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Study of the Toxicological Effects of Acute and Chronic Exposure to Nano Polystyrene on Hematological and Oxidative Stress Indicators in Rats

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ABSTRACT

This study investigated the toxic effects of nano polystyrene plastic particles in laboratory rats using hematological and biochemical analyses. In both acute and chronic exposures, hematological alterations were evident, with a marked increase in white blood cell (WBC) counts, indicating an activated immune response and systemic stress in the fish. In contrast, red blood cell (RBC) counts and hemoglobin (HGB) levels consistently declined, suggesting impaired erythropoiesis, reduced oxygen-carrying capacity, and possible hematological toxicity. These combined changes reflect a shift toward inflammatory and immune activation, alongside anemia-like effects, which may compromise physiological balance and overall health. In both acute and chronic exposures, oxidative stress biomarkers showed significant alterations, as catalase (CAT) activity and malondialdehyde (MDA) levels were markedly increased, reflecting enhanced lipid peroxidation and activation of antioxidant defense mechanisms in the cells. In contrast, superoxide dismutase (SOD) activity decreased, indicating a weakened primary antioxidant response and a reduced capacity to neutralize superoxide radicals. These combined changes suggest an imbalance in the oxidative/antioxidant system, where elevated oxidative damage and insufficient enzymatic defense contribute to cellular stress and potential tissue injury due to exposure to nano polystyrene. This study resulted in some behavioral and physiological alterations, including reduced appetite, weight loss, alopecia, lethargy, sleep disturbances, and heightened aggression, indicating that chronic exposure to nano polystyrene disrupts neurological, metabolic, and systemic functions, indicating its potential classification as a cumulative toxicant with progressive adverse effects. In summary, both acute and chronic exposure to nano polystyrene in rats disrupts hematological function and induces oxidative stress

INTRODUCTION

Microplastics are small particles ranging in size from 1 µm to 1000 µm. These synthetic particles are formed as a result of the gradual breakdown of larger plastic debris into smaller particles due to environmental factors, including physical, chemical, biological, and mechanical characteristics of the debris (Ghosh et al. 2023). They are generally spherical, irregular, cylindrical, or filamentous dark particles. Common sources of microplastics in the environment are the disintegration and degradation of plastic materials, coupled with the release of microplastics in the form of resistant, peel-off, and microporous fillers used in various household care products, such as cleansers, toothpaste, and facial scrubs (Carney Almroth et al. 2018).

The increasing production and disposal of plastics has led to growing concerns regarding the accumulation of microplastics in marine organisms, sediments, and other environments. These particles can disrupt hormones, reproduction, cardiovascular function, and immune responses, and increase oxidative stress through ROS generation. To better understand their physiological impacts, it is

essential to investigate microplastic accumulation in rats at different dosages and assess the related blood parameters (Sulistyorini et al. 2024).

MPs accumulated significantly in the liver and showed a stronger potential for harming rats than lower-level MPs due to reactive oxygen species (ROS). The whole-body physiological function of rats in the blood antioxidant system was activated to maintain balance under stress (Samawi & Werorilangi 2024). ROS accumulation in the brain, liver, and kidneys led to significant organ damage. In addition, MPs' accumulation in the liver affects cholesterol metabolism, inflammation, and immune-related functional lipid oxidation. Bile excretion decreases, leading to oxidative damage and acid accumulation in rats. From the results of these three approaches, MPs have a strong potential to harm rats and could be transferred to a higher-level host through an ecological approach that considers the integrated oxidative effects on the body (Albazoni et al. 2024).

Nano polystyrene was selected for investigation because it represents one of the most common synthetic polymers extensively used in packaging, consumer products, and industrial applications (Abdelkareem et al. 2025). Its widespread production, use, and improper disposal contribute substantially to environmental contamination, where larger plastic debris progressively degrades into micro- and nanosized particles (Vohl et al. 2024). At the nanoscale, polystyrene particles possess unique physicochemical properties, such as increased surface area, high reactivity, and the ability to cross biological barriers, which cause them to accumulate in tissues and interact with cellular systems compared to larger plastic fragments (Kelpsiene et al. 2022). Previous studies have suggested that nano polystyrene can induce oxidative stress, immune disturbances, and metabolic dysfunction in living organisms, highlighting the need for a detailed toxicological assessment. Therefore, focusing on nanopolystyrene allows for a clearer understanding of the potential health risks posed by nanosized plastics and provides a model for evaluating the broader implications of nanoplastics exposure. (Kelpsiene et al. 2022).

