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Nephrotoxicity of Iron Oxide Nanoparticles in Male Mice

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ABSTRACT

Background: The current study was conducted at Al-Nahrain University's Biotechnology Research Center. This study's objectives were to synthesize and characterize iron oxide nanoparticles (Fe_2O_3-NPs) and investigate the effects of varying concentrations on lipid peroxidation, the antioxidant defense system, biochemical parameters and kidney histology in male mice.

Methods: Twenty male Albinos Mice weighing between 25 and 30 g were utilized. The mice were split up into four groups of ten. Group II, Group III and Group IV received oral treatment with Tin oxide NPs at doses of 10, 25 and 50 mg/kg BW/day for four weeks, whereas the first group served as a control. Blood and kidney samples were taken for the examination of various parameters at the conclusion of the experiment.

Result: Creatinine and urea levels dramatically rose following treatment with iron oxide nanoparticles (Fe_2O_3-NPs) at varying doses. Treatment with iron oxide nanoparticles (Fe_2O_3-NPs) at varying concentrations resulted in a significant decrease in the activities of AST, ALT and ALP in mouse kidney homogenates, as well as a significant increase in lactate dehydrogenase (LDH). Histopathological changes were noted, confirming the biochemical perturbations caused by iron oxide nanoparticles (Fe_2O_3-NPs) at varying concentrations in mice kidney.

Key words: Fe_2O_3-NPs, Kidney histopathology, Liver, Mice, SOD, TBARS, Urea.

INTRODUCTION

Nanotechnology is growing quickly. The growing use of nanotechnology goods, particularly in biomedical applications, has sparked worries about the possibility of unanticipated negative health consequences after exposure. Comprehending the toxicological profiles of engineered nanomaterials is essential to guaranteeing their safety for use and responsible development, which maximizes advantages while minimizing hazards. However, the creation of toxicity data is not keeping pace with the research and manufacturing of artificial nanomaterials (Dhakshinamoorthy *et al.,* 2017; Di Bona *et al.,* 2014; Zhang *et al.,* 2017; Yaseen *et al.,* 2025; M Obaid *et al.,* 2025). These organizations have published official documents addressing the need for focused research on suitable methodological tests for determining the toxicity of manmade nanomaterials (Yang *et al.,* 2015; Radu *et al.,* 2015; Yaseen *et al.,* 2024). Iron oxide nanoparticles (IONs) possess significant promise for a range of biological and biomedical uses, including tissue engineering, targeted drug or gene delivery, magnetic resonance imaging (MRI) contrast enhancement, biological fluid detoxification, cancer treatment-induced hyperthermia, cell labeling, cell sorting and immunoassay (Ying *et al.,* 2022). Few toxicological investigations have recently documented the changes in IOMNs' tissue distribution, toxicokinetic characteristics and gene expression in mice or rats following different exposure routes (Kolosnjaj-Tabi *et al.,* 2015; Obaid *et al.,* 2020). The manufacturing process, surface features and particle size are all strongly correlated with the hydrodynamic size of IONs, which in turn influences the magnetic and biological properties, biodistribution, toxicokinetics, elimination and toxicity (De Barros *et al.,* 2014; Obaid *et al.,* 2020). The location of Fe_3O_3 MNPs in the vital organ tissues and their *in vivo* metabolic processes inside the body are still unclear, nevertheless. We used imprinting control region (ICR) mice as an experimental paradigm to synthesize Fe_3O_3 MNPs by chemical coprecipitation. Given the application viewpoint of Fe_3O_3 NMPs, the mice were administered the NMPs intragastrically. Atomic absorption spectrophotometry was used to assess the amounts of Fe_3O_4 MNPs in various organs and tissues at certain time points (Sharma *et al.,* 2018; Salimi *et al.,* 2020; Kareem *et al.,* 2023).

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MATERIALS AND METHODS

Chemicals

The following agents were procured from Sigma-Aldrich (St. Louis, USA).

Animals and experimental design

The experimental protocol was approved by the Local Ethics Committee and Animals Research and twenty male Albino mice weighing 25-30 g were acquired from the Al-Nahrain University animal house.

GP1: Control rats were orally for a period of 4 weeks.

GP2: Fe_2O_3-NPs were administered orally to mice for four weeks at a rate of 50 mg/kg BW/day.

GP3: Mice were treated orally with Fe_2O_3-NPs at a dose of 25 mg/kg BW/day for a period of 4 weeks.

GP4: Mice were treated orally with Fe_2O_3-NPs at a dose of 10 mg/kg BW/day for a period of 4 weeks.

