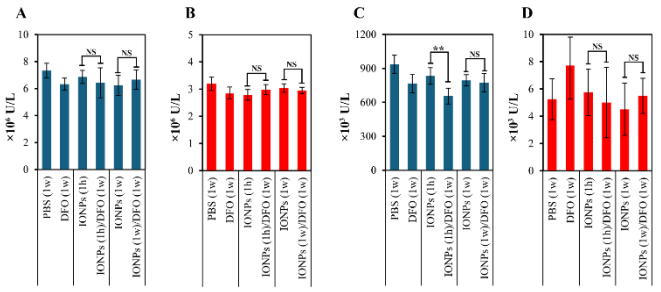

Figure 7: A graph showing RBCs and PLT numbers for albino rat (A and C, respectively) and for domestic pigeon (B and D, respectively), after IV injection of IONPs alone or IONPs IV injection along with DFO subcutaneously. The numbers of RBCs and PLT showed no significant abnormalities in either rat or pigeon after injection of IONPs or DFO. The PLT numbers in rat significantly decreased after 1 hour of IONPs and DFO injection compared to those after 1 hour of IONPs alone. *** and NS indicate p value ≤0.001 and ≥ 0.05 respectively (One-Way ANOVA test).

3.2.3.2. RBCs and PLTs numbers

The injection of IONPs for 1 hour or 1 week alone or simultaneously along with the DFO for 1 week caused no significant change in the numbers of the RBCs in both albino rat and domestic pigeon (Fig. **7A** and **7B** respectively). However, the number of PLT in the albino rat (Fig. **7C**) showed a slight significant decrease after concurrent injection of IONPs for 1 hour and DFO for 1 week compared to that after 1 hour of IONPs single dose. On the other hand, the injection of IONPs alone for 1 week or along with DFO for 1 week did not show any significant change in PLT number. On the same manner, the injection of the IONPs alone or along with DFO under different treatment conditions showed no significant abnormalities in the PLT numbers of the domestic pigeon (Fig. **7D**).

4. Discussion

Recently, magnetic nanomaterials are considered as a corner stone in the nanobiology as well as nanomedicine research fields. However, in the last few years the increased exposure to these magnetic nanoparticles led to harmful effects for both animals and human beings. This exposure resulted from the usage of magnetic nanoparticles in many fields such as medicine, biology, and electronics [**38**-**40**]. IONPs got an approval from the FDA because they are biodegradable and used commercially as an MRI contrasting agent and used in hyperthermia treatment [**5**, **41**, **42**]. Therefore, it is crucial in this study to explores comparatively in detail the toxicity of the IONPs in male albino rats and domestic pigeon after IV injection and the role of DFO as an iron chelator to ameliorate some of induced abnormalities.

In this study the daily IV injection of IONPs (5 mg/kg) alone for 1 week or along with subcutaneous injection of DFO for 1 week caused different biochemical abnormalities. Based on these findings, a higher concentration of iron ions was seen in the blood of rat and pigeon after daily IV injection of IONPs for 1 week, while that levels significantly decreased when DFO was injected simultaneously with IONPs. As a result of higher accumulation of iron (representing IONPs) in the blood, liver, kidney and spleen of both species showed same biodistribution profiles of IONPs in the blood based on AASA analysis. Another study agreed with these findings and reported that IV injection of IONPs with different doses in albino Wister rats caused deposition of the nanomaterial in these organs [**20**, **21**].

In the current study, after daily IV injection of IONPs for 1 week, some parameters representing liver as well as kidney functions in rat and pigeon were changed due to higher accumulation of IONPs in both liver and kidney. While DFO simultaneous injection with IONPs normalized those parameters to be similar with normal species. For example, ALT and AST levels in serum of both rat and pigeon and uric acid in the rat serum significantly increased after 1 week of IONPs injection, while simultaneous injection of DFO with IONPs for 1 week decreased ALT, AST and uric acid levels to be similar with normal species. These results are consistent with those of Awaad [**43**] who reported that intravaginal injection of the magnetic IONPs in albino mice caused a significant increase in the serum levels of ALT and AST after two weeks. As well, some studies informed that the oral administration of IONPs with different doses from 25 µg/gr to 300 µg/gr and 30 mg/kg respectively induced a significant increase in the liver serum enzymes [**18**, **44**]. Contradictory, some researchers studied the effects of IONPs on the broilers and found that administration of IONPs on the birds diet with dose of 80 mg/kg resulted in a significant increase of the liver AST but not ALT [**22**].

The increase in these enzymes is due to the hepatotoxicity which resulted from the aggregation of the IONPs in the liver which led to oxidative stress with generation of the ROS. The injured hepatocyte will have a leaky membrane which releases the liver enzymes and cause the elevation of the liver enzyme levels. However, the normalization of the liver and kidney enzymes after injection with DFO maybe due to its mechanism which gave it the ability to chelate the iron nanoparticles in the form a degradable complexes that could be excreted out of the body through urine or faeces [**29**, **45**]. Also, it was reported that DFO plays an important role in recovering from several diseases in which oxidative stress contribute in pathophysiology, such as the renal and hepatic diseases [**46**].

In our study the HB, HCT value and RBCs count showed non-significant abnormalities in both albino rat and domestic pigeon. These results are in disagreement with Mohammed, D. et al. who reported that the albino rat (Rattus rattus norvegicus) with doses of 1 mg and 10 mg/kg with size (25 and 50nm) showed an elevation in the levels of HB and HCT [**47**]. Also, the number of RBCs increased compared to the control group. However, our results are consistent with those of Shafie, E. H. et al., who studied the effect of IONPs on Blood in male albino rats for 21 days with a single dose of 0.4 mg/day and double dose of 0.8 mg/day and showed non-significant abnormalities [**48**].

5. Conclusion

In conclusion, this study revealed that the IONPs injection for long periods (i.e. 1 week) could induce hepatic, renal and spleen toxicity. This may be due to the aggregation of the IONPs in the liver, kidney, and spleen.

The current study showed that injection of IONPs for 1 week resulted in a significant increase of the liver enzymes and kidney parameters. Also, the iron deposition levels in blood, spleen, liver and kidney in turn compared to the control. However, simultaneous injection of DFO which act as iron chelator caused a significant decrease in the liver and kidney function as well as the iron deposition levels in the blood and other organs in compared to those in animals treated with IONPs for 1 week.

CRediT authorship contribution statement:

Conceptualization, Aziz Awaad. and Ahmed Rushdy methodology, Ahmed Rushdy, Walaa Magdy Abd-Elsamei.; software, Aziz Awaad. and Ahmed Rushdy.; writing—original draft preparation, Aziz Awaad, Ahmed Rushdy, Mohamed A. Adly.; writing—review and editing, Aziz Awaad, Ahmed Rushdy, Mohamed A. Adly.; supervision, Aziz Awaad, Mohamed A. Adly. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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