MATERIALS AND METHODS

The current study included a cohort of 40 female albino rats, each weighing 180–200 g and aged 12 weeks. These subjects were procured from the Housing Animal Facility located within the Faculty of Science at the University of Kufa. The experimental setup involved the use of six plastic cages (Fig. 1), each fitted with metal covers, with dimensions of 43 cm × 27 cm × 15 cm. Plastic bottles with corks featuring metal pipes were used for hydration. The rats were accommodated five per cage (Techiplast, Kettering, UK) with sawdust serving as bedding in an environment with controlled temperature (22 ± 2 °C) and humidity (40 ± 5%). The light-dark cycle was maintained at 12:12 h, with illumination set at 25 lx (Couto & Cates 2019).

Experiment Design: Acute and Chronic Toxicity Assessment of Nano Polystyrene

This study aimed to assess the acute and chronic toxicities of a nano polystyrene emulsion using female rats as an experimental model. The rats were 12 weeks old and weighed between 180 and 200 g. Ethical approval for animal handling was obtained from the Animal Ethics Committee of the University of Kufa, Iraq. were divided into two main categories: acute toxicity and chronic toxicity. Each category included a control group (N = 10). The nano polystyrene, purchased from Sigma-Aldrich, had the following properties: particle size = 100 nm, concentration = 10% (solids), purity = 100%, and density = 10.05 g.cm^{-3}.

Fig. 1: Animals and cages used in the present study.

Acute Toxicity Experiment

In the acute toxicity experiment, rats in the treatment group received a daily dose of 6.783 $mg.kg^{-1}$ of the nano polystyrene emulsion for seven days. In contrast, the control group was administered distilled water to provide a baseline for comparison.

Chronic Toxicity Experiment

For the chronic toxicity experiment, rats in the treatment group were administered a lower daily dose of 2.8 $mg.kg^{-1}$ of the nano-sized polystyrene emulsion over 30 days. As in the acute toxicity experiment, the control group received distilled water. This design aimed to uncover any potential adverse effects of the emulsion in the short and long term.

RESULTS AND DISCUSSION

Morphological and Behavioral Results

Polystyrene nanoparticles can be taken up by the gastrointestinal system and subsequently enter the bloodstream, leading to their accumulation in the cerebral regions of mice, which in turn results in neurobehavioral disturbances (Prust et al. 2020).

Table (1) and Figs (2) and (3) show the changes in the behavior of female rats exposed to varying doses of nanopolystyrene. Most behavioral changes appeared in the last days of the chronic exposure experiments. In addition to the change in stool color, laziness and aggression appeared at the beginning of the acute exposure experiment. The most prominent changes included loss of appetite, weight loss, hair loss, and increased sleep duration.

The observed behavioral and physiological alterations indicate multiple toxicological implications of nano-PS exposure. Increased aggression, lethargy, and prolonged sleep duration suggest possible neurotoxic effects linked to central nervous system disruption and neurotransmitter imbalance (Howard et al. 2024). Reduced appetite, weight loss, and changes in fecal coloration indicate metabolic and gastrointestinal dysfunction, whereas alopecia and overall lethargy reflect systemic stress and impaired homeostasis

Table 1: Behavioral Changes in Female Rats Exposed to Varying Doses of Nanopolystyrene.

Observation	Control group	Acute group	Chronic group
Loss of appetite	-	-	+
Change the color of the feces	-	+	+
Loss of weight	-	-	+
Loss of hair	-	-	+
Refusal of drink	-	-	+
Wounds	-	-	-
Abscesses	-	-	-
Idleness	-	+	+
Aggressiveness	-	+	+
Sleep	-	-	+

Fig. 2: Hair loss in rats in the chronic group.

(Sweetser 2022). Notably, the more severe manifestations in the chronic exposure group highlight the cumulative and progressive nature of these adverse effects, emphasizing their dose- and time-dependent toxicities.

A variety of elements that may influence the neurotoxic effects of micro- and nanoplastics have been identified. The extent of exposure to these particulates is crucial for assessing the possible neurotoxic effects of plastic particles (Wang et al. 2020). However, the existing levels of exposure are significantly lower than those employed under laboratory conditions. Conversely, the duration of exposure in controlled experiments is often considerably shorter than what would be relevant for actual human exposure, despite findings suggesting that the neurotoxic outcomes of micro- and nanoplastics are contingent on the exposure duration (Varo et al. 2019). In addition to the concentration and duration of exposure, the thermal conditions during exposure may also affect the neurotoxicity of micro- and nanoplastics, particularly in aquatic organisms, as increased toxicity has been observed at higher temperatures.

