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STUDY OF IRON OXIDE NANOPARTICLES AND THEIR TOXICOLOGICAL EFFECTS

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Abstract

In the current study, three concentrations of iron oxide nanoparticles (25,125,500) mg /kg, which was injected into the male albino mice inside the peritoneum for 7 and 35 days. Effects on blood parameters included a significant increase in each of the White Blood Cell (W.B.C); Haemoglobin (Hb); packed cell volume (P.C.V); Mean Cell Volume (MCV); Mean Cell Haemoglobin Concentration (MCHC); Mean Cell Haemoglobin (MCH) also the results records significant increase in Red Blood Cell (R.B.C.). The result of Serum showed an increase in AST, ALT after injection for seven and thirty - five days. These particles accumulate in liver, kidney and lung and lead to oxidative stress with a generation of reactive oxygen species. In the case of the lipid profile, all parameters showed a decrease after seven days of exposure to iron oxide NPs. Regarding the histopathological study, the high concentration leads to changes such as focal dispersed necrosis of clusters of hepatocyte cells, sinusoidal dilatation and dispersed necrosis of hepatocyte cells in the liver, Also the high doses (500) mg/kg of Fe_3O_4 caused dense inflammatory cells infiltration with the destruction of alveolar tissue in the lung.

Key words: Fe_3O_4 NPs, Biochemical parameter, bioaccumulation, Histopathology

Introduction

Nanotechnology, one of the leading and most promising technology of the twenty first century. The enormous benefits of the application of nanotechnology in a variety of situations have been publicized. Specifically, nanoparticles are being used in drug delivery, gene transport, medical imaging, molecular diagnostics and cardiac therapy (sahoo *et al*., 2007). As well as in the food, cosmetics and environmental industry (Hood, 2004). Iron found in several different valence states, meaning it exists is a transition metal and can form with other elements such as oxygen various compounds. IONPs come in three main oxidative states: FeO, Fe_2O_3 and Fe_3O_4 (Ohta *et al*., 2012). Some special applications for iron-based *e.g.* in magnetic seals and inks, data storage, and ferrofluids. NP are applied due to their unique characteristics, such as their small size, surface chemistry and magnetic properties (Teja and koh, 2009). Metal inflammatory and metal oxides NPs including IOMNs, which could easily induce response, immune response, and could lead to a change in hematological parameters (like white blood cell count) (Nel *et al*., 2006). Humans can have an impact not only on nanotechnology, but also on the environment chains (Johnson *et al*., 2018). Nanoparticles such as Fe_3O_4 when enter in to the bloodstream, they immediately encounter by the plasma proteins and immune cells. Include pathways numerous activities like reduced number of blood cells, anti-mitotic properties, reduction in cellular antioxidants and stimulation of oxidative stress (Gaharwar and Paulraj, 2015). Identify the liver as a target organ to adverse effects of in vivo exposure to iron oxide NPs When the nanoparticles enter the liver lead to induce Alanine transaminase (ALT) and aspartate aminotransferase (AST) which are considered two of the most reliable markers of hepatocellular injury or necrosis following their exposure, NPs are primarily readily taken up by hepatocytes and Kupffer cells (specialized macrophages located in the liver) (Sadauskas *et al*., 2007). Moreover recent publications have reported that the Fe_3O_4 NPs induced toxicity, exposure of iron nanoparticles to human microvascular endothelial cells increased the cell permeability through reactive oxygen species (ROS) (Apopa *et al*., 2009). Bio-distribution studies have found that two main administration routes for IONPs into the body are intravenous (IV) injection and intraperitoneal (IP) injection, as well as, IONPs are accumulation primarily to the liver and spleen for clearance via mononuclear phagocytes(Bashir *et al*.,

2015; Popa *et al*., 2014; Prodan *et al*., 2013). When mice were exposed to Fe_3O_4 -nanoparticles via intraperitoneal injection caused injuries were monitor in both Hepatic and renal tissues were sliced for physiological observation (Ma *et al*., 2012). The goal of the study is to estimate iron oxide NPs on blood parameters in addition to biochemical parameters, check-up of histopathological changes in liver and lung.

Materials and Methods

Nanoparticles

Iron oxide Fe_3O_4 NPs (73.22 nm) were purchased as black nano powder from Aryj Al-furat company (chemical company in Baghdad, Iraq), spherical morphology. Size average detect by atomic force microscope (AFM) and scanning probe microscope (SPM) Fig. 1.

Animal Housing

Albino male mice were used in the present study between (6-8) weeks and their average weight were about (25±5) gm, obtained from the Biotechnology Research Center. All mice were housed under controlled conditions of temperature, humidity, and feeding.

Toxicity test for Fe_3O_4 NPs

One group was control and there (7 animals per group) is possibility of gaining a direct entry into the body and the purposes of testing for sub-chronic Fe_3O_4 toxicity, were intraperitoneally-injected with (25mg/kg,125mg/kg, and 500 mg/kg) of Fe_3O_4 NPs, two times a week, for 7 and 35 days, After last dosing, blood samples were collected for hematological and biochemical tests.

Hematological parameters

Collected blood samples from mice by heart puncture overall health were evaluated in the present study by using complete blood count, after collected and put in the (EDTA) tube. Measured by using mindray auto hematology analyzer.

Biochemical parameters

Biochemical test was analyzed spectrophotometer, they were placed in the centrifugation instrument (6000 rpm) in (15 min) used for assessment of various biochemical test such as liver function, lipid profile, kidney function and Antioxidant factors.

