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Biochemical and Total Antioxidant Effects of Iron Oxide Nanoparticles on Liver Mice

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ABSTRACT

Background: This study focused on Iron oxide nanoparticles (Fe_2O_3-NPs) from multiple concentrations of Iron oxide nanoparticles (Fe_2O_3-NPs) on oxidation of lipids, the defence system of antioxidants and biochemical measurements in the liver of male mice.

Methods: Twenty male Albino Mice weighing 25-30 g were used in the study. Animals were categorised into four groups, each comprising 10 mice. The initial group served as the control. Groups II, III and IV received oral administration of Tin oxide nanoparticles at 50, 25 and 10 mg/kg weight body per day for four weeks. Blood and liver samples were collected after the experimental period to investigate various parameters.

Result: Treatment with Iron oxide nanoparticles (Fe_2O_3-NPsin) varying concentrations elevated levels of in comparison to the control group, the liver and kidneys exhibited reduced glutathione (GSH) content, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activities, as well as hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS). The protein contents of rats were significantly reduced when they were administered iron oxide nanoparticles (Fe_2O_3-NPs) at concentrations that differed from those of the control group. Treatment with iron oxide nanoparticles (Fe_2O_3-NPs) at varying concentrations substantially increased urea and creatinine. In mice, an increase in urea and creatinine concentration is a substantial indicator of liver dysfunction. In mouse liver homogenates, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly reduced when treated with iron oxide nanoparticles (Fe_2O_3-NPs) at varying concentrations. Conversely, lactate dehydrogenase (LDH) was significantly increased. It is clear that iron oxide nanoparticles (Fe_2O_3-NPs) induce pronounced hazardous effects in the liver of mice in a dose-dependent manner, Biomarkers for the detrimental effects of iron oxide nanoparticles (Fe_2O_3-NPs) may include estimating lipid peroxidation, enzymatic and non-enzymatic antioxidants and biochemical parameters.

Key words: ALT, Fe_2O_3-NPs, GPx, Male mice, SOD, TBARS.

INTRODUCTION

Investigation of nanoparticles is still in its infancy, but it can potentially solve many issues in various fields. By integrating nanotechnology with other scientific disciplines, the concept of synthesizing nanoparticles from their respective metals has been developed, such as chemistry, biology and physics (Omran *et al*., 2024; Hameed *et al*., 2025). Since the 1970s, Fe_3O_4-NPs have demonstrated potential applications due to the advancement of nanotechnology (Ying *et al*,.2022; Jasim and Ali., 2024). The research of Fe_3O_4-NPs has received much attention lately because of its magnetic nature, quantum size effect and very high surface-to-volume ratio, as well as because of their possible uses in bioscience and medicine (Jarockyte *et al*., 2016; Alyasiri *et al.,* 2025). When given to parental mice, Fe3O4-NPs coated with dimercaptosuccinic acid showed no negative impacts on gestation or fetal development, but they did have an impact on the offspring's spermatogenesis process (Kolosnjaj-Tabi *et al.,* 2015; Hmeed *et al.,* 2025). In assessing Fe_2O_3's biocompatibility in rats, toxicity was found in the kidneys, liver and lungs despite its elimination through the urine; however, no toxicities were found in the heart or brain (De Barros *et al*., 2014). Research conducted over the past two decades indicates that the principal mechanism underlying the toxicity of Fe_2O_3-NPs is the concentration-dependent

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overproduction of reactive oxygen species (ROS) (Markides *et al*., 2013 ; Al-Mashhadani *et al.,* 2024). Fe_2O_3-NPs induce oxidative stress in both *in vitro* and *in vivo* settings (Di Bona *et al.,*2014). Fe_3O_4-NPs are non-toxic, biocompatible and biodegradable. They have been used in various biomedical fields, including medication administration, gene therapy, tumour or vascular imaging and *in vivo* monitoring of tagged cells (Yang *et al.,* 2022; Bigini *et al.,* 2012; Ghiath *et al.,* 2025). As soon as these foreign particles are administered *in vivo*, a variety of innate immune systems begin to identify, gather and route them to the body's main elimination channels (Mohd Tamsir *et al*., 2019; Manickam *et al*., 2017). The aim of our study is to show the presence of Fe_3O_4-NPs at different concentrations in the liver of male mice.