In conjunction with the aforementioned exposure characteristics, the intrinsic properties of the particles may markedly affect the neurotoxic potential of micro- and nanoplastics. It is posited that particle size is one of the most significant attributes. Generally, nanoparticles are more readily assimilated and possess a higher toxic potential than microparticles.

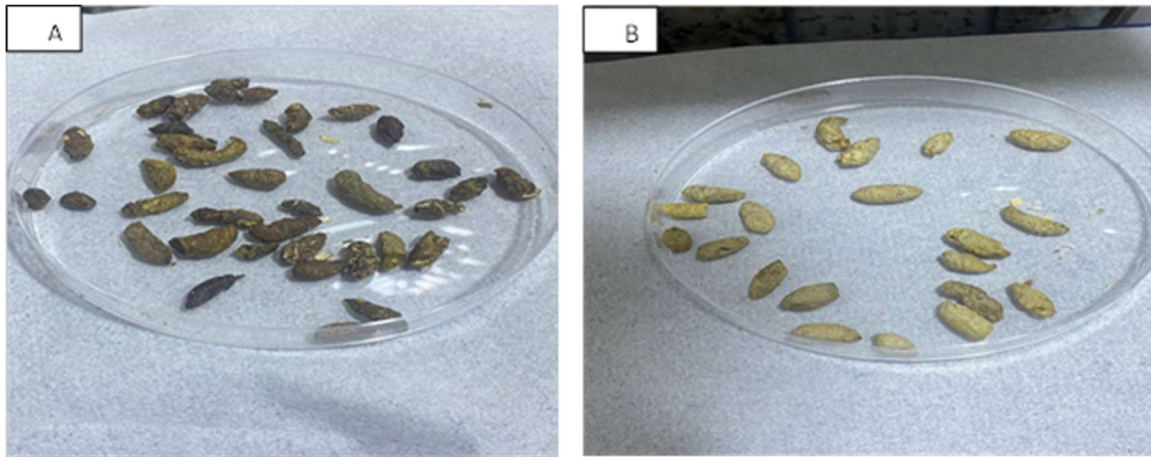

Fig. 3: Change in feces color in the chronic group. (A) control feces, (B) chronic feces.

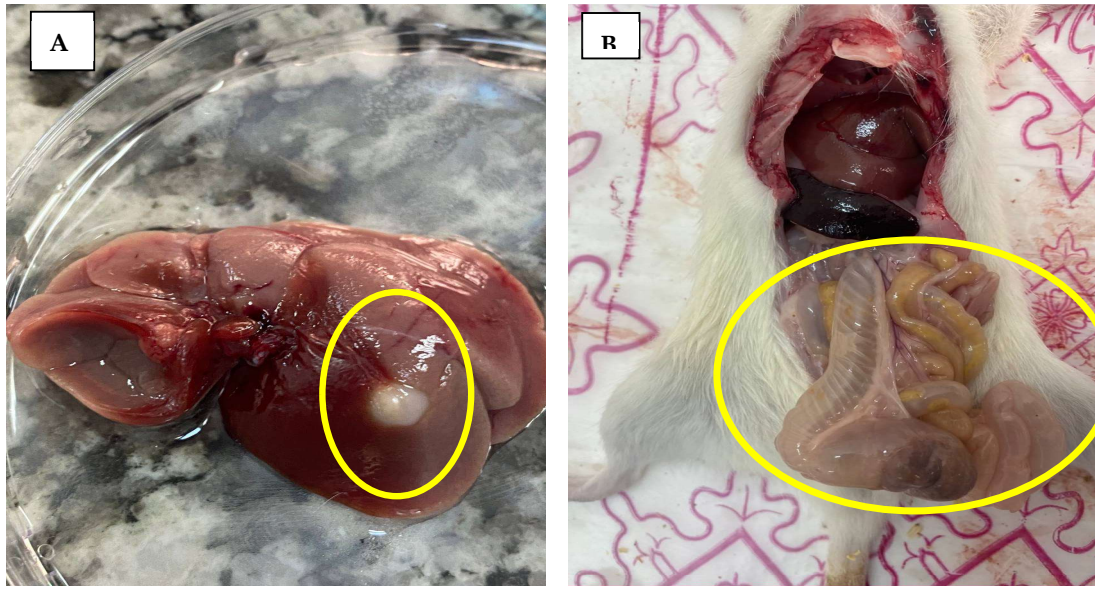

Fig. 4: (A) Liver cyst, (B) Gastrointestinal inflammation.

