Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com
NLM ID:101629416

OPEN ACCESS

Zoology

Characterization of Nickel Oxide Nanoparticles and their Dose-Dependent Effects on Body Weight and Hematological Parameters in Female Albino Rats

Naiha Mehmood[1*], Ijaz Hussain[1], Muhammad Muneer[2], Numera Saif[3], Syeda Ashal Fatima[4], Zoha Zainab[1], Bilal Ahmad[1], Eiman Meer[5], Aimon Munawar[1], Marhaba[6]

[1]Department of Zoology, Government College University Faisalabad, Punjab, Pakistan
[2]Department of Zoology, University of Okara, Punjab, Pakistan
[3]Institute of Zoology, Bahauddin Zakariya University Multan, Pakistan
[4]Department of physiology, University of Agriculture, Faisalabad
[5]Department of Biological and Health Sciences, Pak-Austria Fachhochschule: Institute of Applied Sciences and Technology
[6]Department of Biochemistry, Abdul Wali Khan University, Mardan

DOI: https://doi.org/10.36347/sajb.2025.v13i06.007 | **Received:** 29.04.2025 | **Accepted:** 04.06.2025 | **Published:** 14.06.2025

***Corresponding author:** Naiha Mehmood
Department of Zoology, Government College University Faisalabad, Punjab, Pakistan

Abstract | **Original Research Article**

Commercially widespread nickel oxide nanoparticles (NiO NPs) demonstrate mostly unexplored toxicological features especially when applied to female subjects. This study observed NiO nanoparticles through dose-dependent effects on female albino rats' body weight parameters and hematological data. Transmission electron microscopy (TEM), X-ray diffraction (XRD) together with Fourier-transform infrared spectroscopy (FTIR) investigated properties of NiO nanoparticles. NiO nanoparticles were injected intravenously at three body weight doses of 0.5, 1.5 and 2.5 mg/kg for female rats over 28 days. A fully automatic haematology analyser analyzed weekly body weight changes together with haematological measurements of RBCs, WBCs, haemoglobin, and platelets from each test rat. Per results NiO nanoparticles showed spherical structure and measured 17 nm as their average diameter. Theexperimental rats displayed reduced RBC counts and elevated WBC counts together with body weight decrease that increased proportionally to NiO nanoparticle dose levels. The data demonstrates how NiO NPs manifest dose-dependent harmful effects on female rats which establishes the requirement to conduct supplementary safety evaluations while performing gender-specific studies.

Keywords: Nickel oxide (NiO), Nanoparticles (NPs), Hemoglobin, Transmission electron microscopy (TEM), and RBCs, WBCs.

1. INTRODUCTION

Nanotechnology has emerged as a transformative field, enabling the manipulation of matter at the nanoscale (1–100 nm) and revolutionizing industries such as construction, electronics, medicine, and cosmetics. In recent decades, there has been wide scientific research on the various uses of nanoparticles in construction, electronics, manufacturing, cosmetics, and medicine [1]. It is of paramount importance to be aware of their toxicity for several reasons. Exposure to nanoparticles can occur when handling them, as a result of accidental environmental release, during waste disposal or recycling as they can evade the immune system, enter circulatory apparatus, and reach organs. Being smaller than cells, nanoparticles can penetrate cell walls, enter organelles, and disturb cell physiology [2]. Medical practitioners utilize NPs in medicine for delivering drugs precisely as well as performing image diagnostics and delivering therapeutic solutions. Engineered NPs encompass metal-based NPs as vital components that include metal and metal oxide NPs which serve numerous biomedical

applications [3] in the nanotechnology field. Multiple adverse effects such as immunogenic reactions along with nephrotoxicity and renaltoxicity and hepatotoxicity and neurotoxicity occur after human exposure to metal-based NPs [4]. Nickel oxide NPs stand out in the field because their magnetic and

electrochemical properties match well with battery and sensory needs and biomedical requirements. The rising manufacturing operations and increasing consumption of these nanomaterials generates worries regarding their environmental dangers and toxicological impact [5]. Multiple studies indicate that metal-based NPs, including NiO NPs create hepatotoxicity together with nephrotoxicity and neurotoxicity in animal test subjects, thus requiring full toxicological testing [6]. Nanoparticles demonstrate toxic behaviour that depends on four major aspects: dimension, shape, surface nature, and path of exposure [7]. Cellular damage and oxidative stress develop when small nanoparticles cross cell barriers to build up inside organelles [8]. Nanoparticle toxicity stems mainly from ROS generation which subsequently leads to cellular damage throughout DNA, lipid chains and protein structures [4]. The toxic effects of NPs become worse because they stimulate both inflammatory processes and immune reactions [5]. Nickel oxide NPs have attracted substantial interest because they possess various distinctive attributes like stable thermal behaviour coupled with catalytic properties as well as magnetic functionalities [9]. Nickel oxide NPs serve several applications because of their described characteristics [10]. The potential toxicity in biological systems causes scientists to be cautious about the widespread NPs use. The exposure to NiO particles causes oxidative stress by producing reactive oxygen species, which trigger cellular damage and inflammatory responses [11]. The findings of this research demonstrate that nickel oxide (NPs exposure triggers substantial DNA damage and oxidative stress creation in human lung epithelial cells, thus establishing their genotoxic properties [12].

The environmental consequences of Nio NPs existing in the environment. NiO NPs effects on aquatic organisms at the ecotoxicological level and discovered these particles harmed Daphnia magna growth and reproductive abilities as an important ecotoxicity screening organism [13]. The analysis revealed dose-dependent apoptosis combined with cell death in human hepatoma cells indicating significant hepatotoxic effects [14] through the examination of NiO NPs toxicity levels. The way particles enter the body significantly impacts the extent to which NiO NPs will be toxic. Pulmonary inflammation together with fibrosis develops after NiO NPs exposure through inhalation while gastrointestinal toxicity alongside systemic effects appear through oral ingestion [15]. The researchers used intravenous injection to study systemic toxicity and specific organ effects because this approach allows direct access to the bloodstream [16]. The intravenous administration of NiO NPs into rats led to major changes both in blood cell counts and in liver and kidney markers for oxidative stress. The evaluation process for NiO NPs toxicity requires route of exposure assessment because it affects their toxic reaction outcomes [17].

Nanoparticle toxicity requires more attention because scientists now focus on understanding gender-specific variations in nanoparticle responses. Exposure to nanoparticles produces different reactions between male and female organisms since their metabolic processes and hormonal systems and immune functions work differently [18]. Titania nanoparticles induced stronger oxidative stress and inflammatory responses in female rat subjects than in their male counterparts as documented in [19]. Similarly, the hepatotoxic impact of silver nanoparticles proved stronger against female mice than male mice, thus showing how specific gender factors affect nanoparticle toxicity evaluation [20].

Studies on NiO NPs primarily investigate male subjects, causing a major deficit in knowledge about their impact on female organisms. The requirement for gender-based toxicological studies of NiO NPs was discovered because they discovered dissimilar hematological and biochemical reactions existed between male and female rats exposed to NPs. The immune and hematological response in female rats was stronger than in male rats, according to the data, which showed elevated white blood cells WBC and reduced red blood cells RBC numbers [18].

The toxicity of NiO NPs shows different responses due to hormonal variations that exist between male and female sexes. The research shows that estrogen regulates both oxidative stress and inflammation yet these processes might impact how nanoparticles affect cells [21]. Specific testing of male and female physiology help researchers gain complete comprehension of NiO NPs toxicity mechanisms.

