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RESEARCH ARTICLE
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Investigation of Oxidative Stress and Antioxidative Enzymes in Erythrocytes and Bone Marrow of Albino Rats Treated with Different Concentrations of Copper Ferrite Nanoparticles

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ABSTRACT

Copper ferrite nanoparticles are widely studied for their biomedical applications; however, their potential toxicity to hematopoietic and dental tissues remains unclear. This study evaluates oxidative stress, antioxidative enzymatic contents, and histopathological alterations in albino rats exposed to copper ferrite nanoparticles. Male Wistar albino rats were divided into four groups: Group A (control, 0.0mg/kg), Group B (2.5mg/kg), Group C (5.0mg/kg), and Group D (7.5mg/kg) received copper ferrite nanoparticles intravenously for 15 days. Oxidative stress was assessed by measuring thiobarbituric acid-reactive substances (TBARS) and reactive oxygen species (ROS). At the same time, antioxidant defense was evaluated in terms of the estimation of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) contents. The results showed a dose-dependent increase in TBARS and ROS levels, indicating elevated oxidative stress, along with a significant decline in SOD, POD, and CAT contents, suggesting impaired antioxidant defense mechanisms. Histopathological analysis of teeth revealed structural alterations, including enamel erosion, dentin degeneration, and inflammatory changes, particularly at higher doses. The most severe oxidative and histopathological effects were observed in Group D (7.5mg/kg), indicating potential toxicity associated with exposure to copper ferrite nanoparticles. These findings suggest the need for further research on the long-term effects of copper ferrite nanoparticles on erythrocytes, hematopoietic, and dental tissues, to ensure their safe biomedical use.

Keywords: Albino Rats, Copper ferrite, Erythrocytes, Bone marrow, Oxidative stress, Antioxidative enzymes

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INTRODUCTION

Nanotechnology has transformed numerous scientific domains by allowing for the manipulation of materials at the atomic and molecular levels. Among the diverse nanoparticles, metal oxide nanoparticles have gained a lot of attention for their distinct physicochemical features and potential biological uses (Nikolova & Chavali, 2020). Metal oxide nanoparticles (NPs) belong to a class of nanomaterials and are synthesized from silver, copper, magnesium, zinc, gold, titanium and alginate (Ghonimi et al., 2022). Among these, copper ferrite (CuFe2O4) NPs have attracted Considerable attention due to their remarkable magnetic catalytic, optical, and structural characteristics, making them promising candidates for applications in catalysis, magnetic storage, and biomedicine (Saikova et al., 2023).

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Despite the advantageous properties of copper ferrite, concerns have emerged regarding its toxicological effects on biological systems. Nanoparticles may influence stress response mechanisms and interact with proteins in living organisms. Nanoparticle exposure can cause oxidative stress and disrupt proteins involved in stress response pathways (CAT, SODs, GPXs, GR, PRDXs, PDI and CAT). It is now widely accepted that manufactured NPs cause oxidative stress by producing damaging reactive oxygen species. Nanoparticles' propensity to create ROS is influenced by their physicochemical features, which include size, shape, structure, and metal contents (Cameron et al., 2022). Increased oxidative stress is one of the most important processes in nanoparticle-induced toxicity, described as "a shift in the prooxidant or antioxidant balance in favor of the former" (Horie & Tabei, 2021).

Enhanced levels of ROS lead to pathophysiological outcomes, including inflammation, DNA damage, and fibrosis, which can contribute to the development of various diseases, such as cancer, atherosclerosis, neurological disorders, autoimmune diseases, and type 2 diabetes (Xuan et al., 2023). Due to their small size, nanoparticles can pass through and traverse physiological barriers, traveling via the circulatory system to affect multiple tissues and thereby impact animal and human health (Ghonimi et al., 2022).

Copper ferrite nanoparticles are frequently used in biomedicine, magnetic resonance imaging, magnetic cell separation, as well as energy storage devices, magnetic storage medium and spintronic and electromagnetic appliances (Saikova et al., 2023). Humans are at high risk of exposure to nanoparticles, which can enter the body through various pathways, including the digestive, respiratory, and skin systems, during the production, use, and disposal of nanoparticles. Once deposited in specific areas of the body, nanoparticles are transported to different tissues through the lymphatic or circulatory system. Due to their stability, nanomaterials persist in the body and environment for an extended period, with potential health repercussions from accelerated exposure to nanoparticles that are not yet fully defined. However, there is scarce evidence indicating possible toxic effects (Dobrzyńska et al., 2014).