Some observational changes were observed in the rat organs, including inflammation of the gastrointestinal tract and liver tissue damage in the acute group. In the chronic exposure group, gastrointestinal inflammation, liver steatosis, and liver cysts were observed (Fig. 4).

Hematological Parameters

Figs. 5, 6 and 7 illustrate the impact of acute and chronic exposure on various hematological and biochemical parameters by comparing the exposed groups to the control groups. The white blood cell (WBC) count (10^3.uL^{-1}), presented in Fig. 5, increased in the exposed groups. Acutely, control WBCs were 7.045×10^3.uL^{-1}, while those of the exposed group were 10.466×10^3.uL^{-1}. In the chronic phase, the control WBC count was 6.59 × 103.uL^{-1}, but the exposed WBC count was further elevated to 13.314×10^3.uL^{-1}, indicating a more significant inflammatory or immune response with longer exposure.

Consequently, Red Blood Cells (RBC) count (10^6.uL^{-1}), as shown in Fig. (6), both acute and chronic exposures led to a decrease in red blood cell (RBC count in the exposed groups compared to the controls. In the acute phase, the control RBC count was 6.0483×106.uL^{-1}, whereas the exposed RBC count was 5.281×10^6.uL^{-1}. In the chronic phase, the control RBC count was 6.166 × 106.µL^{-1}, but the exposed RBC count dropped significantly to 4.7975×10^6.µL^{-1}, indicating a more pronounced reduction with prolonged exposure.

Hemoglobin (HGB) levels (g.L^{-1}), depicted in Fig. 7, mirrored the trend of RBCs. In the acute exposure group, the control HGB level was 12.544 g.dL^{-1}, whereas the exposed

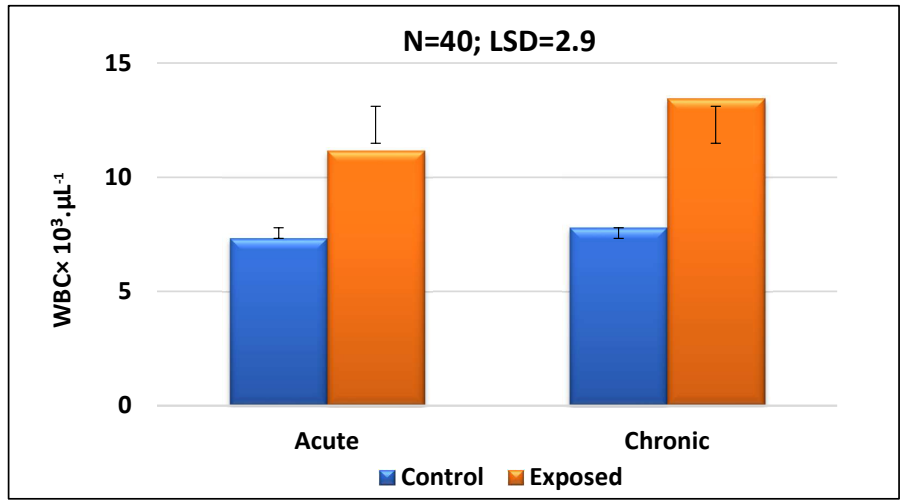

Fig. 5: Comparison of WBCs between the control and exposed groups.

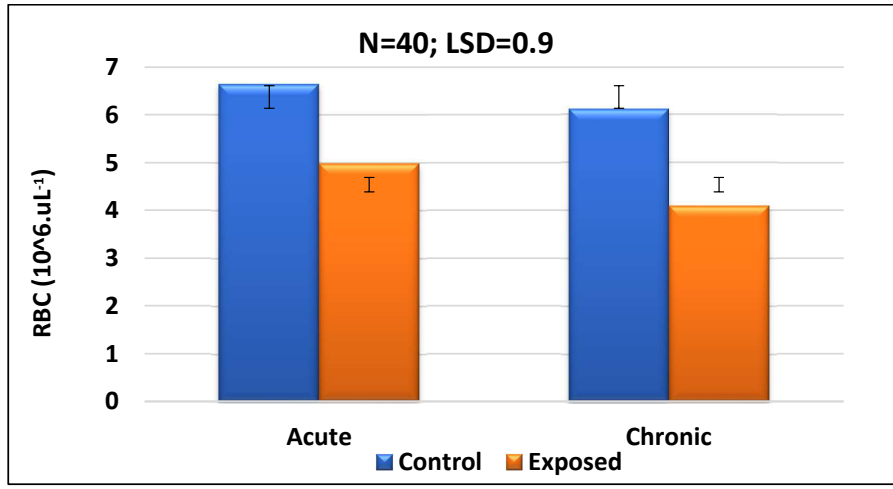

Fig. 6: Comparison of RBC between the control and exposed groups.