The essential indicators of systemic toxicity combined with immune response consist of hematological parameters that include red blood cell (RBC) count and white blood cell (WBC) count alongside hemoglobin levels and platelet count [22]. NiO NPs exposure leads to noticeable changes in blood measurements which demonstrates their capability to induce hematotoxicity. During NiO NPs exposure studies researchers documented a direct relationship between NP dosage and both RBC count and hemoglobin level reduction which might lead to anemic conditions. The WBC count increased because of the nanoparticles' ability to trigger inflammatory or immune responses [23].

Nickel oxides NPs affect blood parameters at the biochemical level. It triggered oxidative stress inside erythrocytes that caused cell breakdown and lowered RBC numbers [24]. and platelet aggregation and activation mechanisms which potentially elevate the danger of blood clotting incidents [25]. In addition to hematological effects, these NPs lead to body weight changes that researchers use as a marker for toxicity [26]. Body weight assessment in female rat subjects revealed NiO NPs dose-response reduction patterns due to

potential systemic toxicity according to research [27]. Similarly, a significant decrease in body weight and food consumption along with reduced health marked rats receiving NiO NPs chemotherapy as observed in [28].

A substantial gap exists in toxicology research regarding NiO NPs since existing studies primarily focus on male subjects while neglecting the investigation of gender-specific toxic effects as well as quantitative dose-response relationships [26, 28]. The mechanisms that explain NiO NPs effects on both hematological systems and physiology have not been sufficiently investigated, leading to a demand for additional research. This research initiative focuses on studying NiO NPs dose-dependent responses regarding body weight changes and hematological assessments in female albino rats. Our study uses transmission electron microscopy (TEM) together with X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR), advanced methods to reveal these NPs physicochemical traits and how these elements relate to the measured toxicity outcomes. This study enhances safety risk assessment comprehension of this NPs, specifically in female bodies thus creating groundwork for gender-based toxicological studies.

2. MATERIALS AND METHODS

This experimental work was conducted in the Muhammad Ali research lab of the Department of Zoology, Government College University, Faisalabad. This study was designed to investigate the dose-dependent toxic effects of nickel oxide nanoparticles (NiO NPs) on female albino rats, with a specific focus on changes in body weight and hematological parameters. Nickel oxide NPs were synthesized and characterized for their physicochemical properties by using advanced techniques, including transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier-transformed infrared spectroscopy (FTIR). This experimental protocol was approved by the institutional ethical review committee, and all procedures were conducted by following international guidelines for care and use of laboratory animals.

2.1 Test Chemical

The test chemical used in this research is Nickel oxide nanoparticle which was purchased from SIGMA-ALDR in the form of Nano Powder (Hummer's Method). Three different concentrations of Nickel oxide nanoparticle have been used as 0.5 mg/kg BW, 1.5 mg/kg BW and 2.5 mg/kg BW in this research.

2.2 Characterization of Nickel Oxide Nanoparticles

The characterization of nanoparticles was done to evaluate the crystal structure and particle size of nickel nanoparticles in this study. X-ray diffraction study was undertaken. The size of the nanoparticles was evaluated by the Debye-Scherrier formula.

$D = k\lambda / \beta \cos \theta$

Where D is the particle size (nm), k = 0.94 is a constant, λ is the X-ray wavelength (0.154 nm), β is the line broadening at half the maximum intensity (FWHM), and θ is the Bragg angle [29]. Transmission electron microscope study was undertaken to know the shape and size of nickel oxide nanoparticles. Also, it confirmed the size of the nanoparticles. A suitable statistical histogram plot was used to determine the mean particle size [30, 31]. Fourier transform infrared (FTIR) spectroscopy was performed to find out the presence of a functional group in NiO-NPs.

2.3 Experimental Model

For the present investigation, a total of 20 female albino rats were obtained from the Department of Physiology at Government College University Faisalabad, Pakistan. All the specimens were stored in a plastic container together with food and thereafter relocated to the animal facility at the Department of Pharmacy, Government College University, Faisalabad, Pakistan, to conduct research. The female rats were housed in standardized cages made from polypropylene with daily bedding changes to maintain a healthy habitat. The rats were maintained under standard lighting circumstances following authorization from the local ethical council of Government College University Faisalabad (GCUF) for animal testing and health care, in compliance with international standards for animal health and care. The rats were given unlimited access to commercial Kent rodent feed (containing 16% protein) and distilled water during the whole research. The temperature of the animal housing was kept at a constant 24 ± 2.5 degrees Celsius. For a period of two weeks, rats were acclimatized and thereafter divided into several groups, such as control and treated groups, by the use of distinct colored permanent markers applied to their tails in a random manner**.**

2.4 Preparation of Exposure Solution of Nickel Oxide Nanoparticles

The nanoparticles of Nickel Oxide were acquired in a powder form that was 99% pure. Prior to exposure, the Nickel oxide nanoparticles were made by dispersing the powder in deionized water. This was done in sterile 50ml falcon tubes at a concentration of 1mg/ml. The mixture was then sonicated for 60 minutes at a temperature of 65°C in an ultrasonic bath. The mechanical agitation was performed by vortexing using a DLAB MX-S instrument from China for a duration of 1-2 minutes. This was done to ensure proper dispersion before exposing the experimental animals.

2.5 Study Design

The toxic effect of a specific form of Nickel, NiO NPs, was evaluated using a dose- time response approach. An experiment was performed using female Albino rats which were weighed and then randomly allocated in four distinct experimental groups, with each group consisting of five rats (n=5). One group, called the control group, received only distilled water. The other

three groups were exposed to Nickel oxide nanoparticles. Three experimental groups were subjected to three distinct doses of Nickel oxide nanoparticles over a period of 28 days, with exposure occurring on alternate days.

Total three doses of Nickel oxide nanoparticles were chosen, categorized as low, medium, and high, with concentrations of 0.5, 1.5, and 2.5 mg/Kg BW respectively, administered to rats every other day for a duration of 28 days. The dose selection was based on the rationale derived from our pilot experiment conducted prior to the current investigation. The Nickel oxide nanoparticles were administered via the intravenous method of exposure. Intravenous injections were administered using 1ml BD insulin-sterilised syringes (Table 1). **Table 1** Grouping of Female Albino rats and their treatment schedule

Table 1

Serial	Groups	Dose	Route of exposure
1	Group 1	Control	Only distilled water
2	Group 2	NiO NPs (0.5 mg/Kg BW)	Intravenous
3	Group 3	NiO NPs (1.5 mg/Kg BW)	Intravenous
4	Group 4	NiO NPs(2.5 mg/Kg BW)	Intravenous

2.6 Body Weight of Rats

The initial body weights of all female albino rats, including the control group, were recorded prior to the commencement of the experiment. Subsequently, the weights were measured every week for a duration of 28 days using a weighing balance, and the values were stated in grams. A graph was created to analyze the relationship between the average body weights of rats and the duration of the experiment in order to notice any fluctuations in body weight.

2.7 Blood Collection and Dissection

Following a 28-day period of exposure to nickel oxide nanoparticles, the animals were subjected to a 24-hour fasting period prior to blood collection. Subsequently, they were sedated using chloroform. From each animal, blood samples were taken via the tail (caudal) vein using 3ml tubes coated with EDTA.K3. For further examination, samples were then stored at 4 °C.

