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In Vivo Toxicological Assessment of Plant Nanofertilizer Exposure on Red Blood Cells and Platelets

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Abstract. Due to the rapid advancement of nanotechnology in agriculture, this research was conducted to investigate the hematological safety of chelated multi-micronutrient nanofertilizer. Thirty-five rats (*Rattus norvegicus*) were divided into five groups: a control group and four experimental groups, which received 62.5, 125, 250, and 500 mg/kg/day for 15 alternate-day doses orally. At 62.5 mg/kg, (MCV) reduced to 38.21±2.09 fL and mean corpuscular hemoglobin concentration (MCHC) and plateletcrit (PCT) increased to 539.71±42.87 g/L and 7.49±1.02, respectively. At a 500 mg/kg dose, RBCs increased to 7.90±2.34 ×10^\12, HCT to 0.42±0.15, and MCV to 52.11±4.46 fL. On the other hand, MCH and MCHC decreased to 18.49±1.46 pg and 355.29±16.27 g/L along with a decrease in MPV to 6.70±0.39 fL. Nanofertilizer produced dose-related hematologic changes, which were severe at both higher and lower doses, with implications for blood safety.

Keywords: Nanofertilizes; Erythrocytes; Blood Platelets; Hematotoxicity; Oxidative stress; Hemocompatibility.

INTRODUCTION

Recent research has shown that a variety of agricultural chemicals—including pesticides, fertilizers, and growth regulators—can induce degradation of DNA in both humans and plants, raising concerns about their long-term biological impact [1]. At the same time, rapid industrial development has intensified the demand for new functional materials with unique physicochemical properties, highlighting the significant role of nanoparticles in modern technological advancement [2,3]. Nanomaterials with particle sizes between 1–100 nm exhibit behaviors and characteristics that differ markedly from those of their bulk counterparts, due to their increased surface-area-to-volume ratio, quantum effects, and high surface reactivity [4]. As a result, nanoparticle research has gained worldwide attention, leading to broad applications across medicine, electronics, agriculture, energy, and environmental remediation [5]. Despite their many advantages, nanoparticles are increasingly recognized for their potential toxicological risks and unintended biological interactions [6,7]. Several studies have demonstrated that nanoparticles can induce oxidative stress, trigger apoptosis, inhibit cell proliferation, and promote inflammatory responses in exposed organisms [8]. In agricultural systems, nanoparticles—often incorporated as nanofertilizers—are used to enhance plant growth, improve nutrient uptake, and increase yield quality and productivity [9]. However, when present at elevated concentrations in plant, animal, or human tissues, these nanomaterials may pose more substantial risks than conventional fertilizers and agrochemicals [10]. The environmental behavior of agricultural nanoparticles is highly complex and influenced by soil conditions, particularly the wetting–drying cycles associated with irrigation practices. These hydrological fluctuations can either stabilize or solubilize sparingly soluble nanoparticles such as ZnO, or promote the formation of soluble ionic species, as has been documented for copper nanoparticles. Moreover, clay minerals in soil can act as carriers, transporting insoluble metallic nanoparticles over long distances. Such mobility increases the possibility of environmental contamination, ecological disruption, and unintended bioavailability to non-target organisms [11]. These factors underscore the necessity for careful evaluation of nanoparticle fate, transformation, and environmental impact prior to their widespread agricultural deployment [12].

A major concern is the ability of nanoparticles to enter the bloodstream of exposed organisms and directly interact with key blood components, particularly red blood cells (RBCs). Their interaction can alter RBC membrane integrity, change mechanical properties, reduce oxygen-transport efficiency, and promote inflammatory cascades, all of which

increase the risk of hemolysis and thrombotic events [13]. Additionally, nanoparticles may influence platelet behavior, either activating or inhibiting platelets, potentially leading to coagulation disorders or life-threatening blood clots [14]. Given these risks, evaluating the safety profile of nanoparticle–blood interactions is crucial to ensure that nanofertilizers and other nanoscale agricultural inputs can be used safely and responsibly [15]. Accordingly, the present study was undertaken to investigate the hemotoxic effects of a chelated multi-micronutrient nanofertilizer on red blood cells and platelets of a non-target organism. By assessing its potential to induce hemolysis, impair platelet function, and disrupt normal blood physiology, this study aims to provide essential insights into the biological risks associated with nanoparticle exposure in agricultural environments.