Bioaccumulation study

After collecting and weighting organs (liver, lung and kidney) from mice in every group, the organ was preserved in a freezing state and digested. The concentrations of iron detected in each sample using a flame atomic absorption spectrophotometer (AAS).

Body weight and organ index

In study period, body weights of animals were recorded, the ratio of organ weight to body weight calculated to determine coefficients of liver, lung and kidneys.

Histopathological study

At the end of the experiments of the toxicological study, liver and lung were collected from animals' groups. and the organs were kept in 10% formalin, then routine histological preparations were carried out. The tissues were further examined using an optical compound microscope that turned for staining by Haematoxylin and Eosin (H&E) stains to detect any abnormal changes in the organ tissues.

Statistical analysis

Analysis of variance (ANOVA(and least significant difference (LSD) were used to explain the differences between means of parameters in treated and control groups ($p \leq 0.05$) ($p \leq 0.01$).

Results and Discussion

Hematological parameters

Test are important to hematological parameters for the assessment of the animal and human physiological

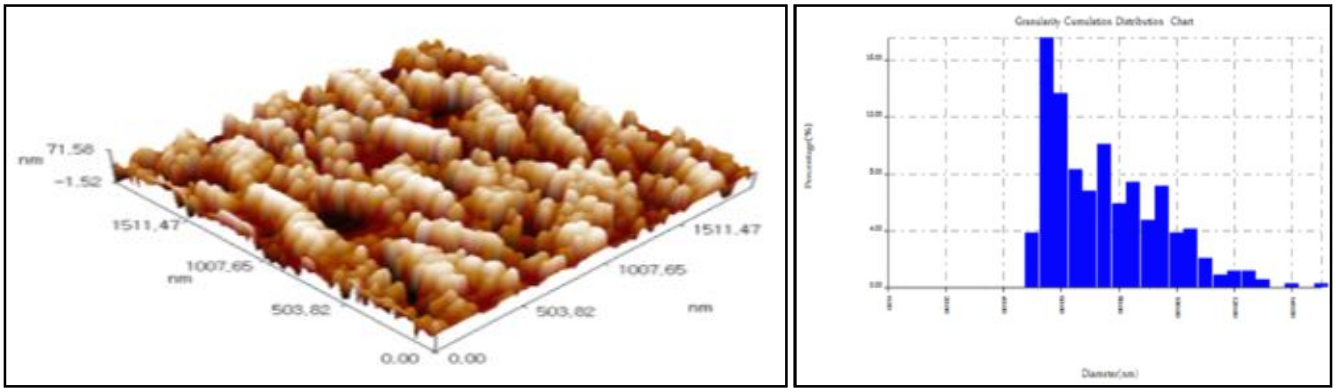

Fig. 1: Characteristics of Fe_3O_4 NPs, spherical shape using AFM (right), granularity distribution chart (left).

Table 1: Mean value ± standard error of hematological parameters in mice exposed to three doses of iron oxide NPs by intra-peritoneal injection after seven and thirty-five days of exposure.

Groups after seven days	Mean ± SE						
	W.B.C10^{9}/l	R.B.C10^{12}/l	Hbg/dl	P.C.V %	M.C.Vfl	M.C.Hpg	M.C.H.Cg\l
Control	3.57±0.10 c	8.90±0.41 a	12.50±0.90 a	37.90±0.40 a	40.62±0.40 c	11.74±0.76 ab	306.50±1.50 bc
25 mg/kg	3.12±0.02 d	8.07±0.05 a	11.00±1.00 a	37.69±0.20 a	40.12±0.10 c	10.76±0.46 b	304.50±3.50 c
125 mg/kg	4.47±0.08 b	8.79±0.09 a	12.30±0.50 a	37.77±0.42 a	43.50±0.40 b	12.10±0.40 ab	313.00±1.00 b
500 mg/kg	5.35±0.08 a	8.99±0.50 a	13.00±0.90 a	38.81±0.07 a	46.05±0.25 a	13.34±0.16 a	323.00±1.00 A
LSD value	0.308 **	1.291 NS	3.325 NS	1.223 NS	1.230 **	1.938 *	7.974 **
Groups thirty five days	**Mean ± SE**						
	W.B.C10^{9}/l	R.B.C10^{12}/l	Hbg/dl	P.C.V %	M.C.Vfl	M.C.Hpg	M.C.H.Cg\l
Control	3.57±0.10 c	8.90±0.41 b	12.50±0.90 ab	37.90±0.40 b	40.62±0.40 b	11.74±0.76 c	306.50±1.50 b
25 mg/kg	5.88±0.06 b	9.55±0.87 ab	11.54±0.36 b	38.60±0.30 ab	40.75±0.55 b	12.75±0.25 bc	312.50±2.50 b
125 mg/kg	5.79±0.55 b	9.34±0.29 ab	12.26±0.04 ab	40.25±1.05 ab	41.67±0.01 ab	13.85±0.25 b	319.00±6.00 ab
500 mg/kg	11.37±0.12 a	11.23±0.27 a	13.70±0.20 a	40.90±0.65 a	47.45±0.95 a	15.70±0.20 a	331.50±2.50 a
LSD value	1.138 **	2.054 *	1.944 *	2.615 *	2.725 *	1.691 **	13.986 *

Different letters in columns refer to significantly different between means at * (P≤0.05), ** (P≤0.01).

status, these indices are closely linked to the animal's response to the environment (Gabriel *et al*., 2004). groups exhibited clear decreases in white blood cells (WBC) count, red blood cells and hemoglobin (Hb) values, along with an increase in the concentration and prolonged injection period in comparison with the control group.