Hematology Analysis

An analysis of blood parameters was conducted using an Auto Hematology analyzer (BC Mindray 3600, Shenzhen, China) to assess changes in all blood parameters [32]. The serum was removed from each blood sample, and different serum biochemical biomarkers of the liver were measured using commercial kits with the help of a chemistry analyzer [33].

Statistical Analysis

SPSS and one way anova was used to find the P value and do the comparison and interpretation of results. Analysis of variance showed significant results. $P< 0.05$.

3. RESULTS

This study investigated the toxic potential of nickel oxide nanoparticles (NiO NPs) in female albino rats following 28 days of intravenous exposure, with a focus on body weight changes and hematological parameters. The assessment of NiO NPs effects occurred by examining both behavioral patterns and weight changes alongside blood cell measurements of RBCs and WBCs and hemoglobin levels and platelet counts. This research examined NiO NPs dose- dependent toxicity while analyzing its effects on the physiological and hematological results of female albino rats.

4. Characterization of Nickel Oxide Nanoparticles

4.1 Spectrum (FTIR) of NiO NPs

The chemical composition together with surface functional groups for nickel oxide nanoparticles (NiO NPs) can be observed with the aid of (Figure 1), which displays their Fourier transform infrared (FTIR) spectrum. A prominent absorption peak appears at 438.1 cm^{-1} in the spectrum because the stretching vibration of Ni-O bonds takes place within the NiO crystal lattice. Nickel oxide exists as a confirmed constituent of these manufactured nanoparticles. The spectrum shows a wide stretching band running from 3380.7 to 3568.9 cm^{-1} which results from O-H bond vibrations. High-surface-area nanoparticles possess surface hydroxyl groups and adsorbed water molecules, which are typical characteristics because they easily attract atmospheric moisture. Analysis of NiO nanoparticles reveals that the peaks between 1323.2 cm^{-1} and 1038.1 cm^{-1} are nonexistent because the material has high purity and minimal organic impurities.

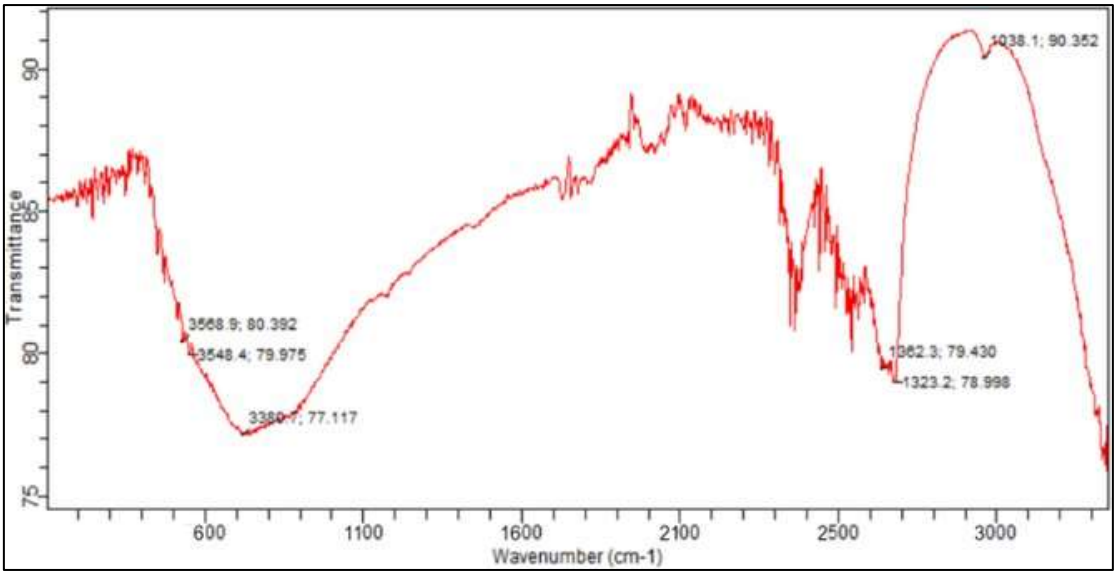

Figure 1: FTIR Spectrum for Nickel Oxide Nanoparticles

*The FTIR spectrum demonstrates the complex molecular composition at nickel oxide nanoparticle surfaces because it determines their catalytic and sensing performance. NiO nanoparticle stability together with reactivity relies prominently on hydroxyl groups present on their surface along with adsorbed water molecules as shown by FTIR spectroscopy. Surface behavior understanding enables development of better functional and technical applications.

4.2 TEM of NiO NPs

Analysis of nickel oxide (NiO) nanoparticles shape and architecture used transmission electron micros copy (TEM) as the primary methodology. Transmission electron microscopy (TEM) provided an image of nanoparticles which appeared from green to black and were smaller than 50 nm (Figure 2). The mean diameter for these nanoparticles reached 17 nm while the measured range extended from 6.2 to 38.1 nm. In cellular medium the nanoparticles showed growth to 31.4 nm in size. The high quality and trace metal purity of the nanoparticles were indicated by their dense clustering nature. The NiO nanopowder density reached 6.67 g/mL under 25°C testing conditions but its bulk density amounted to 0.51 g/mL. The tiny size coupled with high purity of these nanoparticles makes them optimal for battery manufacturing applications.

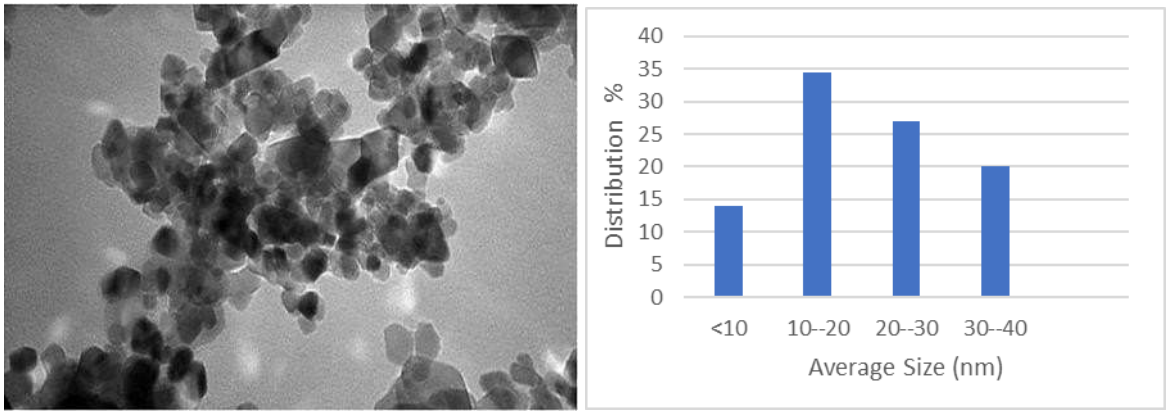

Figure 2: Transmission electron microscopic image and graph of Nickel Oxide Nanoparticles

4.3 XRD of NiO NPs

The X-ray diffraction (XRD) pattern of nickel oxide nanoparticles in (Figure 3) showed a comprehensive structural analysis. The XRD pattern confirms NiO crystallinity through peaks that align with the cubic crystal planes at 2θ positions of 37°, 43° and 63°. The acuity and robustness of these peaks signify a considerable degree of crystallinity, characteristic of nanoparticles within this dimensional range. A wide background and multiple small peaks suggest minor lattice disorder and nanoscale fluctuations. The XRD pattern verifies the phase purity and crystalline characteristics of NiO nanoparticles, crucial for their utilization in electronics and advanced materials.