MATERIALS AND METHODS

Laboratory Animals

Thirty-five male albino rats (*Rattus norvegicus*) of 7-10 weeks old weighing 176-350 g were used in this study. Certified sources: The Iraqi Center for Genetics and Cancer Research/ Al-Mustansiriyah University, National Center for Drug Control and Research (Ministry of Health) provided the animals. All the rats were housed under standard laboratory conditions, kept in a sterilized cages with sawdust bedding and had free access to balanced chow and tap water. Animals were acclimated for 2 weeks before commencing experimental procedures to facilitate physiological stabilization and familiarization with the procedure environment. After identification by weighing, the animals were randomly divided into one control group and four treatment groups (7 rats each). The treatment groups were given graded doses categorized as C1 (62.5 mg/kg), C2 (125 mg/kg), C3 (250 mg/kg) and C4 (500 mg/kg). These groups had been consisted of animals in the age scale 7-10 weeks.

Nanofertilizer and Oral Administration

Glycine-chelated multi-micronutrient nanofertilizer including Fe, Zn, Cu, Mn, B and Mo was being purchased from a certified agricultural market at Baghdad. The reference condition for foliar application was the manufacturer's recommendation of 1 g/L (for 1 kg of water). Experimental doses of 62.5, 125, 250 and 500 mg/kg were selected on this consideration. The single dose for each group was calculated based on the average body weight of the animal, as recommended by [16]. The doses used were established in a previous experiment before carrying out the major trial. Doses determined were then given to experimental groups (C1-C4). All rats were administered a single oral dose via gavage, which was performed with a 38 mm long 16-gauge ball-tipped stainless steel feeding needle.; there was an interval of 24 hours between doses. Animals were then anesthetized with chloroform and dissected 24 hour after the last dose. Blood was obtained by cardiac puncture for hematological analysis.

Hematological Analysis

Blood samples were gathered into 2 mL tubes pretreated with the anticoagulant ethylenediaminetetraacetic acid tripotassium salt (EDTA-K_3). Tubes were carefully inverted once immediately following collection to promote proper mixing of blood with the anticoagulant. A 15 μL aliquot was directly aspirated using a metal sampling probe connected to a BHA-5000 veterinary automatic hematology analyzer (Weemed, China). The analyzer was based upon flow cytometry, using two reagents: a diluent and lysing solution. Hematological variables were measured automatically, and the final results were available, printed within about 1 min.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (ver. 23). An overall one-way ANOVA was used to explore initial group differences. When a significant effect was identified, pairwise comparisons were conducted using the Least Significant Difference (LSD) method to test for specific group differences. Moreover, Pearson's correlation coefficient was used to determine the strength and direction of linear relationships between the variables examined. The results, presented clearly and concisely as the mean ± SD, and with a significance threshold of $p \leq 0.05$, ensure the ease of understanding our findings.

RESULTS AND DISCUSSION

Comparison of RBC parameters (Table 1) revealed that group C4 displayed a significant increase in RBC count compared to the control, whereas groups C1-C3 showed no significant difference; hemoglobin, on the other hand, remained largely unaltered, although a decrease was noted in C2, and slight increases were observed in the other groups. Red cell indices exhibited more pronounced alterations, since MCV decreased in group C1, remained within the normal range in groups C2 and C3, and increased significantly in group C4. In contrast, MCH remained unaltered in groups C1-C3 but showed a significant reduction in group C4, and MCHC demonstrated an apparent dose-dependent response, increasing in C1 and decreasing in C4.

The platelet parameters (Table 2) revealed non-significant changes, with a slight increase in platelet numbers in groups C1-C4, especially evident in C1 and C2, and a slight decrease in C3 and C4. Meanwhile, PCT increased significantly in C1 but decreased in the other groups toward basal levels. Moreover, MPV was reduced markedly only in C4, compared to both the control and C1 groups. While in C2 and C3, which mimicked a slightly damaged condition, the values remained close to those of the control.

Correlation analysis (Table 3) revealed a significant positive correlations of RBC, Hb, and HCT, as well as between HCT and MCV; MCV and MCHC, while a negative correlation was observed between HCT and MCHC; platelet indices were poorly correlated to each other, with the sole exception of an inverse link between PCT, indicating that mass volume variability was more significant than number.