Table 2: Mean value ± standard error (SE) of liver function test in mice exposed to iron oxide NPs by intra-peritoneal injection for seven and thirty five days.

Groups after 7, 35 days	Mean ± SE			
	ALT/ 7U\l	AST/7U\l	ALT/35U\l	AST/35U\l
Control	30.00±1.73 b	28.67±2.02 c	30.00±1.73 c	28.67±2.02 a
25 mg /kg	28.67±1.85 b	34.67±3.17 bc	38.00±1.52 bc	30.33±0.88 b
125 mg /kg	33.67±3.52 b	39.67±1.45 b	40.33±3.28 b	36.00±1.52 bc
500 mg /kg	49.00±1.00 a	49.67±2.84 a	50.00±2.88 a	51.67±2.40 c
LSD value	7.271 **	8.061 **	8.062 **	5.879 **

Different letters in columns refer to significantly different between means at** (P≤0.01).

Regarding P.C.V, the result in first dose (25 mg/kg) and second dose (125 mg/kg) after seven days showed significant decrease in mean value (37.69±0.20% and 37.77±0.42%) respectively, compared with control (37.90±0.40%). After thirty five days intraperitoneal injection of Fe_3O_4 NPs in male mice, the results of all parameters showed value increase, The results of the statistical analysis showed that there were significant differences between the total numbers in most study periods and at the probability levels (P≤0.05)

Table 3: Mean value ± standard error (SE) of lipid function test of mice exposed to iron oxide NPs by intra-peritoneal injection for (seven) and (thirty five) days.

Groups 7 days	Mean ± SE				
	TCmg/ dl	Trig.mg/ dl	LDLmg/ dl	HDLmg/ dl	VLDLmg/ dl
Control	84.67±1.45	81.33±1.85	37.67±2.90	32.00±3.51	16.26±0.37
25 mg /kg	86.00±.05	81.67±2.40	38.00±2.88	27.67±4.48	16.33±0.48
125 mg/ kg	89.33±2.33	88.67±3.17	40.67±1.20	31.33±0.88	17.80±0.69
500 mg/ kg	88.00±1.15	82.67±2.33	41.33±2.33	30.67±3.28	16.46±0.48
LSD value	6.960 NS	8.116 NS	7.932 NS	10.816 NS	1.694 NS
NS: Non-significantly.					
Groups 35 days	**Mean ± SE**				
	TCmg/ dl	TGmg/ dl	LDLmg/ dl	HDLmg/ dl	VLDLmg/ dl
Control	84.67±1.45 a	81.33±1.85 a	37.67±2.90 b	32.00±3.51 a	16.26±0.37 bc
25 mg /kg	89.33±2.60 b	78.67±0.88 b	36.33±1.85 b	37.67±1.33 a	15.73±0.17 c
125 mg/ kg	91.00±2.64 b	87.33±2.02 c	41.33±1.45 b	36.00±1.00 a	16.46±0.40 b
500 mg/ kg	108.33±4.91 b	96.67±2.90 bc	55.33±2.90 a	32.67±2.18 a	19.33±0.58 a
LSD value	10.313 **	6.679 **	7.725 **	7.271 NS	1.335 **

Different letters in columns refer to significantly different between means at ** (P≤0.01).

Table 4: Mean value ± standard error (SE) of antioxidant factors test of mice exposed to iron oxide NPs by intra-peritoneal injection for (seven) and (thirty five) days.

Groups	Mean ± SE			
	CAT/7U/L	SOD/7U/L	CAT/35U/L	SOD/35U/L
Control	4.72 ±0.48 a	11.00 ±0.43 a	4.72 ±0.48 c	11.03 ±0.39 b
25 mg /kg	4.44 ±0.22 a	9.69 ±0.26 b	6.22 ±0.11 b	11.73 ±0.09 ab
125 mg /kg	4.66 ±0.57 a	9.87 ±0.35 ab	7.11 ±0.17 ab	12.22 ±0.36 ab
500 mg /kg	5.13 ±0.21 a	10.17 ±0.08 ab	7.62 ±0.26 a	13.25 ±0.56 a
LSD value	1.588 NS	1.212 *	1.155 **	1.541 *

Different letters in columns refer to significantly different between means at * (P≤0.05), ** (P≤0.01).

and P≤0.01), table 1. Ability of these nanoparticles interaction with many components of blood specially WBC, platelets and coagulation factors in addition to plasma proteins and RBC may have significant effect on distribution of nanoparticles (Hagens *et al*., 2007).