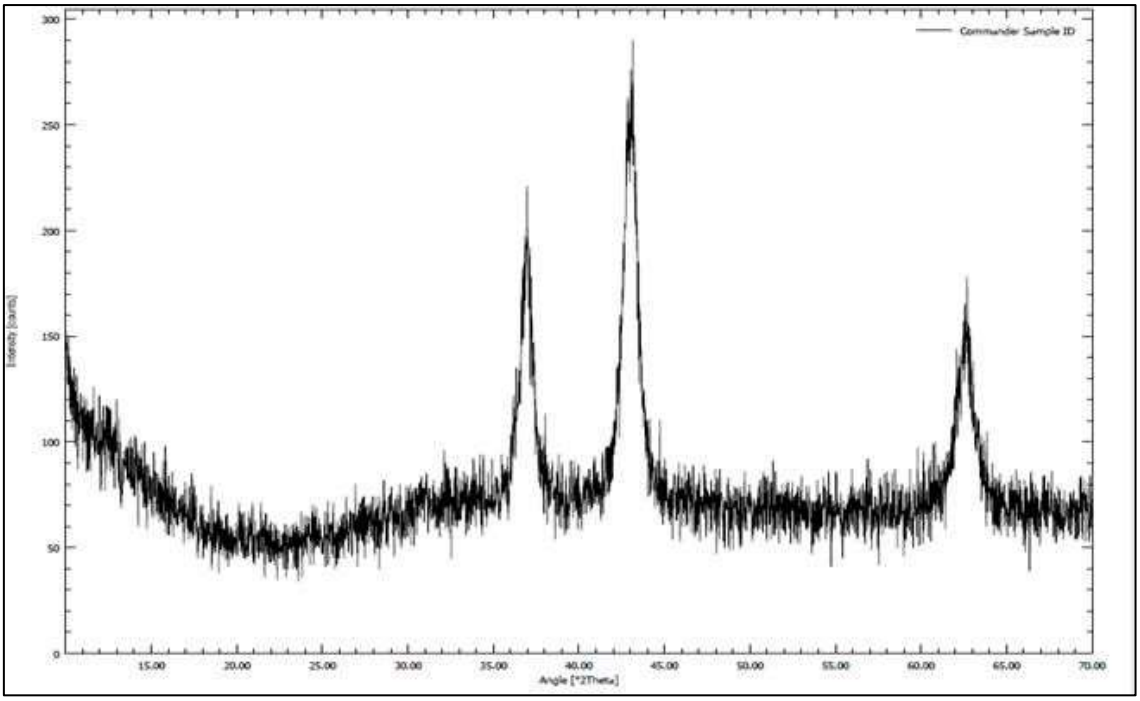

Figure 3: X-ray Diffraction image Nickel Oxide Nanoparticles

4.4 General Observations

No animals died or showed any signs of disease during the course of the trial. Daily exams found no significant variations in physical condition, mobility, nutritional intake, or exploratory behaviour between the control and treatment groups. Nonetheless, the rats experienced a brief period of lethargy and decreased mobility following the initial dose of nickel oxide nanoparticles (NiO NPs). The effects were quickly neutralized, and the rats resumed their regular activity levels.

4.4.1 Body Weight

Weekly body weight measurements of female albino rats were recorded throughout the experimental period. Table 2 demonstrates a consistent trend of weight reduction across the weeks, alongside notable variations in body weight over time. Weeks three and four demonstrated the most substantial reductions, highlighting the adverse effects of nickel oxide nanoparticles (NiO NPs). The substantial effect of NiO nanoparticles on rat health was demonstrated by the ineffectiveness of vitamin C injection in preventing or reversing weight loss (Figure 40) illustrates the mean body weight and standard deviations for each group, offering a clear visual representation of these variances.

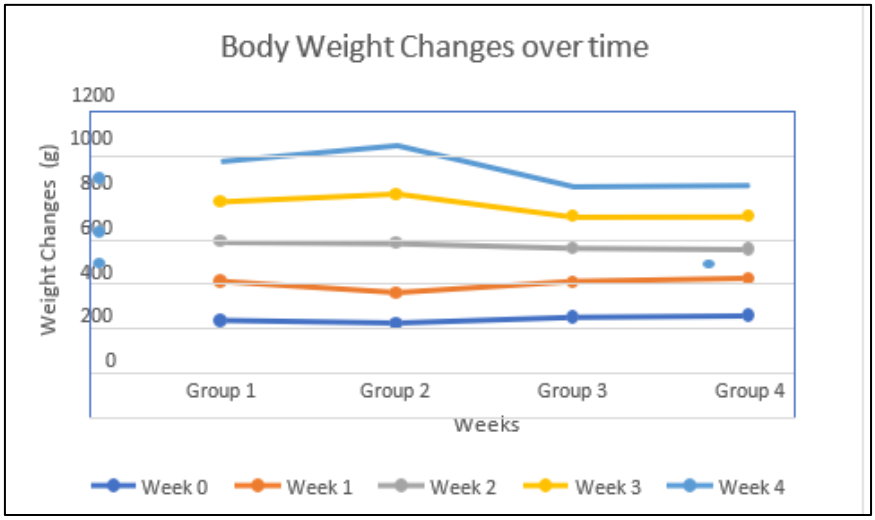

Figure 4. Effects of different NiO NPs concentrations on final body weight (grams) of female albino rats. G1 (Control treated with distilled H2O), G2 (treated with 0.5 mg/Kg BW NiO NPs), G3 (treated with 1.5mg/Kg BW NiO NPs), G4 (treated with 2.5 mg/Kg BW NiO NPs)

Graph of body weight changes according to time duration also tells the relation between the body weight changes and respective toxicity of nanoparticle concentration. Analysis of variance also shows that body weight changes are significant (P<0.05) in table 3.1 below.

Table 2: One way Anova (Analysis of variance) of weekly body weight changes during 30 days of trial

	Source of Variation	SS	df	MS	F	P-value	F crit
Week 0	Between Groups	5833.36	5	1166.67	6.315445	0.0007***	2.62
	Within Groups	4433.6	24	184.7333			
	Total	10266.9	29				
Week 1	Between Groups	6235.366	5	1247.07	5.5631524	0.001***	2.620
	Within Groups	5380	24	224.1666			
	Total	11615.3	29				
Week 2	Between Groups	8005.3666	5	1601.07333	5.135760492	0.002**	2.620
	Within Groups	7482	24	311.75			
	Total	15487.366	29				
Week 3	Between Groups	9497.3666	5	1899.47333	9.2252226	5.30081E-05***	2.620
	Within Groups	4941.6	24	205.9			
	Total	14438.96	29				
Week 4	Between Groups	11011.766	5	2202.35333	11.8896167	7.31414E-06***	2.620
	Within Groups	4445.6	24	185.233333			
	Total	15457.366	29				

*Non-significant (P>0.05); **Significant (P<0.05); *** Highly Significant (P<0.001)

Table 3: Analysis of variance of hematological parameters in female albino rats injected NiO NPs intravenously during trial

4.5 Hematological Analysis

Significant differences in the hematological parameter values (p<0.05) shown in (Table 3). In comparison to the control group, the treated groups had significantly higher levels of white blood cells (WBC), mean corpuscular hemoglobin (MCH), and platelets (PLT). There were also considerably decreased levels of red blood cells (RBC), hemoglobin (HB), MCV (Mean Corpuscular Volume), the mean concentration of corpuscular hemoglobin (MCHC), and hematocrit.