MATERIALS AND METHODS

Chemicals

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

Animals and experimental design

The Local Ethics Committee and Animals Research approved the experimental protocol and twenty male Albino mice weighing 25-30 g were acquired from the Al-Nahrain University animal house. After two weeks of acclimatization, the mice were randomly assigned to four groups of ten animals each and the NIH Guide for Laboratory Animal Welfare was used to manage them.

GI

A ball-tipped curved intubation needle was employed to administer distilled water orally to rats for four weeks.

GII

Mice were treated orally with Fe2O3-NPs at 50 mg/kg BW/ day for 4 weeks.

GIII

Mice were treated orally with Fe2O3-NPs at 25 mg/kg BW/ day for 4 weeks.

GIV

Mice were treated orally with Fe2O3-NPs at 10 mg/kg BW/ day for 4 weeks.

Mice were fasted overnight, put to sleep and then dissected after 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 placed in non-heparinized glass tubes. The serum was then separated by centrifugation at 3000 rpm for 15 minutes. Before analysis, the collected serum was kept at -18°C (Abdula *et al.,* 2024; Kadhim *et al.,* 2024; Abass *et al*., 2025).

Biochemical analysis

AST, ALT and ALP levels were evaluated utilizing a commercial assay developed by Systems Diagnostic Laboratories (Texas, USA), using the specified methodology (Hasan *et al.,* 2024; Yahya *et al.,* 2024; Elsamie *et al*., 2021).

Antioxidants parameters estimations

The methods described were used to estimate the antioxidant factors in the testis homogenize (Hasan *et al.,* 2024; Razooki *et al*., 2025).

Ethical approval

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

Statistical analysis

The organosomatic index (OSI) is the mean 6 SD of six mice in each group. We used multiple Tukeys comparison tests and one-way analysis of variance (ANOVA) to look at all the data, with a significance level of $P<0.05$.

RESULTS AND DISCUSSION

The effect of different concentrations of Iron oxide nanoparticles on mice liver

The liver homogenate of male rats treated with varying concentrations of Iron oxide nanoparticles exhibited a concentration-dependent increase in thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2) compared to the control and other Iron oxide nanoparticle groups, as indicated by the data in Fig 1. However, the glutathione content (GSH) was significantly reduced compared to group control.

The impact of different levels of iron oxide nanoparticles on the liver of mice

Fig 2 presents data regarding the functions of hepatic antioxidant enzymes (SOD, CAT, GPx, GR and GST). Compared to the control group, the antioxidant enzyme activity was significantly reduced ($P<0.05$) in various rat groups treated with iron oxide nanoparticles.

Effect of different concentrations of Iron oxide nanoparticles on mice liver

As shown in Fig 3, AST, ALT, LDH, ALP and Protein were significantly decreased in Iron oxide nanoparticle-treated groups at different concentrations compared with controls.

Mice administered different concentrations of iron oxide nanoparticles in the current study demonstrated a significant decrease in GSH levels (Gaharwar *et al*., 2020; Ansari *et al.,* 2019). It is commonly known that GSH has antioxidant properties and that it influences associated enzymes' redox state. The liver homogenate's reduced glutathione (GSH) levels fell after iron oxide nanoparticle treatment. The rise in lipid peroxidation appears to be