Biochemical parameters

Liver function test

Iron oxide (Fe_3O_4) nanoparticles and magnetic field cause significant increasing of ALT after 30 day of injection in male Wister rats. Therefore, nanoparticles have toxic effect but magnetic fields may correct this effect to some extent , thus, increasing of the ALT enzyme shows damaging effect of iron oxide nanoparticles on liver cells (Jarahian *et al*., 2018). Many previous studies (Christ-Crain *et al*., 2004; Popova and Buravkova, 2006; Vozarova *et al*., 2002) (demonstrated that excess iron oxide administration can result in liver damage and leakage from the liver cytosol to blood flow caused increasing of these enzymes activity and it may be due to increasing of anabolism and reducing of catabolism AST, ALT. Also, the results showed a considerable decline (p≤0.01) in means of ALT (28.67±1.85 U/L) after exposure dose (25 mg/kg) through the seven days. In seven days, there are slight increase in second dose (33.67±3.52 (UL) and in third dose (500 mg/kg). Statistical analysis of the results of ALT indicated that two doses of exposure show marked significant elevation in ALT (P≤0.01). Another report, noted increased levels of (AST/ALT) enzymes in serum and this increase associated with hepatic multiples/toxicity, and may denote stress on the liver imposed by the NPs (Aliahmad *et al*., 2016). Furthermore, AST and ALT enzyme raised after all three period and exposure to Fe_3O_4 nanoparticles in thirty-five days of study compared with mean value of control, showed in table 2.

lipid profiles

The results varied between slight increase and decrease in all parameters (TC, TG, LDL, HDL and VLDL) for the three concentrations (25, 125, 500) mg/kg used in this study after one week of exposure to Fe_3O_4 NPs. No significant difference compared with the control group. Data current suggested that the high exposure of Fe_3O_4 after 35 day can change in all values of lipid profiles table 3. In addition, LDL play the main role in causing the progression of atherosclerosis and, in specific, coronary sclerosis(Rifai *et al*., 1992).

Antioxidant factors

A statically significant and dose-dependent increase

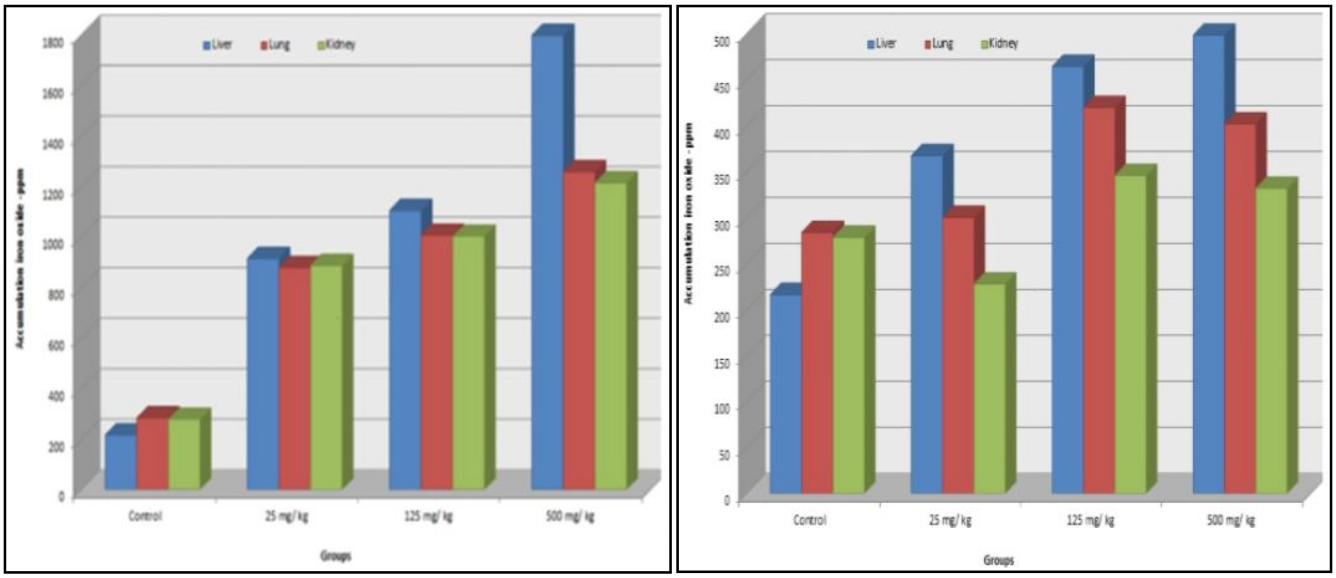

Fig. 2a, b: Accumulated iron oxide in liver, lung and kidney of mice after seven (a) and thirty five day from exposed to concentration (25,125,500) mg / kg (b).