	Source of Variation	SS	Df	MS	F	P-value	F crit
WBC	Between Groups	353.386307	5	70.677261	55.20446	2.219E-12***	2.620654
	Within Groups	30.72676	24	1.2802817			
	Total	384.113067	29				
RBC	Between Groups	109.639	5	21.9278	50.4229	5.9E-12***	2.6206541
	Within Groups	10.4371	24	0.43488			
	Total	120.076	29				
HB	Between Groups	110.11312	5	22.02262	51.92994	4.322E-12***	2.62065415
	Within Groups	10.178	24	0.424083			
	Total	120.29112	29				
HCT	Between Groups	2786.66667	5	557.33333	30.877193	1.046E-09***	2.62065415
	Within Groups	433.2	24	18.05			
	Total	3219.86667	29				
MCV	Between Groups	2884.266667	5	576.85333	43.590932	2.849E-11***	2.620654148
	Within Groups	317.6	24	13.23333			
	Total	3201.866667	29				
MCH	Between Groups	121.15103	5	24.230206	59.1593872	1.0394E-2***	2.620654
	Within Groups	9.8298	24	0.409575			
	Total	130.98083	29				
MCHC	Between Groups	159.5399767	5	31.907995	45.123663	1.969E-11***	2.62065415
	Within Groups	16.97096	24	0.7071233			
	Total	176.5109367	29				
PLT	Between Groups	1685707.9	5	337141.58	75.219278	7.2268E-14***	2.6206541
	Within Groups	107570.8	24	4482.1167			
	Total	1793278.7	29				

*Non-significant (P>0.05); **Significant (P<0.05); *** Highly Significant (P<0.001)

The hematological analysis revealed significant alterations in key blood parameters following exposure to nickel oxide nanoparticles (NiO NPs) in female albino rats. These changes were observed in a dose-dependent manner, with notable differences between the control and nanoparticle-treated groups. The findings indicate that NiO NPs can potentially harm human blood cells through oxidative stress and inflammation and directly injure blood-producing tissues. Graphical representations of the data analyse the effects of NiO NPs on independent hematological parameters.

4.5.1 Red Blood Cells (RBC)

The red blood cell (RBC) count exhibited a substantial decline in every nanoparticle exposure group versus the control group which potentially indicated the development of anemia combined with impaired erythropoiesis. The most severe RBC count reduction occurred when using NiO NPs at the highest tested concentration. Human blood cells showed decreased levels of red blood cells because nanoparticle toxicity likely exerted oxidative stress on erythrocyte precursor cells in the bone marrow or led to an increase in blood cell damage as (Figure 50) indicates. NiO NPs demonstrate toxicities to normal blood cell production at dosages which ultimately lead to development of anemia based on these laboratory results.

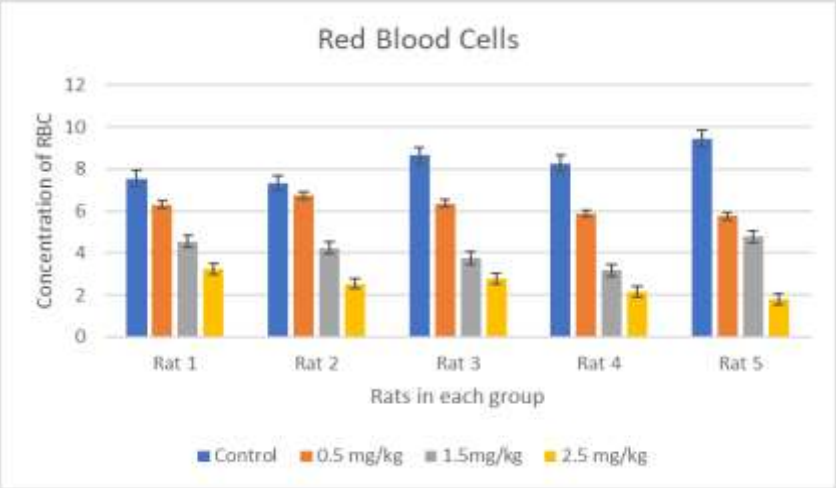

Figure 5: Dose-dependent effect of NiO NPs in female albino rats on Red blood cell concentration

4.5.2. White Blood Cells (WBC)

White blood cell (WBC) numbers increased proportionally with NiO NPs exposure levels which indicates that NiO NPs stimulate immune or inflammatory responses. WBC levels increased because the body works toward fighting NiO NPs toxicological effects through immune cell activation like neutrophils and lymphocytes (Figure 6). The nanoparticles usually initiate tissue damage through oxidative stress and provoke systemic inflammation. The elevated WBC levels demonstrate NiO NPs have the ability to disturb immune system balance so it leads to stressful conditions within the blood cells.

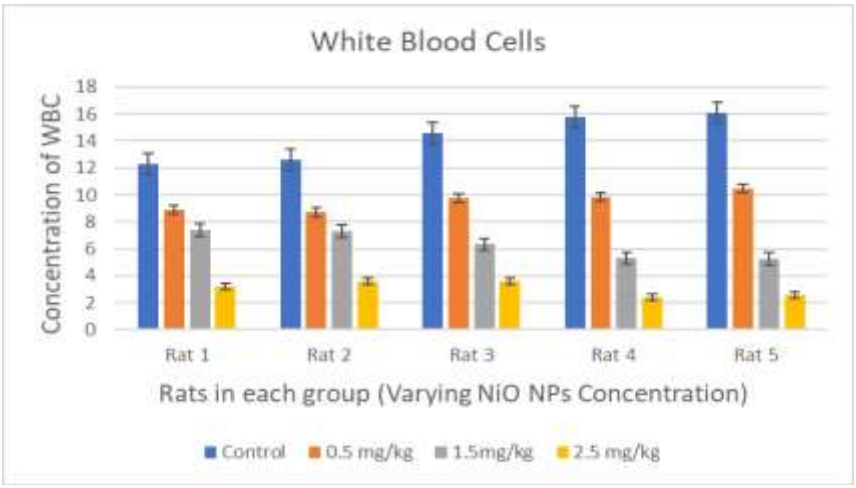

Figure 6: Dose-dependent effect of NiO NPs in female albino rats on (WBC) concentration

4.5.3. Hemoglobin (Hb)

The groups treated with nanoparticles showed lower Hemoglobin (Hb) levels which confirmed the development of anemia. The lowering of Hb quantities corresponds with reduced RBC numbers because red blood cells depend on hemoglobin to transport oxygen. The presence of NiO NPs seems to reduce Hb levels since hemoglobin synthesis gets impaired or because oxidative stress increases the breakdown of red blood cells (Figure 7). These results demonstrate NiO NPs' effect on blood oxygen transportation capabilities and their ability to disrupt blood cell health.