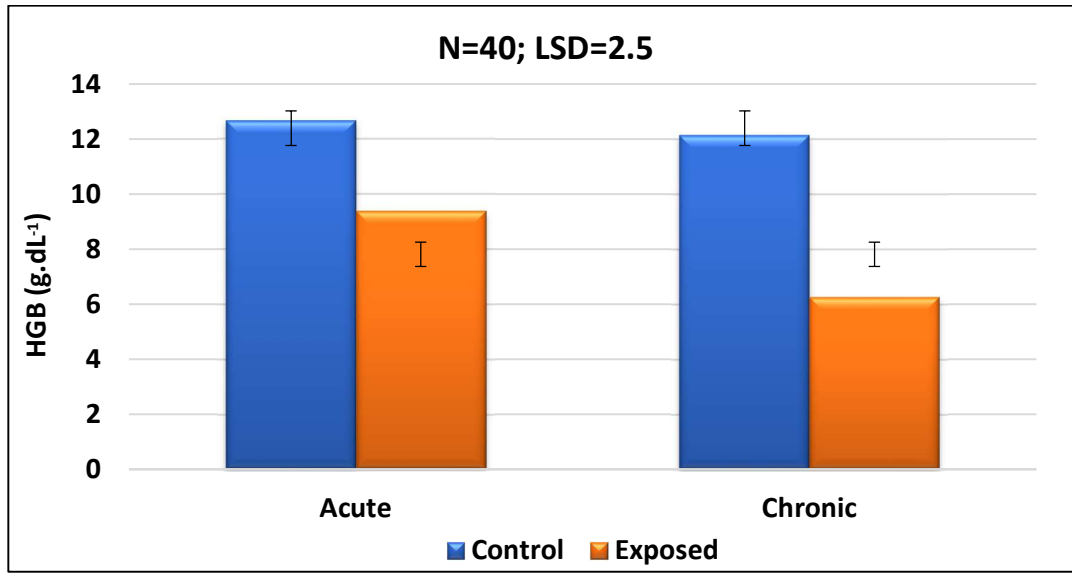

Fig. 7: Comparison of HGB levels between the control and exposed groups.

group had an HGB level of 11.282 g.dL^{-1}. Chronically, control HGB was 11.329 g.dL^{-1}, but exposed HGB showed a substantial decrease to 6.264 g.dL^{-1}, suggesting a severe impact on the oxygen-carrying capacity with chronic exposure.

Conversely, compared to the control group, those exposed to nano polystyrene exhibited significantly elevated white blood cell (WBC) counts. Conversely, hemoglobin (HGB) and red blood cell (RBC) levels were higher in the control group than in the exposed group. There was a notable reduction in the average RBC and HGB levels in both acute and chronic compared to those who were not exposed.

The observed increase in white blood cell counts suggests an immunological response, likely triggered by oxidative and inflammatory stress induced by nano polystyrene exposure (Dey Chowdhury et al. 2025). Conversely, significant reductions in RBC and hemoglobin levels indicate the development of anemia, potentially resulting from oxidative damage to erythrocytes or impaired erythropoiesis. The simultaneous elevation of WBCs and suppression of RBC and Hb levels reflect a dual hematological stress response characterized by immune activation and reduced oxygen-carrying capacity (Spinelli et al. 2025). This hematological profile, characterized by leukocytosis and anemia, highlights the combined effects of immune and oxidative stress on the body's homeostasis, particularly under chronic exposure conditions. These findings underscore the importance of monitoring both immune and red cell parameters in early toxicological assessments, as they serve as integrative markers of systemic physiological stress in fish.

Abdel-Zaher et al. (2023) investigated the hemotoxic properties of polyethylene microplastics (MPs) in murine models. They found that MP concentrations of 60 µg.mL^{-1} and 600 µg.mL^{-1} significantly affected red blood cells (RBCs) and hemoglobin (Hbs) ($p < 0.05$). Even during recovery periods, these hematological parameters, including RBCs, HCT, and Hb levels, remained sensitive to MP exposure at both concentrations. Compared to the control and 6 µg.mL^{-1} MP groups, mice exposed to MPs exhibited notable differences in their hematological profiles. While significant changes in red blood cells and HCT were observed post-recovery, other hematological parameters did not show substantial changes. These findings suggest that individual variations among participants may influence the observed effects. Importantly, the study indicated that MP concentration is a critical factor in determining adverse effects, with a 15-day recovery period positively impacting the improvement of hematological parameters.