Mice were fasted overnight, put to sleep and then dissected at the conclusion of the experiment. For biochemical tests, blood samples were extracted from the aorta and placed in glass tubes devoid of anticoagulants. Each rat's abdominal cavity was opened and the kidney and liver were removed. Histological and biochemical analyses were performed on these tissues.

Blood and serum samples

Each rat's aorta was used to draw blood, which was then inside glass tubes that have not been heparinized. Centrifugation was used for 15 minutes at 3000 rpm to separate the serum. Before being examined, the collected serum was kept at -18°C.

Tissue samples

The kidneys of the scarified mice were immediately removed and cleansed with cold saline. After that, they were weighed and cleaned using a cooled saline solution at a concentration of 0.9%. The tissues were chopped and homogenized (10% w/v) in a Potter-Elvehjem-type homogenizer with 1.15% KCl in ice-cold sodium phosphate buffer (0.01 M, pH 7.4). For 20 minutes at 4°C, the homogenates were centrifuged at 10,000 × g. The various enzyme activity, free radicals and biochemical parameters were analyzed using the resulting supernatants.

Determination of thiobarbituric acid-reactive substances content

The technique was used to quantify thiobarbituric acid-reactive compounds (TBARS) in liver and kidney homogenate of Ohkawa *et al.* (1979).

Determination of hydrogen peroxideand educed glutathione content concentration

In an ice bath, 100 mg of sample tissue was extracted using 5 ml of TCA (0.1%, w/v) and it was centrifuged for 15 minutes at 12,000 × g in order to determine the hydrogen peroxide (H_2O_2) concentration (Velikova *et al.,* 2000; Altemeemi *et al.,* 2021).

Determination of urea and creatinine concentration

A commercial kit provided by Diamond, Egypt, was used to measure the levels of creatinine and urea in the serum. The Patton and Crouch technique was used to estimate urea (1977).

Histopathological examination

Hematoxylin and eosin stain (Bancroft and Stevens, 1990) was used to prepare serial paraffin slices of kidneys that had been preserved in Bouin's solution for histological analysis. These sections were then seen under a light microscope According to (Al-Khuzaay *et al.,* 2024; Yahya *et al.,* 2024; Hameed *et al.,* 2025; Abd El-Rahmana *et al.,* 2024).

Ethical approval

This research was initiated after receiving the consent of the Medical Research Ethics Committee, Al-Nahrain University/Biotechnology Research Center.

Statistical analysis

Each measurement was performed twice in different experiments for each treatment. The data were expressed as mean ± standard error (SE). SPSS 17 was utilized to perform the statistical analyses, which included one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Effect of different concentrations of Iron oxide nanoparticles in mice kidney

According to the data in Table 1, the kidney homogenate of male rats treated with varying concentrations of iron oxide nanoparticles demonstrated a concentration-dependent

Table 1: Effect of different concentrations of Iron oxide nanoparticles in mice kidney.

Parameters	Experimental groups			
	Cont.	Fe_2O_3-NPs 50 mg/kg BW/day	Fe_2O_3-NPs 25 mg/kg BW/day	Fe_2O_3-NPs 10 mg/kg BW/day
TBARS (nmol/g tissue)	21.44±0.66^{d}	28.22±0.733^{c}	31.80±2.22^{b}	36.70±0.801^{ab}
H_2O_2 (µmol/g tissue)	41.60±2.70^{d}	55.01±2.39^{c}	61.10±2.77^{bc}	68.11±4.22^{ab}
GSH (mmol/mg protein)	3.44±0.011^{a}	2.14±0.055^{b}	1.80±0.055^{b}	1.70±0.044^{c}

significant increase in hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) when compared to the control and other iron oxide nanoparticle groups. However, compared to the control, the reduced glutathione level (GSH) was much lower.

Effect of different concentrations of Iron oxide nanoparticles in mice kidney

Table 2 displays information on the activity of the kidney antioxidant SOD, CAT, GPx, GR and GST enzymes. In comparison to the control group, the activity of antioxidant enzymes was significantly ($P<0.05$) reduced in a number of rat groups treated with iron oxide nanoparticles.

Effect of different concentrations of Iron oxide nanoparticles in mice kidney

Iron oxide nanoparticles at varying concentrations dramatically reduced the protein content of the rat kidney while considerably increasing serum urea and creatinine, as seen in Table 3, as compared to the control group.