caused by depleted GSH stores, which may otherwise control LPO levels (Reddy *et al*., 2017). A reduced level of GSH exacerbates the negative effects since it is essential for detoxifying ROS. GSH is an essential component of cells' antioxidant defences and a cofactor for antioxidant enzymes like GSH peroxidases (Averill-Bates *et al.,* 2023). Glutathione-related enzymes utilize glutathione to detoxify peroxides generated by elevated lipid peroxidation in oxidative stress (Seiler *et al*., 2008). Given the study's findings of increased lipid peroxidation and decreased GSH activity, it is plausible that the heightened peroxidation is caused by reduced GSH storage. Fe_2O_3-NPs decreased AST, ALT and ALP activity, according to the study's findings, which is in line with The claim that all groups treated with Fe_2O_3-NPs had significantly higher ALT activity compared to the control group (Obidah Abert *et al.,* 2022; Al-Maliki *et al.,* 2024). The cytotoxicity of contaminants, clinical practice and cellular damage can all be detected by the enzyme LDH. The transition from anaerobic glycolysis to aerobic respiration is indicated by LDH activity. Significant cellular damage may cause changes in dehydrogenase activity in rats given acetamiprid and abamectin benzoate. This would increase dehydrogenase release and impact the metabolism of proteins and carbohydrates (Chakroun *et al*., 2016; Talleh *et al*., 2020; Zedan *et al*., 2022). The liver protein level of mice given different doses of Fe_2O_3-NPs was considerably

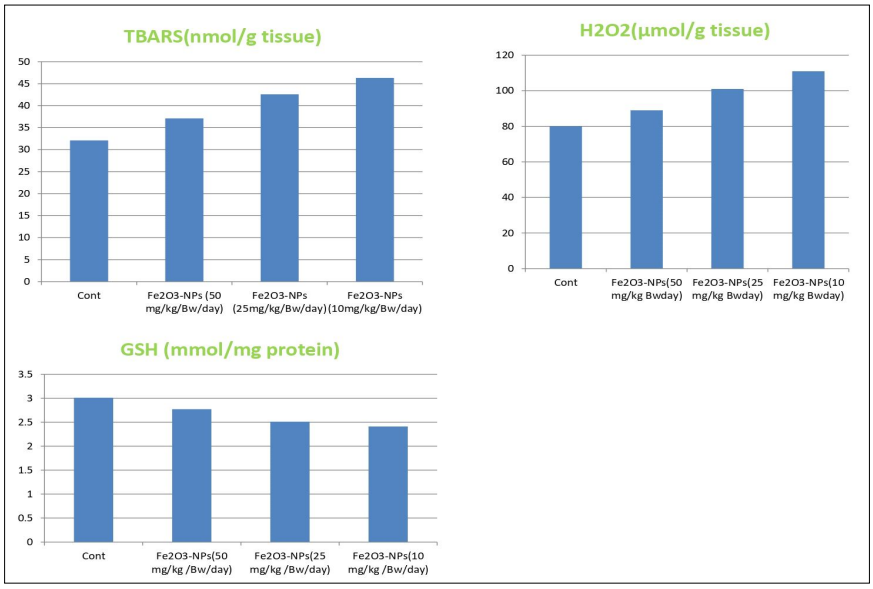

Fig 1: Different concentrations of iron oxide nanoparticles have different effects on the livers of mice.