In a separate preliminary study, Sun et al. (2021) assessed the impact of µPS on hematological parameters and gene expression in mice. This study revealed that polystyrene microparticles (MPs) adversely affect white blood cell populations, including monocytes, lymphocytes, neutrophils, eosinophils, and basophils. This could potentially weaken the immune system and lead to more serious health issues. The observed harmful effects included DNA damage, altered cytokine expression, heightened oxidative stress, changes in immune responses, modifications in antigen processing pathways, and degranulation, all of which contribute to inflammatory and stress responses. A significant reduction in leukocyte counts was specifically noted in C57BL/6 mice

exposed to 0.5 mg of 5 µm PSMP, reinforcing the established detrimental effects of PSMP.

Oxidative Stress Test

Figs 8, 9, and 10 illustrate the impact of acute and chronic exposure on key biochemical markers, including catalase (CAT) activity, Superoxide Dismutase (SOD) activity, and malondialdehyde (MDA) levels, comparing the exposed groups to the control groups. In the acute phase, CAT activity in the exposed group (78.286 µmol min^{-1} mg^{-1} protein) was elevated compared to that in the control group (63.7317µmol min^{-1} mg^{-1} protein), suggesting an initial compensatory response to oxidative stress. However, under chronic exposure, CAT activity in the exposed group (55.367 µmoL.in^{-1}.mg^{-1}.protein) significantly declined below that in the control group (68.362µmol min^{-1} mg^{-1} protein), indicating a potential depletion or impairment of this antioxidant enzyme over time. SOD activity consistently

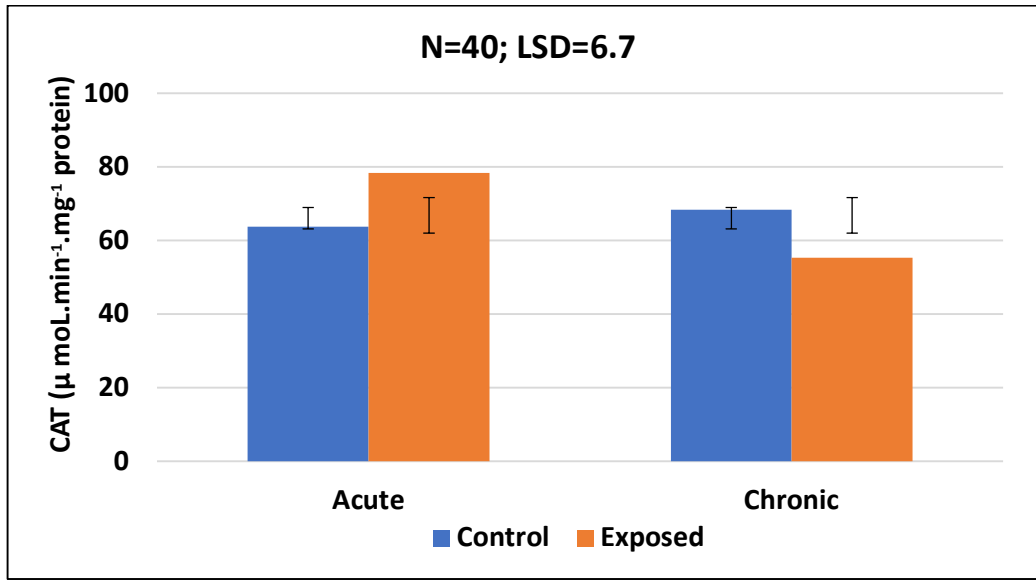

Fig. 8: CAT activity in exposed vs. control groups.