Earlier studies have demonstrated the harmful effects of copper ferrite nanoparticles in human cell culture, highlighting significant alterations in cellular morphology and survival (Ahmad et al., 2016). Additionally, a study on Wistar rats demonstrated that intraperitoneal injections of $CuFe_2O_4$ nanoparticles can disrupt antioxidant activities and blood plasma parameters in a gender-specific manner, indicating overall toxicity (Riaz et al., 2020). Exposure to metal oxide NPs, including silver and copper nanomaterials, has been shown to adversely affect survival, body weight, size and structure in aquatic species, signifying potential environmental and health hazards (Ostaszewska et al., 2016). Erythrocytes are highly susceptible to nanoparticles, which can induce oxidative stress, thereby impairing their function and lifespan. This study investigates the oxidative stress induced by copper ferrite nanoparticles, as well as the antioxidant mechanism, particularly in erythrocytes and bone marrow, and the histopathological changes in the teeth of albino rats, aiming to gain a deeper understanding of the potential risks associated with their widespread applications and exposure.

MATERIALS & METHODS

This research was approved by the Board of Studies, Department of Zoology and Advanced Studies, and the Advanced Studies and Research Board, the Islamia University of Bahawalpur (IUB), Pakistan.

Study Animal

Research was conducted in the Animal Rooms at Baghdad ul Jadeed Campus, Department of Agriculture, IUB. Twenty male adult albino rats free of any clinical ailments were used in this study. The rats were obtained from IUB, Baghdad ul Jadeed campus (Pakistan). Each rat was housed in a metal wire cage with a regular dark/light interval and constant temperature. Water and food were accessible every day. The CuFe2O4 NPs were obtained from the Institute of Physics, IUB.

Experimental Design and Treatment

The rats were divided into four treatment groups: A, B, C, and D. Each group consisted of five rats. Group A was named the control group. All rats were fed commercial chicken feed. The weekly body weight of every male in each group was determined using a digital weight computerized weight balance. For 15 days, rats in categories B, C and D were given daily injections of CuFe2O4 NPs. Rats in group B were administered CuFe2O4 NPs at a dose of 2.5mg/kg BW. Rats in group C received 5.0mg/kg while rats in group D were administered CuFe2O4 NPs @7.5mg/kg BW. The nanoparticles were administered intraperitoneally daily for 15 days. The rats were keenly observed daily. Sampling was conducted on days 5, 10, and 15 of the experiment.

Sample Processing and Biochemical Analysis

Erythrocytes and bone marrow cells were collected from all experimental rats at days 5, 10, and 15th of the research trial. The cells were then washed with phosphate buffer saline and lysed with distilled water to release their contents. The lysates were centrifuged at 3000rpm for ten minutes and the resulting supernatants were stored at -20˚C. TBARS and ROS were measured at absorbance of 532 and 505nm according to previous protocol (Akram et al., 2021). Other parameters such as CAT, POD and SOD were measured at absorbance of 240, 470, and 560nm respectively, using ultraviolet spectrophotometer in erythrocytes and bone marrow of rats following the previous protocol (Ghazanfar et al., 2018).

Histopathology Analysis

For histopathological analysis, at day 15, the different teeth were carefully extracted from each rat. The teeth were fixed in 10% formalin. Following decalcification, the teeth were then embedded in paraffin and thin blocks and sections were cut using a microtome and stained using Hematoxylin and Eosin. These stained sections were observed for microscopic changes (Nursanti et al., 2017).

Statistical Analysis

The collected data were statistically evaluated by using the ANOVA test with the IBM statistical software package (SPSS). Tukey's test was employed to compare the means of oxidative and antioxidant factors between nanoparticles. $P<0.05$ was used as the level of significance.

RESULTS

Behavioral and Physiological Effects

Rats given copper ferrite nanoparticles ($CuFe_2O_4$ NPs) during the experiment showed physiological changes including watery feces and signs of depression as well as behavioral changes like lethargy. On the other hand, the untreated control rats remained active throughout the trial.

The administration of copper ferrite nanoparticles indicated no significant changes in body mass of each group. Rats in groups B, C and D did not exhibit progressive increase/decrease in body weight corresponding to the escalating dosages of copper ferrite nanoparticles (Table 1).