Kidney histopathology

Fig 1 shows the morphological and histological changes in kidney tissues in all different study groups. The results showed the following: G1: The photomicrograph of the kidney sections stained with H and E in the control mice showed normal histological characteristics of the renal tubules (Tb) and glomruli (g) in the cortical portion. G2: A photomicrograph of the kidney sections stained with H and E after receiving 50 mg/kg BW/day of Fe_2O_3-NPs revealed mild to severe vascular degeneration in the renal tubule lining epithelial cells. G3: Photomicrographs of the kidney sections stained with H and E after receiving a dose of Fe_2O_3-NPs at a rate of 25 mg/kg BW/day revealed pyknotic nuclei, considerable localized necrosis and an increased incidence of vacuolar degeneration. G4: A photomicrograph of the kidney sections stained with H and E after receiving a dosage of Fe_2O_3-NPs at a rate of 10 mg/kg BW/day showed pyknotic nuclei, significant localized necrosis and an increased incidence of vacuolar degeneration.

These enzymes function independently and are kept up properly in concert and in concert to preserve the integrity and proper operation of tissues and cells under typical physiological settings. In the current work, rats fed Fe_2O_3-NPs showed elevated levels of TBARS and H_2O_2 and lower levels of antioxidants such GSH, indicating enhanced oxidative stress in kidney tissues (Ghaznavi *et al.,* 2022. The higher use of GSH to fight potassium free radicals or the binding of the -SH group to nanoparticles may be the cause of the drop in GSH levels. These results were in agreement with both (Reddy *et al.,* 2017; Luo *et al.,* 2024; Abd El-Aziz *et al.,* 2024). In order to keep ROS at appropriate levels, An impressive array of defense mechanisms is provided by antioxidant enzymes. Even little variations (Jomova *et al.,* 2024). Because it catalyzes the conversion of superoxide radicals into H_2O_2, (Pereira *et al.,* 1994; Gusti *et al.,* 2021; Al-Maliki *et al.,* 2025). Together with glutathione peroxidase, the ubiquitous enzyme catalase is a key component of the antioxidant defense system and catalyzes the breakdown of H_2O_2 into H_2O. Mice given Fe_2O_3-NPs at varying dosages for three weeks in the current study demonstrated a considerable reduction in protein content and alkaline phosphatase (ALP) activity, but a rise in lactate dehydrogenase activity. Following kidney damage, these enzymes are released into the bloodstream, which

Table 2: Effect of different concentrations of Iron oxide nanoparticles in mice kidney.

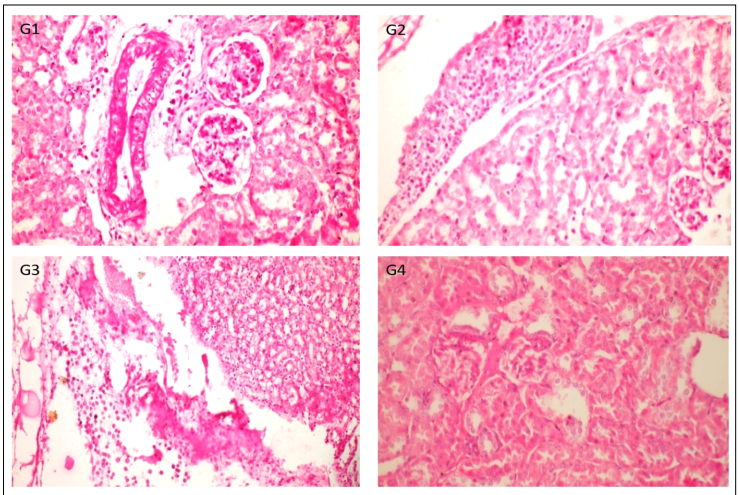
Parameters	Experimental groups			
	Cont.	Fe_2O_3-NPs 50 mg/kg BW/day	Fe_2O_3-NPs 25 mg/kg BW/day	Fe_2O_3-NPs 10 mg/kg BW/day
SOD (U/mg protein)	59.66±3.22^{a}	51.77±2.44^{b}	44.33±3.66^{c}	41.17±2.31^{cd}
CAT (µmol/hr/mg protein)	60.11±2.33^{a}	48.11±3.22^{b}	43.33±2.11^{c}	39.22±0.77^{d}
GPx (U/mg protein)	27.66±0.88^{a}	23.04±0.732^{b}	20.21±0.55^{c}	17.22±0.45^{cd}

Means ± SE are used to express values; each treatment group has five members. ABCD significant differences were observed between mean values within a row that did not share a common superscript letter ($p<0.05$).

Table 3: Effect of different concentrations of Iron oxide nanoparticles in mice kidney.