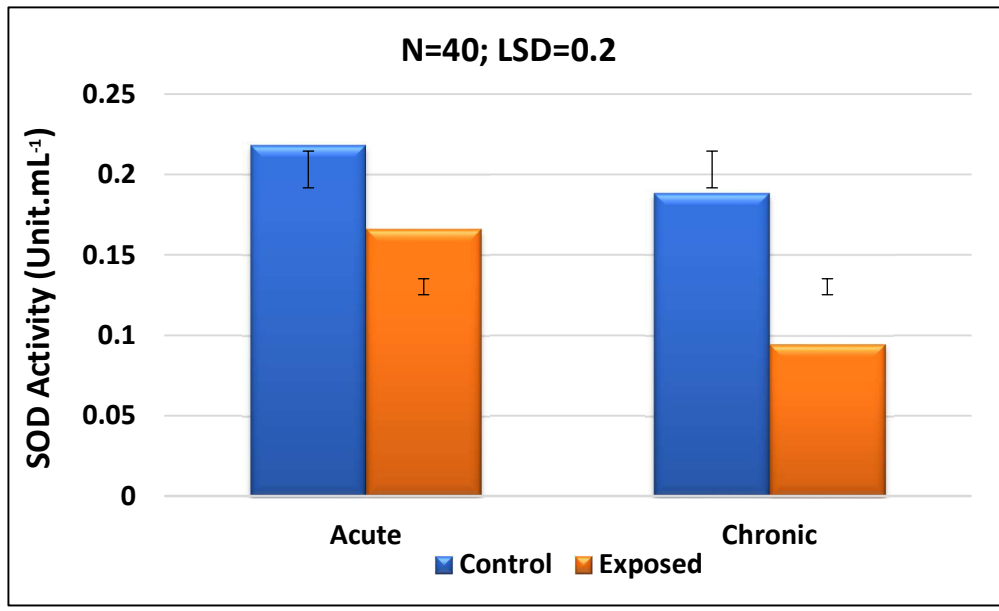

Fig. 9: SOD activity in exposed and control groups.

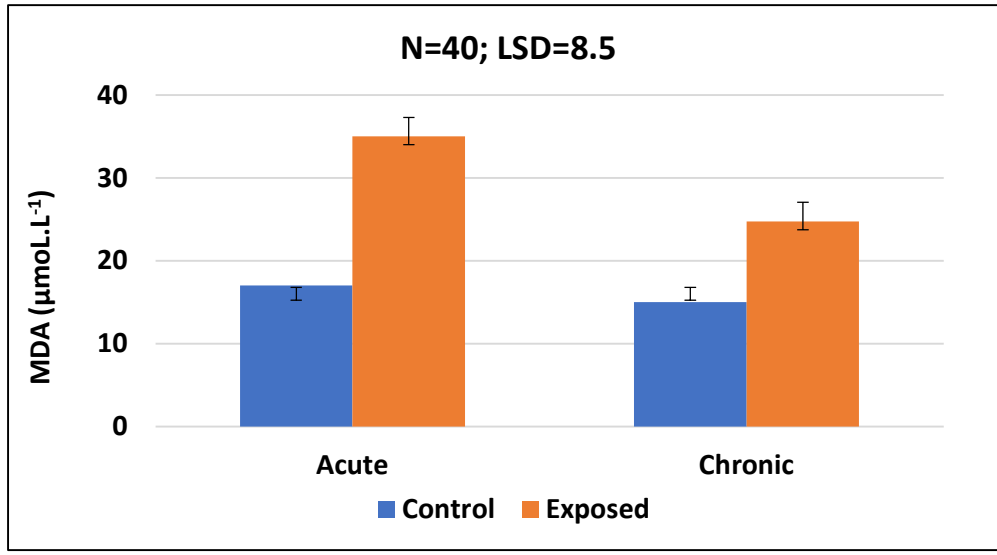

Fig. 10: MDA activity in exposed and control groups.

decreased in the exposed groups for both acute (0.166 Unit.mL^{-1} vs. control 0.2181 Unit.mL^{-1}) and chronic (0.0944 Unit.mL^{-1} vs. control 0.201 Unit.mL^{-1}) exposures, with the chronic reduction being more pronounced, suggesting a sustained suppression of this critical antioxidant defense. Conversely, MDA levels, an indicator of lipid peroxidation and oxidative damage, were markedly increased in the exposed groups under both acute (35.0054 µmoL.L^{-1} vs. control 17.034 µmoL.L^{-1}) and chronic (24.759 µmoL.L^{-1} vs. control 15.29 µmoL.L^{-1}l) conditions. This consistent elevation of MDA strongly implies that exposure leads to significant oxidative stress and cellular damage, with the initial antioxidant responses (such as the acute CAT increase) seemingly insufficient to counteract the damage, especially during prolonged exposure when key antioxidant enzymes such as SOD and CAT are compromised.