Table 1: Body weight (g) of albino rats treated with different doses of copper ferrite nanoparticles

Experimental Days	A (0.0)	B (2.5mg/kg)	C (5.0mg/kg)	D (7.5mg/kg)
5	132.3±1.86	132.4±1.81	133.2±2.38	129.9±1.52
10	133.2±2.61	131.3±3.60	131.1±1.24	128.4±1.40
15	134.5±2.22	130.2±2.91	129.2±1.75	127.5±1.47

Oxidative Stress and Antioxidant Response

The levels of TBARS and ROS in erythrocytes were markedly different compared to the control group. Specifically, groups B, C and D exhibited notably higher TBARS and ROS levels than group A. The results also demonstrated a statistically significant variation in contents of POD, CAT and SOD in erythrocytes compared to control group. Groups B, C and D displayed significantly lower CAT, POD and SOD contents than the control group (Table 2).

Table 2: Oxidative and antioxidant profile in erythrocytes of albino rats treated with different doses of copper ferrite nanoparticles

Parameters/ Days	Groups			
	A (0.0)	B (2.5mg/kg)	C (5.0mg/kg)	D (7.5mg/kg)
Erythrocytes				
Reactive oxygen species (ROS) contents				
5	0.58± 0.03	0.59±0.02	0.60±0.03	0.61±0.04
10	0.57±0.05	0.62±0.07	0.63±0.08	0.74±0.05*
15	0.58±0.04	0.63±0.04	0.69±0.06*	0.83±0.03*
Thiobarbituric acid reactive substances (TBARS) content (nmol/TBARS formed/mg protein/min)				
5	0.69±0.05	0.71±.0.04	0.73±0.03	0.74±1.42
10	0.67±0.04	0.72±0.02	0.75±0.03	0.86±0.08*
15	0.68±.0.07	0.73±0.06	0.82±0.08*	0.94±0.07*
Antioxidant enzymes				
Superoxide dismutase SOD (units/mg protein)				
5	0.34±0.06	0.32±0.08	0.31±0.04	0.29±0.07
10	0.35±0.05	0.31±0.03	0.28±0.04	0.27±0.02*
15	0.35±0.02	0.29±0.05	0.23±0.05*	0.21±0.02*
Catalase CAT (units/min)				
5	0.20±0.07	0.21±0.06	0.19±0.04	0.17±0.06
10	0.21±0.03	0.20±0.07	0.19±0.02	0.15±0.01*
15	0.21±0.05	0.19±0.05	0.16±0.03*	0.14±0.04*
Peroxidase POD (units/min)				
5	0.57±0.03	0.55±0.05	0.53±0.04	0.52±0.04
10	0.54±0.05	0.52±0.07	0.51±0.03	0.49±0.02
15	0.56±0.04	0.51±0.03	0.41±0.06*	0.36±0.07*

Values (mean±SD) bearing asterisk within a row differ significantly ($P<0.05$).

TBARS and ROS levels in bone marrow differed significantly from the control group. Groups B, C and D had higher ROS and TBARS levels than the untreated group. Groups B, C and D showed significantly reduced CAT level in rats administered higher doses of nanoparticles as compared to the control group. POD levels were significantly reduced in groups C and D relative to the control group. The lowest POD level was recorded in group D, which received the highest dose of $CuFe_2O_4$ NPs. The findings indicate a statistically substantial difference in SOD level compared to the control group. SOD level was notably lower in groups C and D relative to the control group (Table 3).

Table 3: Oxidative and antioxidant profile in bone marrow of albino rats treated with different doses of copper ferrite nanoparticles

Parameters/ Days	Treatments			
	A (0.0)	B (2.5mg/kg)	C (5.0mg/kg)	D (7.5mg/kg)
Bone Marrow				
Reactive oxygen species (ROS) contents (optical density)				
5	0.34±0.02	0.37±0.07	0.38±0.05	0.41±0.09
10	0.34±0.04	0.38±0.09	0.39±0.04	0.51±0.05
15	0.33±0.03	0.41±0.06	0.48±0.02	0.55±0.05
Thiobarbituric acid reactive substances (TBARS) content (nmol/TBARS formed/mg protein/min)				
5	0.33±0.07	0.36±0.07	0.37±0.07	0.42±0.05
10	0.31±0.07	0.37±0.08	0.41±0.09	0.48±.0.04*
15	0.32±0.05	0.38±0.10	0.52±0.03*	0.62±0.02*
Antioxidant enzymes				
Superoxide dismutase SOD (units/mg protein)				
5	0.31±0.02	0.28±0.03	0.27±0.03	0.26±0.02
10	0.33±0.03	0.27±0.02	0.23±0.01*	0.21±0.02*
15	0.31±0.03	0.26±0.02	0.21±0.01*	0.19±0.03*
Catalase CAT (units/min)				
5	0.43±0.04	0.42±0.09	0.39±0.08	0.38±0.07
10	0.44±0.02	0.41±0.08	0.38±0.07	0.34±0.06*
15	0.45±0.02	0.39±0.07	0.33±0.06*	0.27±0.05*
Peroxidase POD (units/min)				
5	0.37±0.02	0.36±0.03	0.34±0.01	0.32±0.09
10	0.36±0.03	0.34±0.02	0.32±0.04	0.27±0.08*
15	0.36±0.04	0.33±0.08	0.26±0.07*	0.19±0.07*