These results are in agreement, yet also partially differ from, previous ones reported for NPs, which have revealed diverse and even contradictory hematological parameters as a function of both the administration route and dose: for example molybdenum nanoparticles intraperitoneal exposure failed to cause significant changes in RBC count, hemoglobin or hematocrit compared to those noted after oral exposure to molybdenum trioxide NPs [17]; zinc oxide nanoparticles (ZnO NPs) decreased RBCs and hemoglobin content especially under chronic high-dose conditions [7], while Ramadan *et al.* [18] demonstrated a significant declines in RBC and hematocrit with concurrent increases in MCV, MCH, and MCHC, while intraperitoneal ZnO NPs increased hemoglobin and hematocrit in male rats [19] and oral administration to rabbits resulted no marked changes [20]; copper oxide nanoparticles (CuO NPs) decreased hemoglobin, RBCs, MCV values, hematocrit levels, and platelet count after oral delivery [21], but only raised the level of an erythrocyte index such as MCV among males as well as MCH among females following intraperitoneal injection of the nanoparticles [22]; Conversely iron oxide nanoparticles greenly synthesized from parsley extract restored blood parameters in lead acetate-induced anemic rats [23] Additionally, following intraperitoneal treatment with these nanoparticles, there were no significant changes in hematological parameters, such as hemoglobin concentration and platelet counts, which remained at similar levels to those of the control group [24].

TABLE 1. Alterations in red blood cells parameters (Mean±S.D.)

Groups / Parameters	Control	C1	C2	C3	C4	P-Value	LSD
RBCs Count 10^12/L	6.27±0.36	7.11±0.59	5.46±0.81 b	6.93±2.02	7.90±2.34 ac	0.045*	1.59
Hb g/L	129.14±9.44	145.71±4.92	116.86±16.64	146.29±41.34	147.00±49.28	0.25	32.85
HCT	0.32±0.04	0.27±0.02	0.25±0.01	0.30±0.08	0.42±0.15 abcd	0.005**	0.09
MCV fL	46.19±4.21	38.21±2.09 a	46.46±6.03 b	43.83±7.14 b	52.11±4.46 abcd	<0.001**	5.55
MCH pg	20.20±1.11	20.61±1.93	21.44±0.97	21.27±1.43	18.49±1.46 abcd	0.004**	1.55
MCHC g/L	446.0±41.65	539.71±42.87 a	467.29±54.90 b	492.57±54.47	355.29±16.27 abcd	<0.001**	48.37

[1] $P \leq 0.05$
[2] Lowercase letters indicate significance as: a to control, b to C1, c to C2, and d to C3

TABLE 2. Alterations in platelets parameters (Mean±S.D.)

Groups / Parameters	Control	C1	C2	C3	C4	P-Value	LSD
Platelets 10^9/L	606.86±277.46	859.71±126.34	814.00±211.80	667.00±200.57	692.71±310.63	0.3	255.79
PCT ml/L	5.09±1.08	7.49±1.02 a	5.16±1.21 b	5.48±1.77 b	5.17±2.62 b	0.05*	1.8
MPV fL	7.94±1.27	8.27±0.59	7.40±0.55 b	7.39±0.41 b	6.70±0.39 ab	0.004**	0.79

[1] $P \leq 0.05$

[2] Lowercase letters indicate significance as: a to control, b to C1

TABLE 3. Correlation matrix of blood parameters

Parameters	RBC	HB	HCT	Platelets	MCV	MCH	MCHC	MPV	PCT
RBC	1	**0.941****	**0.824****	0.127	-0.019	-0.323	-0.096	-0.152	**-0.354***
HB		1	**0.756****	0.127	-0.045	-0.005	0.072	-0.092	**-0.375***
HCT			1	0.085	**0.477****	-0.285	**-0.522****	-0.277	**-0.553****
Platelets				1	-0.044	0.083	0.103	0.066	-0.182
MCV					1	0.008	**-0.867****	**-0.389***	**-0.452****
MCH						1	**0.448****	0.21	0.058
MCHC							1	**0.408***	**0.401***
MPV								1	0.191

[1] *$P \leq 0.05$,
[2] ** $P < 0.01$

CONCLUSION

Taken together, the results suggest distinct stages of hematological adaptation. At low exposure (C1), early adaptive responses were evident, including smaller RBC size, elevated MCHC, and increased platelet volume. By contrast, groups C2 and C3 represented a relatively stable stage with limited alterations. At the highest dose (C4), however, pronounced deviations emerged: elevated RBC count, increased MCV, but reduced MCH and MCHC, together with platelet dysfunction characterized by reduced volume and a slightly increased count. These changes may reflect either osmotic or oxidative stress leading to swollen RBCs with lower hemoglobin content, or the release of larger reticulocytes containing less hemoglobin per cell.

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