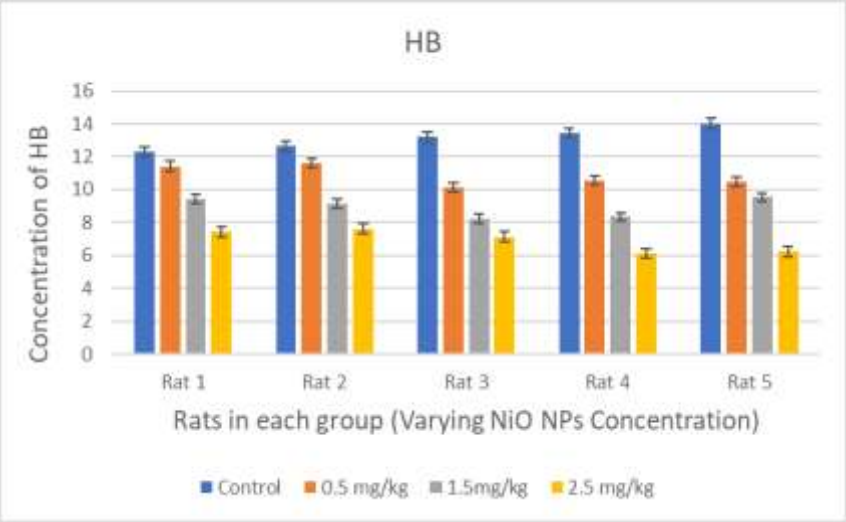

Figure 7: Dose-dependent effect of NiO NPs in female albino rats on (HB) concentration

4.5.4 Mean Corpuscular Volume (MCV)

The mean corpuscular volume (MCV) decreased significantly in groups receiving nanoparticle treatment (Figure 8). The measurement presents evidence of microcytic anemia since red blood cells display abnormally small dimensions. NiO NPs-induced oxidative stress would likely worsen either iron deficiency or impaired hemoglobin synthesis because microcytosis develops as a result of these conditions. The decrease in MCV extends the evidence that NiO NPs interrupt the normal processes of erythropoiesis and red blood cell maturation.

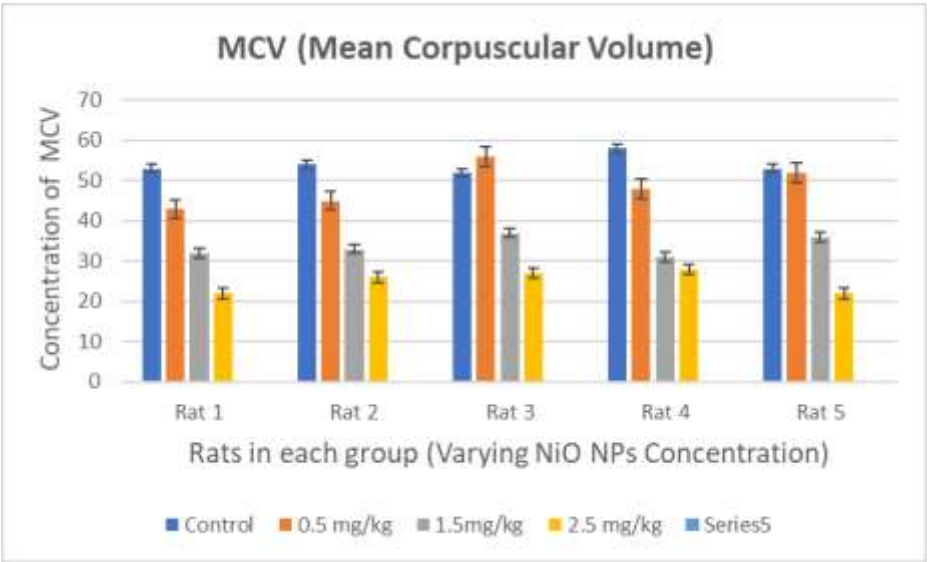

Figure 8: Dose-dependent effect of NiO NPs in female albino rats on (MCV) concentration

4.5.5. Mean Corpuscular Hemoglobin (MCH)

The nanoparticle treatment groups showed significant elevations in mean corpuscular hemoglobin in blood cells (Figure 9). Red blood cell compensation through increased hemoglobin levels marks a possible response to anemia while trying to preserve oxygen supply capacity. The compensatory response shown by the body is insufficient to counteract the general reduction in RBC count together with Hb levels. The increase in MCH indicates NiO NPs cause multiple effects on red blood cells which may impact their regular functional capabilities.

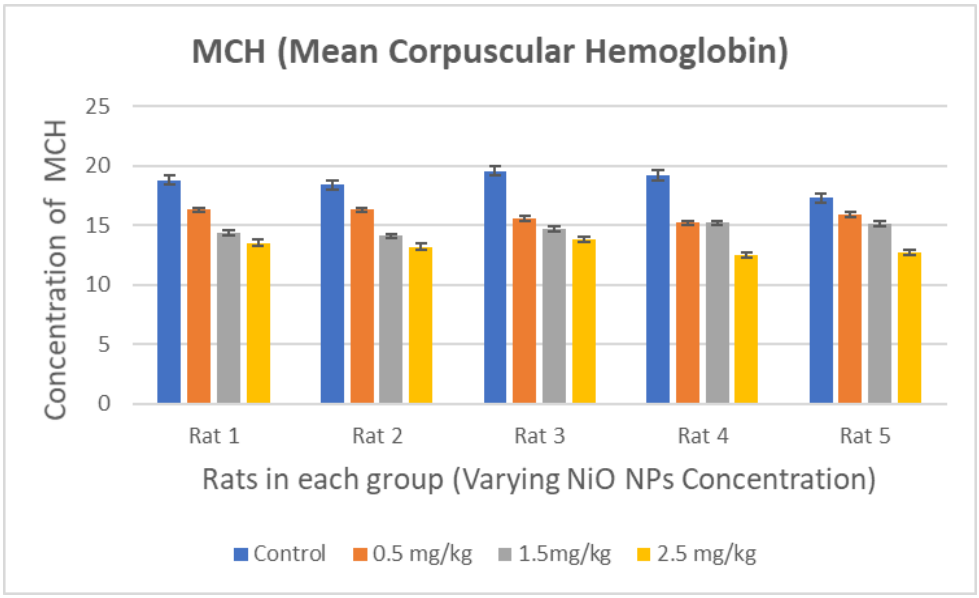

Figure 9: Dose-dependent effect of NiO NPs in female albino rats on (MCH) concentration

4.5.6 Mean Corpuscular Hemoglobin Concentration (MCHC)

The mean corpuscular hemoglobin concentration (MCHC) levels decreased significantly in blood from participants who received nanoparticle treatments (Figure 10). Lower concentration of hemoglobin in red blood cells defines hypochromic anemia so some medical condition leads to poor hemoglobin synthesis or iron deficiency. The MCHC levels decrease in parallel with decreased Hb content and decreased red blood cell count thus demonstrating anemia as the cause. The research indicates that NiO NPs show hematological toxicity by negatively impacting hemoglobin synthesis mechanisms.