Values (mean±SD) bearing asterisk within a row differ significantly ($P<0.05$).

Histopathology Evaluation

Microscopic analysis of dental structures revealed normal histological structures of the teeth of rats of group A. However, rats of groups B and C treated with 2.5 and 5.0mg/kg BW nanoparticles displayed mild and moderate microscopic changes in their teeth at days 5, 10, and 15th of trial in groups B and C. These changes were pulp calcifications, inflammatory responses, changes in dentin bridge thickness, resorption of dentin, reduced vascularization in the pulp tissue, thickness of inner enamel epithelial cells, increased cellularity of fibrocytes, thickened periodontal tissue, periodontal tissue, necrosis of odontoblasts, elevated percentage of osteoclasts and delayed growth of the periodontal tissues. Conversely, mild to moderate effects were observed in the teeth of group B rats treated with 2.5mg/kg CuFe2O4 NPs. These alterations were severe in rats of group D at day 15 of trial (Table 4 and Fig. 1).

DISCUSSION

With the advancement of science and technology, as well as the rapid growth of nanotechnology, NPs are becoming increasingly prevalent in various fields such as medical, agricultural environment, energy production and

materials research. Different features including synthesis, characterization, size, nanocomposites, nanomaterials and route of exposure play important role in the pathogenesis of induction of toxic effects in target and non-target animals

Table 4: Severity of histopathological ailments in teeth of albino rats treated with different doses of copper ferrite nanoparticles

Histopathological Lesions	TREATMENTS			
	A(0.0 mg/kg)	B(2.5 mg/kg)	C(5.0 mg/kg)	D(7.5 mg/kg)
Inflammatory reactions	-	++	++	++++
Pulp calcifications	-	++	++	++++
Increase in cellularity of fibrocytes	-	++	+++	+++
Increased resorption of dentin	-	++	++	+++
Hyaline necrosis	-	++	+++	++++
Thickness of periodontal tissue	-	++	+++	++++
Necrosis of odontoblasts	-	++	+++	++++
Disrupted fibers	-	++	++++	+++
Decrease vascularization of pulp tissue	-	++	+++	++++
Decrease of the mineral contents	-	++	+++	++++
Increased dentin bridge thickness	-	+	+++	++++
Elevated percentage of osteoclasts	-	++	+++	++++