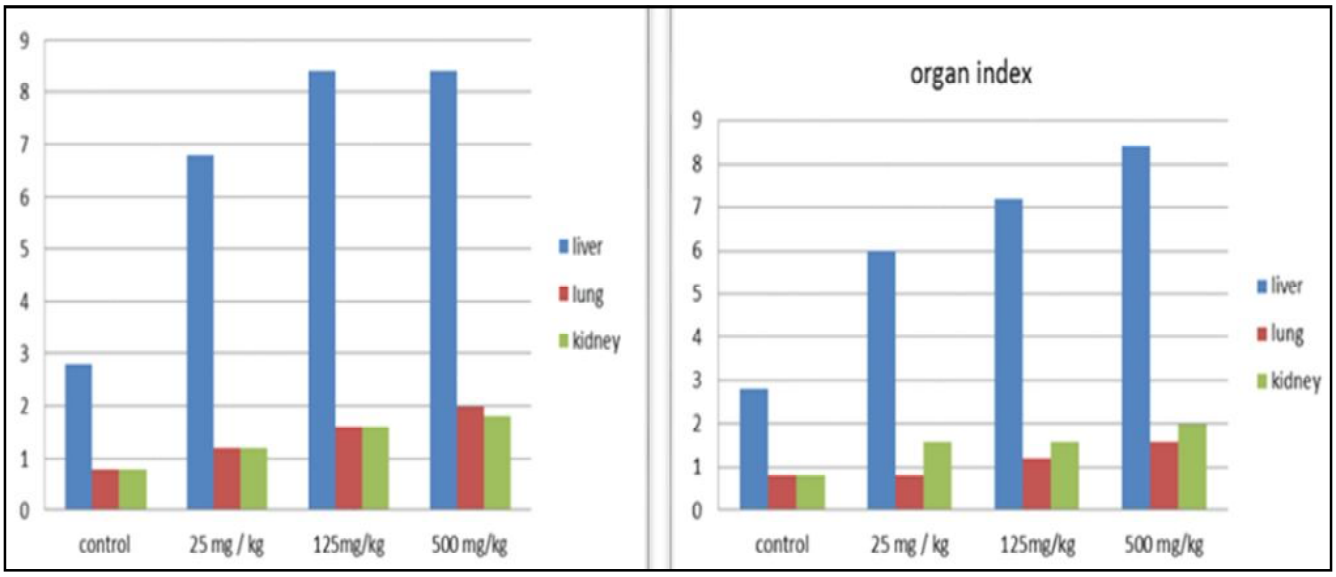

Fig. 3a, b: Organ index of mice exposed injection after 7 (right) and 35 (left) days.

($p \leq 0.05$) in serum mean levels of CAT and SOD enzymes was also observed in the treated animals after 7 days. This increase could be related to the increase of iron nanoparticles by intra -peritoneal in different doses to mice in oxidative stress, lead to generate ROS (Dashtipour *et al.*, 2015; Samal and Paulraj, 2010). On the other hand, in case of Catalase enzyme, highest mean value in 35 days mean value (7.62±0.26 U/L) compared with control (4.72±0.48 U/L). The analysis of variance showed significant differences between values at ($P \leq 0.01$) table 4.

Previous studies have reported an increase in oxidative stress with an increase in free iron (Puntarulo, 2005; Mehta *et al*., 2004).

Table 5: Mean value ± standard error (SE) of accumulated in liver, lung and kidney of mice exposed to iron oxide NPs by intra-peritoneal injection for(seven) and (thirty five) days.

Groups 7	Mean ± SE		
	Liverppm	Lungppm	Kidneyppm
Control	216.06 ±473 d	283.49 ±14.17 b	278.36 ±9.48 bc
25 mg/ kg	367.00 ±12.00 b	300.00 ±10.00 b	227.50 ±27.50 c
125 mg/ kg	464.00 ±20.00 a	419.50 ±30.50 a	345.50 ±12.50 a
500 mg/ kg	498.00 ±4.00 a	401.53 ±1.35 a	331.56 ±1.56 ab
LSD value	47.38 **	68.93 **	62.23 **
Groups 35	**Mean ± SE**		
	Liverppm	**Lungppm**	**Kidneyppm**
Control	216.06 ±4.73 d	283.49 ±14.17 d	278.36 ±9.48 d
25 mg/ kg	914.59 ±14.59 c	878.00 ±2.00 c	886.69 ±7.47 c
125 mg/ kg	1104.29 ±2.73 b	1006.31 ±4.01 b	1002.95 ±1.45 b
500 mg/ kg	1797.41 ±47.30 a	1258.00 ±25.00 a	1213.50 ±2.50 a
LSD value	97.77 **	57.10 **	24.37 **

Different letters in columns refer to significantly different between means at ** ($P \leq 0.01$).

Bioaccumulation of iron oxide nanoparticles

The unique properties for nanoparticles have a tendency to aggregate. As well as, Nanoparticles accumulation gradually in blood and different organs and their gradual removal from urine and feces. As well as, the most prevalent organs in the accumulation are liver, kidneys and spleen regardless route of exposure, nanoparticles physiochemical properties and animal models (Wu and Tang, 2018). After injection by 25 mg/ kg of Fe_3O_4 NPs in mice and analysis tissue of liver, lung and kidney, the result showed no accumulated in Kidney (227.50±27.50 ppm), slight increase accumulation in both liver and lung (367.00±12.00 ppm, 300.00±10.00 ppm) compared with control (Kidney, 278.36±9.48ppm, liver, 216.06±473 ppm, lung, 283.49±14.17 ppm) which appearance in table 5 and Fig. 2a. In other study concluded after injected Fe_3O_4-nanoparticles to mice, that the nanoparticles are rapidly delivered by blood to lung, liver and spleen, as well as, that metabolic rate of Fe_3O_4 NPs is affecting of these organs by their toxicity and possibly they remained in organs for a long time (Zhao *et al*., 2012). Recorded significant levels of Fe , especially after 35 days of injection mice in first dose (25 mg/kg) at the same period of exposure, the levels were 914.59±14.59 ppm, 886.69±7.47 ppm, 878.00±2.00 ppm, in liver, kidney and lung respectively which observed in Fig. 2b.