Parameters	Experimental groups			
	Cont.	Fe_2O_3-NPs 50 mg/kg BW/day	Fe_2O_3-NPs 25 mg/kg BW/day	Fe_2O_3-NPs 10 mg/kg BW/day
Urea (mg/dl)	40.22±3.29^{d}	44.07±1.29^{c}	47.11±2.33^{bc}	50.06±4.22^{ab}
Creatinine (mg/dl)	0.633±0.011^{d}	0.766±0.012^{c}	0.790±0.033^{bc}	0.841±0.041^{ab}
LDH (U/mg protein)	879±40.70^{d}	933±19.11^{c}	1089±30.05^{b}	1148±41.08^{ab}
ALP (U/mg protein)	181±7.11^{a}	177±3.33^{b}	157±4.03^{c}	140±6.66^{d}
Protein (mg/g tissue)	70.66±3.50^{a}	61.05±4.06^{b}	55.41±3.33^{b}	45.11±3.45^{c}

Means ± SE are used to express values; each treatment group has five members. ABCD significant differences were observed between mean values within a row that did not share a common superscript letter ($p<0.05$).



G1: Normal histological features of the glomruli (g) and renal tubules (Tb) in the cortical part were seen in the photomicrograph of the kidney sections stained with H and E in the control mice. G2: Following the treatment of 50 mg/kg BW/day of Fe_2O_3-NPs, a photomicrograph of the kidney sections stained with H and E showed mild to severe vascular degeneration in the renal tubule lining epithelial cells. G3: Following the administration of a dosage of Fe_2O_3-NPs at a rate of 25 mg/kg BW/day, the kidney sections' photomicrographs stained with H and E showed significant localized necrosis, pyknotic nuclei and an elevated incidence of vacuolar degeneration. G4: Following the administration of a dosage of Fe_2O_3-NPs at a rate of 10 mg/kg BW/day, a photomicrograph of the kidney sections stained with H and E revealed significant localized necrosis, pyknotic nuclei and an elevated incidence of vacuolar degeneration.

Fig 1: Hematoxylin and Eosin-stained photomicrographs of kidney slices in each group.

causes an increase in their activity in serum samples (Mishra *et al.,* 2017; Al-Maliki *et al.,* 2024). It's common knowledge that lipid peroxidation weakens cell membranes, allowing cytoplasmic enzymes to leak out (Bagchi *et al.,* 1995). Thus, liver, kidney and lung necrosis may be the cause of the lower activity of these enzymes in serum shown in our investigation (Wang *et al.,* 2010; Zedan *et al.,* 2023). Xinobiotics have been shown to alter the activities of several enzymes in various tissue organs. Normal histological structures of the glomruli and renal tubules in the cortical and medullary regions were found in the control rat group's kidney sections stained with hematoxylin and eosin. Bowman's capsule and proximal and distal convoluted tubules encircle the glomerulus, which is free of inflammatory alterations. A basic squamous epithelium is formed by cells in Bowman's capsule's outer or parietal layer. Because of their very diverse shapes, cells in the inner layer are invisible to histological routine staining. The glomerulei have an oval or circular form. The kidney segment showed modest to severe vascular degradation in the cortical zone of the renal tubule lining epithelial cells with pyknotic and karyolyzed nuclei following the injection of iron oxide nanoparticles. On the other hand, following the administration of iron oxide nanoparticles, kidney sections showed numerous abnormalities in the cortex and medulla renal structures, including the loss of the characteristic Malpighian corpuscles and the appearance of renal tubules with a wide lumen and degenerated epithelium, as well as pyknotic nuclei, increased incidence of vacuolar degeneration and noticeable congestion in the renal blood vessels (Fig 1). Numerous high-quality nanomaterials have surfaced as a result of the quick development of nanotechnology and because of their special biological characteristics, they are widely used in medicine, cosmetics, coatings, new materials, catalysts and many other fields to enhance existing products or create ones with new functions (Balas *et al.,* 2021; Saleh *et al.,* 2024). Exposure to nanomaterials is getting more likely, but the safety concern is also growing more acute. According to early toxicological research, nanoparticles may have a negative effect on both the environment and human health (Tate *et al.,* 2009; Ghiath *et al.,* 2025; Zedan *et al.,* 2022).

CONCLUSION

Iron oxide nanoparticles (Fe_2O_3-NPs) clearly had significant negative effects on mice's kidneys in a dose-dependent manner. Biomarkers for the detrimental effects of iron oxide nanoparticles (Fe_2O_3-NPs) might include biochemical measures, lipid peroxidation estimation and enzymatic and non-enzymatic antioxidants.

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Conflict of interest

All authors declared that there is no conflict of interest.

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