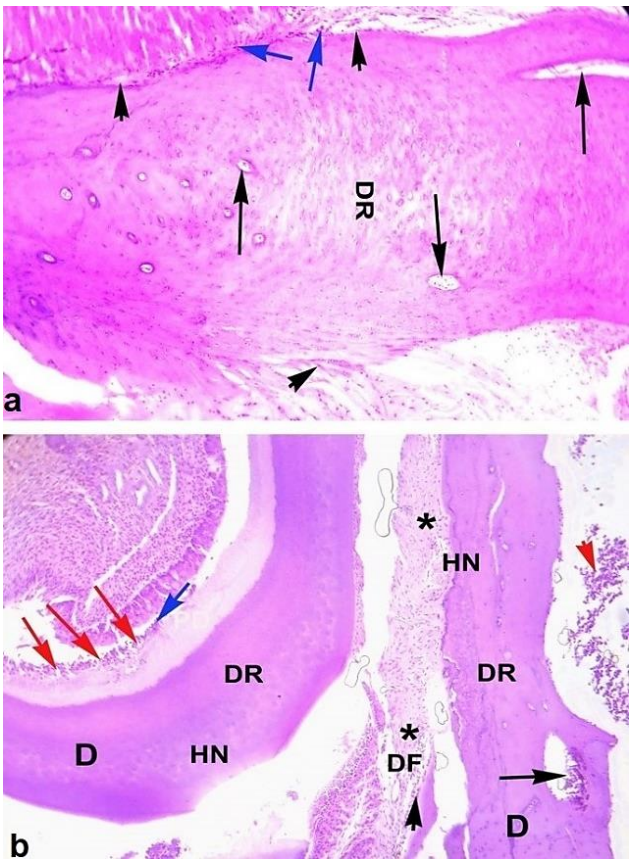

Fig. 1: Photomicrograph of teeth of albino rats of group C (a) and D (b) treated with higher doses of nanoparticles at day 15 indicating various histopathological lesions. Dentin (D), increased osteoclast and resorption lacunae (red arrows), increased fibrocytes (*) disrupted fibers (DF), inflammatory material (black arrow), mononuclear cell infiltration (red and black arrow heads) increased thickness of peridentin (PD), necrosis of odontoblasts (blue arrow) and hyaline necrosis (HN). H & E stain; 400X.

from the macroscopic to the microscopic level. Because of this shift in properties, NPs and nanotechnology are incredibly significant for a variety of applications including health management (Xuan et al., 2023). Copper ferrite nanoparticles (CuFe2O4NPs) are the most significant and important ferrites that exhibit point transitions, changes in semiconductor nature, tetragonality variation and electrical switching under various situations. Research on the potential hazards of spinel ferrite nanoparticles has grown considerably in recent years. Given their wide range of applications, it is crucial to evaluate their toxicity on different tissues. The extensive utilization of $CuFe_2O_4$ nanoparticles in biological fields has gained significant scientific interest; however, their associated toxicity and environmental risks must also be carefully considered (Srikanth & Nutalapati, 2022).

Prolonged contact to nanoparticles presents potential risks to animal life and human health, requiring though assessment and examination of environmental pollutants to minimize their adverse impacts (Ali et al., 2024). Numerous studies have highlighted concerns regarding the deleterious effects of manufactured nanomaterials in living species primarily focusing on exposure levels (Anwar et al., 2023). A key issue is the induction of oxidative stress (Cao et al., 2020) in different tissues when exposed to NPs which are utilized in (Pandey & Saha, 2023), surface modification (Oliva et al., 2023), lubrication, stabilization, cellular delivery (Dang et al., 2014; Hasan et al., 2013) and energy harvesting (Samy et al., 2022). Due to their nanoscale dimensions and elevated surface area relative to volume, nanoparticles can interact with biological molecules (Hussain et al., 2023). They also readily penetrate nuclear and cell membranes, causing indirect damage such as oxidative stress and inflammatory responses (Hasan et al., 2021; Javed et al., 2023; Zafar et al., 2020).

Previously, potential concerns linked with the applications of nanomaterials, including genotoxicity mechanisms and health risks, have gained significant attention (Ghouri et al., 2023). These nanoparticles can generate reactive oxygen species either through direct and indirect pathways, leading to genotoxicity, cellular damage, and cell death (Huang et al., 2022; Huang et al., 2023). Numerous studies examining various nanomaterials such as copper iron oxide, calcium nanoparticles, copper nanoparticles, nickel-iron oxide, and zinc-iron oxide have reported harmful effects on multiple species, including human cells, primarily through the induction of oxidative stress. With nanotechnology rapidly advancing across medicine, industry and nutrition, understanding and mitigating these effects has become increasingly important. A previous study also reported an increase in ROS production as well as DNA damage on exposure to the ZnO nanomaterials (Iqbal et al., 2024). ROS levels in cells range from low to high, leading to a variety of effects, including apoptosis, autophagy, and necrosis (Younas et al., 2024). Different types of nanoparticles, insecticides and herbicides are known to trigger oxidative stress by generating intracellular reactive oxygen species (Horie & Tabei, 2021; Kanwal et al., 2024; Shafqat et al., 2024)

The considerable rise in oxidative damage indicators and diminished levels of several antioxidant proteins in bone marrow and erythrocytes of CuFe2O4 nanoparticles exposed rats in this study might be attributed to the activation of an immune response (Zhuo et al., 2024). Earlier, different investigations (Srisuvetha et al., 2020; Zhuo et al., 2024) demonstrated that interaction with nanoparticles stimulates NLRP3 complexes resulting in cellular

impairment and the activation of inflammatory processes in tissues producing oxidative stress.