Organ index and body weights

Slight decreasing illustrated in body weight were noted after daily intraperitoneal injection with Fe_3O_4 NPs by used the doses: 25, 125 and 500 mg/kg for 7 days, In this study, the organ index of liver, kidney

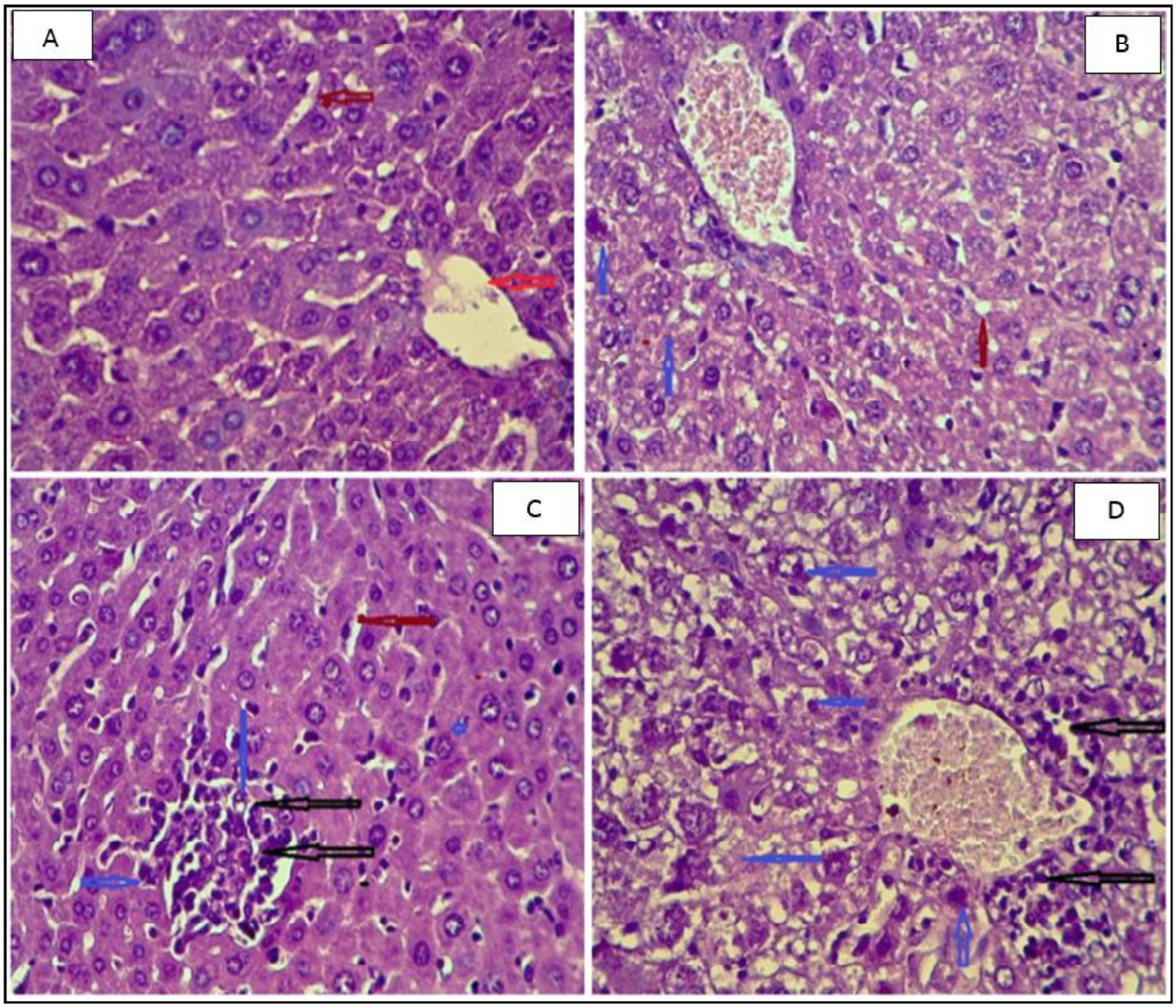

Fig. 4: Section of liver, control (A), slight dilatation of sinusoid (blue and red arrows) (B), focal dispersed necrosis of clusters of hepatocyte cells (blue arrow) with abundant inflammatory cells infiltration (black arrow), sinusoidal dilatation (red arrow) (C), dispersed necrosis of hepatocyte cells (blue arrows) with abundant infiltration of inflammatory cells (D) (black arrows) (X40) (H & E).

and lung increased significantly after injection with (25,125,500 mg/kg of iron nanoparticles) respectively were showed in Fig. 3a, b. In addition, after mice treated and measured the organs such as liver and kidney these organs did not show any increase in weight compared with control (Remya *et al*., 2016). Also, rats treated with IONPs after one, three and four weeks showed significant loss in body weight (Reddy *et al*., 2017).

Histopathological changes in tissues

Liver

Histolopathological examination of liver in control group showed normal liver of mice. Iron oxide nanoparticles induced various pathological change in liver of mice injected intraperitoneal with third dose (500 mg/kg) of Fe_3O_4 after seven days included few changes in central venule . In the current study, the results of a liver tissue examination after a 35 day of exposure to iron oxide NPs, showed organ damage in (125,500) mg/kg of iron oxide doses as in the figures below Fig. 4A, B, C, D. Histological checking to the livers of the rats ,which injected intraperitoneally by dose of (500 mg/kg) iron oxide NPs through 5 weeks, showed the lobules was damaged because a large amount of iron containing pigment as conglomerates in periportal zones, intralobular and, occasionally, centrilobular, and as separate particles in sinusoids (Katsnelson *et al*., 2011).