In both acute and chronic exposure to nano-polystyrene, oxidative stress markers demonstrated pronounced dysregulation. Catalase (CAT) activity and malondialdehyde (MDA) levels were significantly elevated, reflecting an adaptive response to excessive hydrogen peroxide generation and increased lipid peroxidation within the cell membranes. The increase in MDA levels further indicates that nano polystyrene particles induce oxidative damage by promoting the breakdown of polyunsaturated fatty acids, leading to structural alterations in cellular and subcellular membranes (Bovolin et al. 2023). Conversely, superoxide dismutase (SOD) activity was reduced, suggesting that prolonged or excessive production of superoxide anions overwhelmed the primary line of antioxidant defense. The decline in SOD may result from the direct interaction of nano polystyrene with the enzyme, depletion of essential cofactors, or oxidative modification that impairs its activity (Babaei et al. 2022). Together, these changes highlight that nanopolystyrene exposure disrupts the delicate balance between pro-oxidants and antioxidants, driving cells toward a state of oxidative stress. This imbalance compromises redox homeostasis and may trigger downstream consequences, such as mitochondrial dysfunction, protein oxidation, and DNA damage, ultimately contributing to systemic toxicity in both acute and chronic conditions (Patel et al. 2024).

Overall, these results indicate that exposure, whether acute or chronic, induces significant oxidative stress, leading to cellular damage. While there may be an initial compensatory antioxidant response (increased CAT acutely), prolonged exposure appears to overwhelm and eventually deplete antioxidant defense mechanisms (decreased SOD and chronically decreased CAT), leading to persistent and detrimental lipid peroxidation.

This investigation is consistent with the observations of Hou et al. (2021), who analyzed the impact of µPS on the initiation of pyroptosis and apoptosis in ovarian granulosa cells of rodent models, specifically through the NLRP3/Caspase-1 signaling pathway. Their longitudinal study, spanning 90 days, involved 32 healthy female Wistar rats subjected to diverse concentrations of 0.5 µm PS microplastics dispersed in deionized water. These results substantiate that the presence of nanoparticles increases MDA concentrations while undermining the organism's antioxidant defenses. The results showed that PS microplastics instigated oxidative stress in rats, resulting in reduced antioxidant capacity and subsequent oxidative injury (Li et al. 2020, Wang et al. 2020).

These antioxidant enzymes are pivotal biomarkers for evaluating the initial oxidative damage caused by exogenous agents. SOD levels were recorded, while CAT activity decreased in mice exposed to microplastics (Deng et al. 2017). Numerous studies have highlighted changes in the antioxidant capabilities of aquatic species due to exposure to microplastics. (Xie et al. 2020). The infusion of microplastics into the biological design of an organism may result in the emergence of Reactive Oxygen Species (ROS), initiating an immune response that releases these compounds (Das 2023). The relationship between oxidative stress and inflammation is deeply rooted, and microplastics may induce tissue inflammation in areas of their accumulation. The inflammatory mechanisms may cause the emergence of reactive oxygen species (ROS) and activate pathways linked to oxidative stress (Hu & Palić 2020). The unique pathways activated and the level of oxidative stress provoked by microplastics may fluctuate based on different variables, such as plastic type, particle size, exposure time, and physiological responses of the impacted organism (Solomando et al. 2020).

CONCLUSIONS

The findings of this study revealed that nano-polystyrene (NPS) has significant potential to induce alterations in hematological parameters. Acute exposure increases the white blood cell (WBC) count, suggesting an immune or inflammatory response. Conversely, red blood cell (RBC) and hemoglobin (Hb) levels decreased in the subjects under investigation, indicating a potential risk for the development of anemia. Chronic exposure resulted in even more pronounced elevations in WBC counts, along with further reductions in RBC and hemoglobin concentrations. Together, the hematological alterations observed in this study not only signal immune and inflammatory stress but also raise concerns regarding anemia-related complications. Thus, these parameters serve as critical biomarkers for evaluating the systemic impact of nano-PS exposure. Evidence of oxidative stress was apparent, with early and consistent alterations in oxidative stress biomarkers, particularly elevated malondialdehyde levels and suppressed antioxidant enzyme activities, positioning them as valuable early warning indicators. Their responsiveness to both acute and chronic exposures makes them crucial tools for preemptive toxicological screening and risk assessment in environments where nano-polystyrene and similar agents are present. This decrease in antioxidant enzyme levels suggests an acceleration of the apoptotic process, which may ultimately lead to cell necrosis. Behavioral and physiological changes were observed in the exposed rats, including reduced appetite, weight loss, alopecia, prolonged sleep duration, changes in fecal coloration, lethargy, and increased aggression, which were especially prominent in the chronic exposure group. Future studies should focus on long-term and environmentally relevant exposures, including chronic low-dose regimens and recovery groups, to better assess cumulative and delayed effects. Comparative assessments of different nanoplastic types, sizes, and shapes are recommended, with rigorous physicochemical characterization. The inclusion of both sexes will help identify sex-specific responses, while expanding endpoints to histopathology, oxidative stress, inflammation, genotoxicity, and molecular pathways will provide broader toxicological insights.

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