Therefore, oxidative stress, which elevates the production of reactive species and causes the death of numerous cellular organelles, may be the cause of the reduced levels of diverse antioxidant biomarkers in rats' teeth. According to several research in previously published literature, oxidative stress causes damage to various cells and can interfere with regular physiological processes, resulting in tissue necrosis through programmed cell death (Ali et al., 2024; Samim et al., 2023).

There have been several histological abnormalities found in rats' teeth as a consequence of oxidative stress induction (Yaman et al., 2018). CuFe2O4 NPs may cause histological and oxidative illnesses in the current investigation due to excessive free radical release, antioxidant depletion and activation of signaling cascades. Free radicals have been shown to engage immediately with many micro and macromolecular structures, such as lipids, proteins and DNA present in different types of cells, resulting in oxidative stress-associated damages. The significantly elevated levels of oxidative stress indices (TBARS and ROS) in rat erythrocytes and bone marrow in this study show that CuFe2O4 NPs caused pathological alterations in cell membrane integrity, resulting in reduced antioxidant levels.

Furthermore, it has been reported that oxidative damage caused by nanoparticles lowers the levels of enzymatic antioxidants, a widely recognized secondary defensive mechanism (Ghaffar et al., 2021; Sanati et al., 2022; Wang et al., 2022). CAT and SOD neutralize superoxide free radicals and detoxify H_2O_2, whereas POD scavenges lipid hydroperoxides (Hussain et al., 2018; Kumar et al., 2023). Therefore, the reduction of these proteins produces oxidative impairments in various organs.

Reactive oxygen species (oxygen-based free radicals) are highly reactive and can cause damage to various biological structures, including DNA. Bone marrow and erythrocytes are vital parts of the blood-forming system, and they are particularly vulnerable to reactive damage. Investigating the harmful effects of $CuFe_2O_4$ nanoparticles (NPs) is crucial to identifying possible hazards associated with their applications (Samy et al., 2022). Extensive research has explored the cytotoxic effects of these NPs using various animal models (Chong et al., 2021; Han et al., 2016). While some investigations have demonstrated that these NPs exhibit dose-dependent cytotoxicity, others have reported minimal to no adverse effects on cells (Chen et al., 2019; Hanley et al., 2009; Namvar et al., 2015). Our results align with earlier research that has reported increased levels of oxidative stress indicators (ROS and TBARS) and reduced antioxidant enzymatic antioxidants activity in tissues such as the bone marrow and teeth. Histopathological examination of the treated rats' muscle (gum) tissues revealed atrophied cell, muscular fiber degeneration and the presence of inflammatory components (Mahmood et al., 2024).

Conclusion

This study demonstrates that copper ferrite nanoparticles induce oxidative stress and alter the antioxidant defense system in the erythrocytes and bone marrow of albino rats in a dose-dependent manner. The significant increase in TBARS and ROS levels, coupled with the depletion of antioxidative enzymes (SOD, POD and CAT), suggests that copper ferrite nanoparticles disrupt redox homeostasis, leading to oxidative damage. The highest dose (7.5mg/kg) exhibited the most pronounced toxic effects, indicating potential risks associated with prolonged or high-dose exposure. These findings highlight the importance of evaluating the biocompatibility and toxicity of copper ferrite nanoparticles before their biomedical applications. Further in-depth studies, including long-term exposure assessments and molecular mechanisms of toxicity, are essential to establish safe dosage limits and explore possible protective strategies against nanoparticle-induced oxidative stress.

DECLARATIONS

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Data Availability: All the data is included in this manuscript and can be obtained from the corresponding author on reasonable request.

Ethics Statement: This study was approved by the Board of Studies of the relevant department and conducted following the guidelines established by the Bioethics Committee of Islamia University of Bahawalpur, Pakistan.

Author's Contribution: Muhammad Asif: Execution, Investigation, Methodology, manuscript preparation. Gulnaz Afzal and Riaz Hussain: Supervision and data analysis. Roshan Riaz and Hafiz Muhammad Nouman: Conceptualization, involved in manuscript writing and formal analysis: Moeen Afzal, Ur-Til-Wusqa, Mubeen Talib and Shanzab Noor: Writing of initial manuscript, data collection and Investigation: Arooj Ali: Data Curation, Methodology, Validation. Rashid Iqbal and Konul Ahmadova: Formal Analysis and Resources. Riaz Hussain: Conceptualization, Writing – R.

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