Lung

Lung is complex system, due to response to foreign

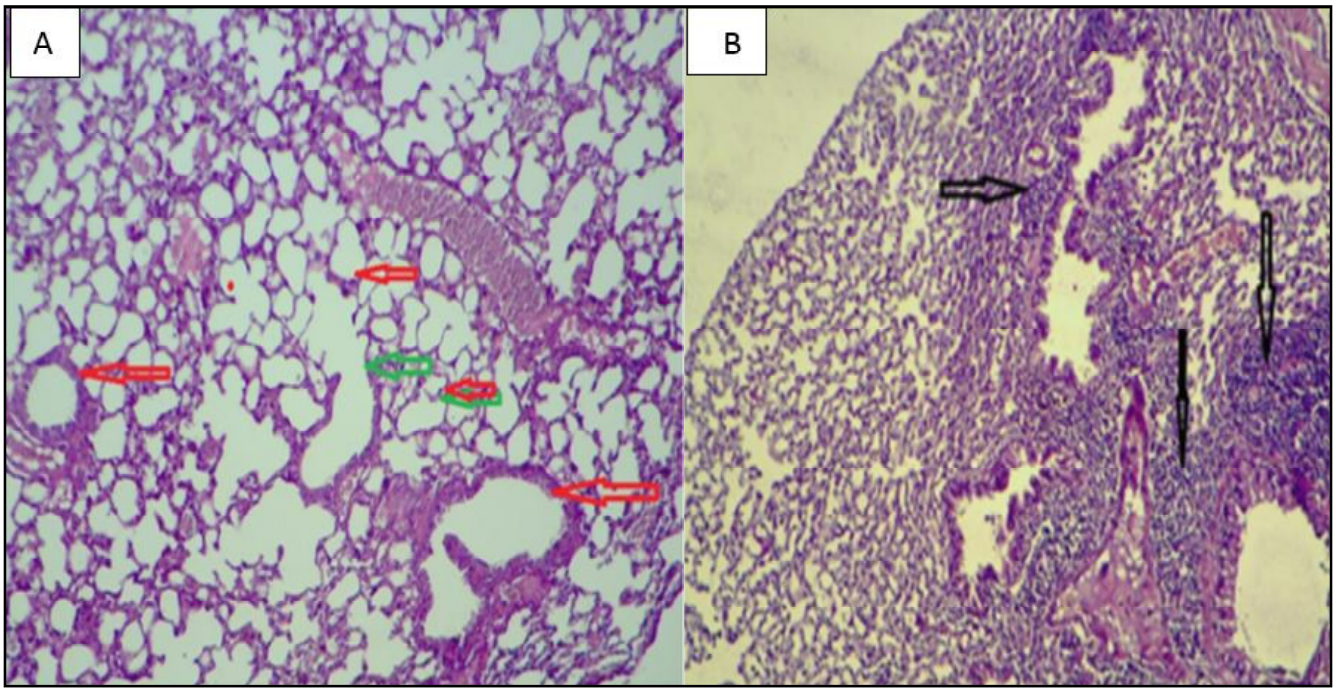

Fig. 5: Section of lung, control (A), heavy inflammatory cells infiltration with destruction of alveolar tissue (black arrows) (B) (X10) (H & E).

particle exposure relies on chemical interactions and communications between a vast array of cell types (Kornberg *et al*., 2017). The change of histopathological in mice that exposed to (500 mg/kg) of Fe_3O_4 NPs after 35 days show heavy inflammatory cells infiltration with destruction of alveolar tissue Fig. 5 A, B.

Conclusions

The result of doses Fe_3O_4 NPs demonstrated that 35 days repeated, caused adverse sign and symptoms of toxicity in high dose intraperitoneally-injected in mice, these changes it appeared clearly in the blood parameter and accumulating in both the liver, kidney and lung this is related to tissue changes.

References

Aliahmad, M., A. Rahdar, F. Sadeghfar, S. Bagheri and M.R. Hajinezhad (2016). Synthesis and Biochemical effects of magnetite nanoparticle by surfactant-free electrochemical method in an aqueous system: The current density effect. *Nanomedicine Research Journal,* **1(1):** 39-46.

Apopa, P.L., Y. Qian, R. Shao, N.L. Guo, D. Schwegler-Berry, M. Pacurari and V. Castranova (2009). Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling. *Particle and fibre toxicology,* **6(1):** 1.

Bashir, M.R., L. Bhatti, D. Marin and R.C. Nelson (2015). Emerging applications for ferumoxytol as a contrast agent in MRI. *Journal of Magnetic Resonance Imaging,* **41(4):** 884-898.

Christ-Crain, M., P.R. Huber, U. Keller, C. Meier, B. Müller, J. Puder and J.J. Staub (2004). Changes in liver function correlate with the improvement of lipid profile after restoration of euthyroidism in patients with subclinical hypothyroidism.

Dashtipour, K., M. Liu, C. Kani, P. Dalaie, A. Obenaus, D. Simmons and M. Zarifi (2015). Iron accumulation is not homogenous among patients with Parkinson's disease. *Parkinson's Disease, 2015*.

Gabriel, U., G. Ezeri and O. Opabunmi (2004). Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). *African Journal of Biotechnology,* **3(9):**.

Gaharwar, U.S. and R. Paulraj (2015). Iron oxide nanoparticles induced oxidative damage in peripheral blood cells of rat. *Journal of Biomedical Science and Engineering,* **8(04):** 274.

Hagens, W.I., A.G. Oomen, W.H. de Jong, F.R. Cassee and A.J. Sips (2007). What do we (need to) know about the kinetic properties of nanoparticles in the body? *Regulatory toxicology and pharmacology,* **49(3):** 217-229.

Hood, E. (2004). Nanotechnology: looking as we leap: National Institue of Environmental Health Sciences.