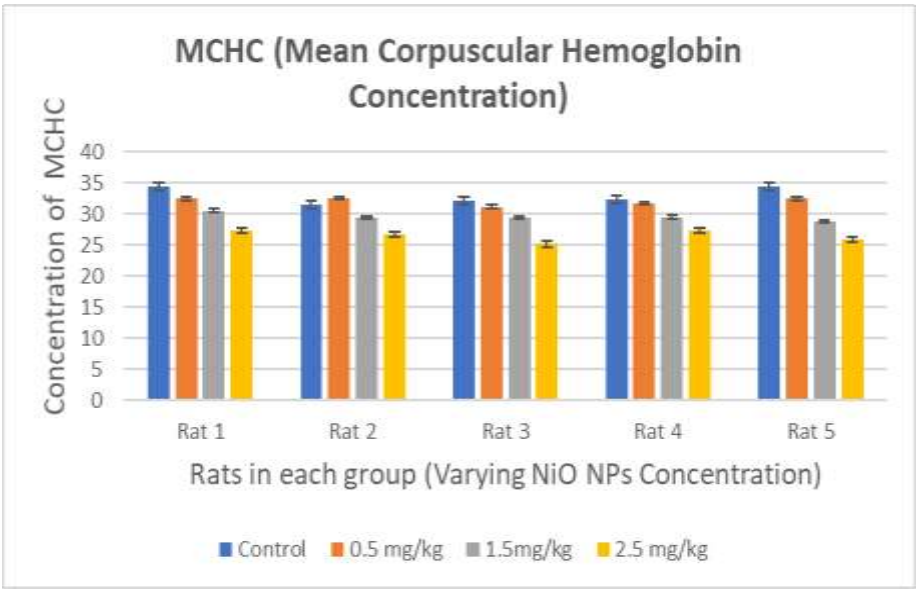

Figure 10: Dose-dependent effect of NiO NPs in female albino rats on (MCHC) concentration

4.5.7 Hematocrit (HCT)

The proportion of blood occupied by red blood cells known as hematocrit (HCT) showed significant reduction in groups with nanoparticle administration. The observed decrease in RBC count and Hb levels confirms the occurrence of anemia because it matches the recorded HCT reduction. The HCT reduction occurs because NiO NPs cause decreased red blood cell production as well as increased destruction of blood cells through oxidative stress damage (Figure 11). This shows NiO NPs can reduce red blood cell volume which demonstrates their ability to cause blood cell deteriorating.

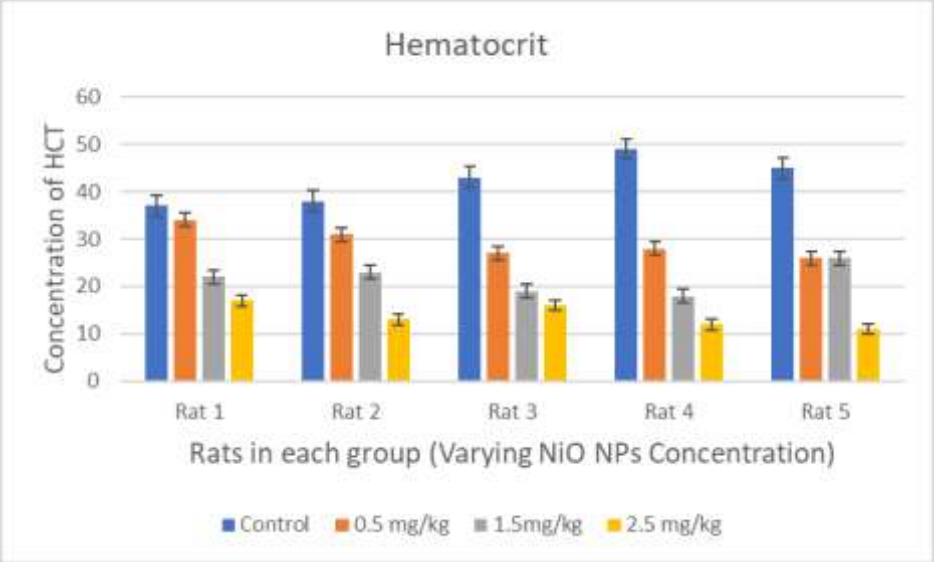

Figure 11: Dose-dependent effect of NiO NPs in female albino rats on (HCT) concentration

4.5.8 Platelets (PLT)

The nanoparticle treatment groups showed significantly higher counts of platelets which indicates NiO NPs might trigger a thrombocytic effect (Figure 12). The increase in platelet numbers indicates two possible outcomes; either an inflammatory process or a stress reaction because platelets serve as key players in both inflammation and tissue repair. The elevated PLT counts function as a protective response to minimize NiO NPs-induced vascular damage and microbleeding. The research indicates NiO NPs create disturbances that affect healthy platelet equilibrium while triggering adverse changes to the blood system.

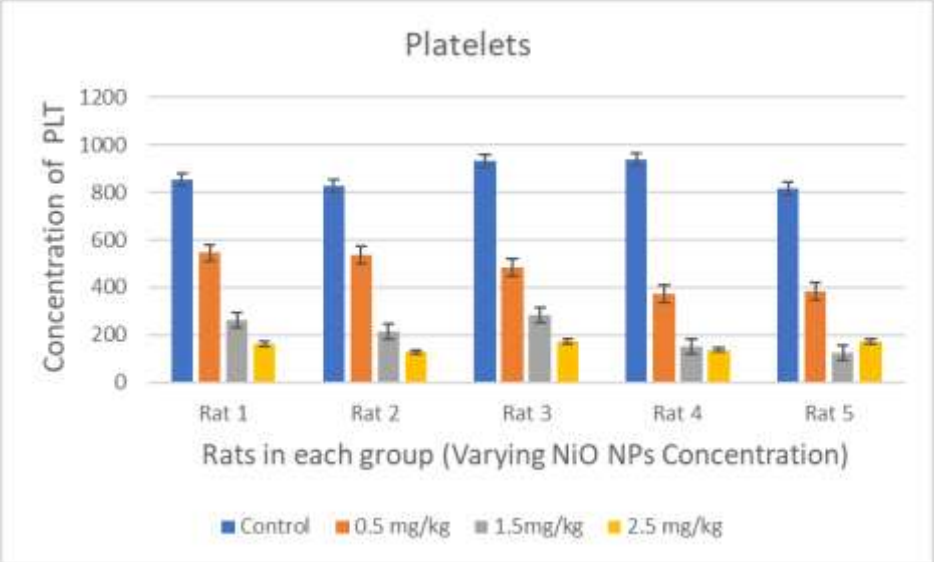

Figure 12: Dose-dependent effect of NiO NPs in female albino rats on (Platelets) concentration

5. DISCUSSION

Nickel oxide nanoparticles (NiO NPs) are usually used in different industrial and biomedical applications due to their unique physicochemical properties [9]. These nanoparticles' stable structure, wide surface area, and reactive nature make them useful in a variety of industries, including electronics, energy storage, catalysis, and medical devices. [34]. But their increasing usage has sparked worries about potential impacts on the environment and human health [35]. This study assessed NiO NPs toxic effects in female albino rats through thorough nanoparticle evaluation and measurements of bodily weight alterations and blood study results to understand NiO NPs toxicity pathways while determining their health risks. The studies about NiO NPs toxicity is limited and especially lack knowledge about female models [36]. Many previous studies investigated NiO NPs toxicity in male rats without studying how these toxic effects differ between genders [37]. For example, [38] studied NiO NPs toxicity in male rat subjects failed to examine gender-based impacts. Similarly Hematological changes occurred in

male rats treated with NiO NPs according to research findings but details about female rat response to the NPs remain unavailable [39].