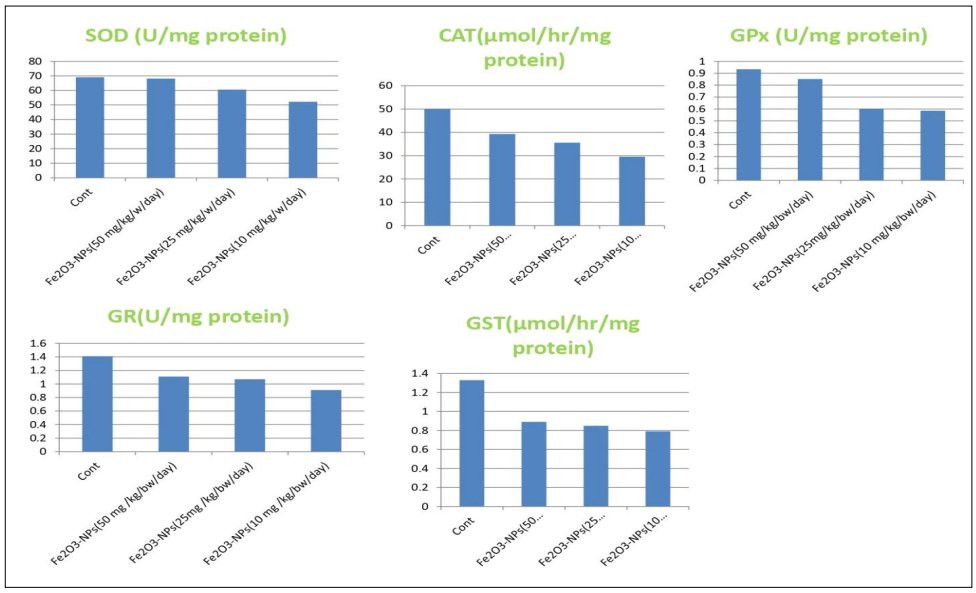

Fig 2: Effect of different concentrations of Iron oxide nanoparticles in mice liver.

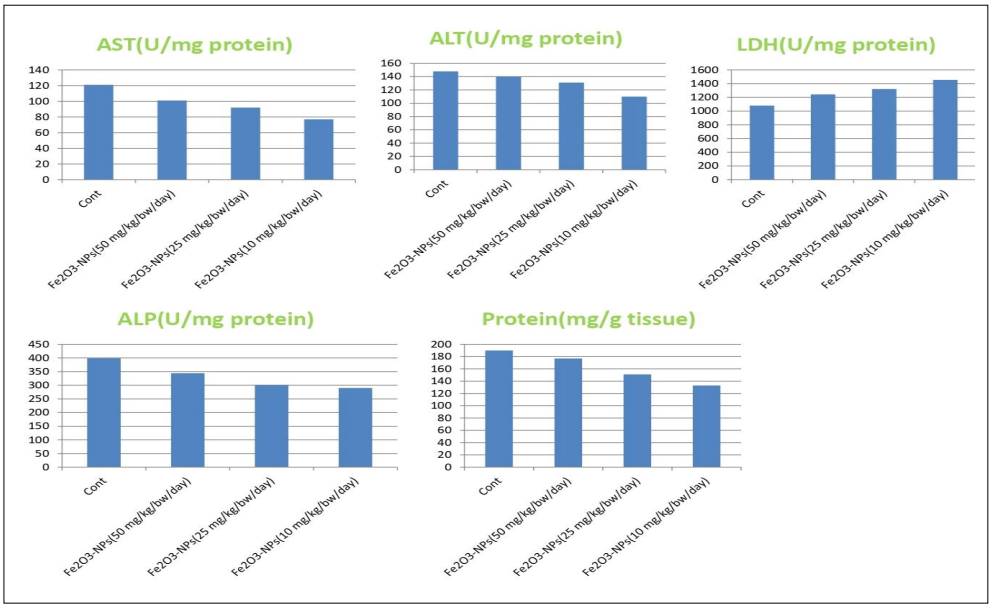

Fig 3: The liver of mice is affected by different levels of iron oxide nanoparticles.

($p<0.05$) lower than that of the control group. Protein is one of the main biological components that free radicals may damage. Both the protein degradation caused by ROS generated by Fe_2O_3-NPs and the negative effects of the nanoparticles on liver cells may account for the reduction in liver proteins caused by Fe_2O_3-NPs. Consequently, The production of chemically unstable metabolites may be the cause of the toxicity of Fe_2O_3-NPs. These results are quite similar to those of other writers who found that living organisms exposed to xenobiotics had less liver protein (Askri *et al.,* 2019; Al-Hamadany *et al*., 2023; Saleh *et al*., 2024). Most likely, the live organism's natural reaction to the stress caused by the pesticide is to reduce its protein level in order to overcome it (Amin *et al.,* 2021; Fırat *et al*., 2011; Al-Dulimi *et al*., 2023).

CONCLUSION

Iron oxide nanoparticles (Fe2O3-NPs) produced significant adverse effects on mice livers in a dose-dependent manner. Biomarkers for the detrimental effects of iron oxide nanoparticles (Fe2O3-NPs) may be established by estimating lipid peroxidation, enzymatic and non-enzymatic antioxidants and biochemical parameters.

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Conflict of interest

The authors declare the absence of any conflict of interest.

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