Jarahian, A., S. Fatahian and K. Shahanipour (2018). Investigation the effect of Fe3O4 nanoparticles on liver and stress oxidative parameters at the presence of magnetic field in rat. *Nanomedicine Journal,* **5(2):** 96-101.

Johnson, N.W., B. Gupta, D.J. Speicher, C.S. Ray, M.H. Shaikh, N. Al-Hebshi and P.C. Gupta (2018). Etiology and risk

factors. *Oral and Oropharyngeal Cancer*.

Katsnelson, B.A., T.D. Degtyareva, I.I. Minigalieva, L.I. Privalova, S.V. Kuzmin, O.S. Yeremenko and M.Y. Khodos (2011). Subchronic systemic toxicity and bioaccumulation of Fe3O4 nano-and microparticles following repeated intraperitoneal administration to rats. *International Journal of Toxicology,* **30(1):** 59-68.

Kornberg, T.G., T.A. Stueckle, J.A. Antonini, Y. Rojanasakul, V. Castranova, Y. Yang and L. Wang (2017). Potential toxicity and underlying mechanisms associated with pulmonary exposure to iron oxide nanoparticles: Conflicting literature and unclear risk. *Nanomaterials,* **7(10):** 307.

Ma, P., Q. Luo, J. Chen, Y. Gan, J. Du, S. Ding and X. Yang (2012). Intraperitoneal injection of magnetic Fe3O4-nanoparticle induces hepatic and renal tissue injury via oxidative stress in mice. *International journal of nanomedicine,* **7:** 4809.

Mehta, S.H., R.C. Webb, A. Ergul, A. Tawak and A.M. Dorrance (2004). Neuroprotection by tempol in a model of iron-induced oxidative stress in acute ischemic stroke. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* **286(2):** R283-R288.

Nel, A., T. Xia, L. Mädler and N. Li (2006). Toxic potential of materials at the nanolevel. *Science,* **311(5761):** 622-627.

Ohta, K., R.E. Cohen, K. Hirose, K. Haule, K. Shimizu and Y. Ohishi (2012). Experimental and theoretical evidence for pressure-induced metallization in FeO with rocksalt-type structure. *Physical review letters,* **108(2):** 026403.

Popa, C., E. Andronescu, M. Stoicea, P. Le Coustumer, S. Galaup, M. Beuran and A. Prodan (2014). Fabrication, Characterization, toxicity and biocompatibility evaluation of iron oxide nanoparticles. *Digest Journal of Nanomaterials & Biostructures (DJNB),* **9(1):**.

Popova, J. and L. Buravkova (2006). Blood biochemical parameters in women during long-term simulated hyperoxic diving up to 8 ATA. *Undersea Hyperb Med.,* **33(3):** 211-216.

Prodan, A.M., S.L. Iconaru, C.S. Ciobanu, M.C. Chifiriuc, M. Stoicea and D. Predoi (2013). Iron oxide magnetic nanoparticles: characterization and toxicity evaluation by *in vitro* and *in vivo* assays. *journal of Nanomaterials, 2013*.

Puntarulo, S. (2005). Iron, oxidative stress and human health. *Molecular aspects of medicine,* **26(4-5):** 299-312.

Reddy, U.A., P. Prabhakar and M. Mahboob (2017). Biomarkers of oxidative stress for *in vivo* assessment of toxicological effects of iron oxide nanoparticles. *Saudi journal of biological sciences,* **24(6):** 1172-1180.

Remya, N., S. Syama, A. Sabareeswaran and P. Mohanan (2016). Toxicity, toxicokinetics and biodistribution of dextran stabilized Iron oxide Nanoparticles for biomedical applications. *International journal of pharmaceutics,* **511(1):** 586-598.

Rifai, N., G.R. Warnick, J.R. McNamara, J.D. Belcher, G.F. Grinstead and I.D. Frantz Jr. (1992). Measurement of low-density-lipoprotein cholesterol in serum: a status report. *Clinical chemistry,* **38(1):** 150-160.

Sadauskas, E., H. Wallin, M. Stoltenberg, U. Vogel, P. Doering, A. Larsen and G. Danscher (2007). Kupffer cells are central in the removal of nanoparticles from the organism. *Particle and fibre toxicology,* **4(1):** 10.

Sahoo, S., S. Parveen and J. Panda (2007). The present and future of nanotechnology in human health care. *Nanomedicine: Nanotechnology, Biology and Medicine,* **3(1):** 20-31.

Samal, N. and R. Paulraj (2010). *Combined role of magnetic iron oxide nanoparticles and 2.45 GHz microwave radiation on antioxidant enzymes of mice*. Paper presented at the 2010 International Conference on Electromagnetics in Advanced Applications.

Teja, A.S. and P.Y. Koh (2009). Synthesis, properties and applications of magnetic iron oxide nanoparticles. *Progress in crystal growth and characterization of materials,* **55(1-2):** 22-45.

Vozarova, B., N. Stefan, R.S. Lindsay, A. Saremi, R.E. Pratley, C. Bogardus and P.A. Tataranni (2002). High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *diabetes,* **51(6):** 1889-1895.

Wu, T. and M. Tang (2018). Review of the effects of manufactured nanoparticles on mammalian target organs. *Journal of Applied Toxicology,* **38(1):** 25-40.

Zhao, S., X. Lin, L. Zhang, L. Sun, J. Li, W. Yang and Z. Sun (2012). The in vivo investigation of Fe3O4-nanoparticles acute toxicity in mice. *Biomedical Engineering: Applications, Basis and Communications,* **24(03):** 229-235.