One important deficiency in existing literature exists regarding the scarcity of all- encompassing research on NP-dose dependency relationships. By utilizing only one concentration of NiO NPs restrict researchers are restricted from determining the link between NP dosage and toxic effects [40]. For instance, the NiO NPs toxicity at one dose level hindered the utility of their research findings [36].

Little scientific evidence exists about the specific mechanisms that cause NiO NPs to induce harmful effects on blood cells. Scientists have established a connection between oxidative stress and inflammation, yet they still need to clarify how NiO NPs contribute to anemia and leukocytosis through specific mechanisms. The study identified oxidative stress as a main toxicity factor of NiO NPs but failed to provide extensive details about its effects on blood cell measurements [41]. Our research aimed to resolve three essential knowledge gaps in existing literature regarding NiO NPs toxicity since current research lacks gender-specific toxicity testing and proper dose-response examinations and mechanistic understanding of NP-induced hematology toxicity. The study delivers in-depth information about NiO NPs toxicity and associated health and safety risks when focusing on female subjects. The characterizing work of NiO NPs through TEM, XRD, and FTIR analysis produced vital information about particle dimensions alongside shape characteristics and chemical properties according to study documentation. [42]. Cell research data and NiO NPs TEM imaging and XRD pattern data showed NiO NPs have spherical shapes and cubic forms. The spherical NiO nanoparticles possessed an initial diameter of 17 nm before increasing to 31.4 nm after incubation in cell culture medium based on TEM observations. The observed phenomenon matches scientific evidence which shows that smaller nanoparticles become more toxic and reactive because of their large surface area [43]. The X-ray diffraction data revealed high crystallinity of NiO NPs through peaks appearing at 37°, 43° and 63° which confirmed their cubic crystal structure. The findings show that NiO NPs made using green synthesis show equivalent XRD patterns [44].

According to the FTIR test results researchers observed a distinct Ni-O bond peak at 438.1 cm^{-1} together with water molecules or surface hydroxyl groups showing multiple absorption bands spanning 3380.7 to 3568.9 cm^{-1}. A high surface area among nanoparticles leads them to absorb environmental moisture according to previous research [45]. Similarly strong peaks at 438.1 cm^{-1} in the FTIR spectrum, corresponding to the Ni-O bond [46]. But there are variations in nanoparticle size and purity, which may be due to differences in synthesis methods [47- 49].A range of analyses demonstrate that NiO NPs exhibit minimal organic contamination and high purity because there are no distinct peaks observed between 1323.2 cm^{-1} to 1038.1 cm^{-1} [50]. This is supported by nanoparticle quality stands essential for toxicological investigations [51]. NiO NPs exhibit reactivity as well as toxicity levels that directly depend on their size and shape together with surface properties because small-sized nanoparticles with extensive surface area have a higher potential to induce cellular damage and generate oxidative stress [52]. A direct relationship exists between NiO nanoparticle dimensions and purity level which shows that synthesis parameters structurally define nanoparticles [53].

The body weight losses observed in NiO NPs-treated groups demonstrate systemic toxicity because nanoparticles cause oxidative stress and inflammation [25]. Siddiqui's work confirmed these finding through their weight loss results in rats exposed to NiO NPs over extended periods [54]. Similarly, the study conducted by Ali in 2020 revealed that oxidative stress functions as a crucial factor in nanoparticle-caused toxicity, which decreases metabolic efficiency and results in weight reduction [55]. While male rats experienced weight increase at each dose level yet female rats only gained weight at the highest dose level [56].

The weight decrease in animals treated with nanoparticles appears linked to food intake reduction and digestion problems because research established reduced food consumption in these animals [57]. The systematic toxicity hypothesis is strengthened by short-term observations of physical symptoms, including lethargy and mobility impairment, which appear right after the initial dose. Research findings demonstrate that rats that encounter metal oxide nanoparticles display comparable behavioral effects [58]. Body weight measurement and identification of physical reactions in toxicity tests support the assessment of nanoparticle- related systemic effects. Strict regulatory guidelines become essential because the observed toxicity effects show dependency on exposure concentration [59].

The blood analysis showed substantial variations in seven vital blood markers: RBC, WBC, Hb, MCV, MCH, MCHC and HCT along with PLT. Hematological toxicity from NiO NPs exposure displays signs through blood parameter alterations that scientists attribute to oxidative stress together with inflammatory responses [60]. The significant reduction in RBC, an anemic conditions, developed in NiO NPs-treated groups because of either hindered red blood cell production or increased cell rupture known as hemolysis [61]. This study presented evidence that NiO NPs exposure led to reduced RBC count findings because such NPs damaged erythrocyte precursors through oxidative mechanisms [62]. This work is supported by High amounts of anemia developed in animals exposed to nanoparticles as

indicated by two research studies that linked this effect to oxidative stress toxicity [24, 63].

The raised WBC count reveals an inflammatory or immune response, as reported by a study that NiO NPs activate immune cells, leading to systemic inflammation [64]. Another similar study demonstrated that nanoparticles induce the release of pro-inflammatory cytokines, contributing to leukocytosis [61, 65]. The decrease in Hb levels added supports the observation of anemia, as hemoglobin is a critical component of red blood cells responsible for oxygen transport [54, 66]. This is consistent with the observation of impaired hemoglobin synthesis in nanoparticle-treated animals [67].

The reduction in MCV suggests the presence of microcytic anemia, where red blood cells are smaller than normal [68]. This is in line with the results of a study which observed microcytosis in rats exposed to metal oxide nanoparticles [69]. The increase in MCH may indicate compensatory mechanisms in response to anemia, as reported some similar changes in nanoparticle-treated animals [40, 70]. The decrease in MCHC indicates hypochromic anemia, where red blood cells have lower hemoglobin concentration [68]. The reduction in HCT levels aligns with the observed reductions in RBC count and Hb levels, further supporting the presence of anemia [71]. The increase in platelet count may reflect a thrombocytic response to vascular damage or inflammation caused by NiO NPs, as reported by researcher who observed elevated platelet levels in nanoparticle-treated animals [72].

These findings collectively suggest that NiO NPs induce hematological toxicity through mechanisms involving oxidative stress, inflammation, and disruption of erythropoiesis [73]. The results highlight the need for further research to elucidate the molecular pathways underlying these effects.

6. CONCLUSION

This study confirmed that nickel oxide nanoparticles (NiO NPs) caused significant dose-dependent toxicity in female albino rats. The characterization using TEM, XRD and FTIR validated the spherical morphology, high purity, and crystalline structure of nickel oxide nanoparticles, averaging 17nm in size. The body weight of female albino rats decreased by 12% with low dose treatment, and reached 18% with medium dose treatment and almost 24% with high dose exposure compared to unexposed controls. The blood analysis from high-dose tested subjects showed decreased red blood cells by 25% and reduced hemoglobin by 30%, together with elevated white blood cells by 40%. The 15% reduction in mean corpuscular volume and the 10% decrease in mean corpuscular hemoglobin concentration in blood cells showed characteristic signs of small-sized pale anemia known as microcytic hypochromic anemia. The observed 20% increase in blood platelet numbers indicates possible blood clotting response. NiO nanoparticles induced toxic effects throughout the body and blood cells according to these results through mechanisms involving oxidative stress and inflammatory processes. The research tackles female rat subjects to address knowledge shortages regarding sex-specific toxicity effects. Future research needs to investigate extended NiO nanoparticle exposures along with their biological mechanisms of harm in order to develop safer usage methods for this material throughout industries.

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