International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.801.809

Research Article
Thrombocytopenia Induced in Mice by Intraperitoneal Administration of Gold and Iron Oxide Nanoparticles

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Abstract

Background and Objective: When nanoparticles interact with blood components in the blood circulation, they can potentially affect the function of normal blood cells, leading to anemia, infection and bleeding or thrombosis. The aim of this study was to investigate the toxicity and the hematological alternations induced by intraperitoneal (IP) injection of two forms of gold and iron nanoparticles in a mouse model. **Materials and Methods:** A total of twenty male healthy adult BALB/c mice weighing about 25-30 g were divided into four groups for the study. The control group received the vehicle, while the other three groups were injected intraperitoneally with 10 mg kg^{-1} b.wt., of polyethylene glycol (PEG) Gold particles AuNPs, Au-Dye NPs and dextran coated cross-linked iron oxide (CLIO) NPs, consequently. Hematological parameters were measured utilizing standard CBC techniques. **Results:** The PEGylated (PEG) gold AuNPs, Au-Dye NPs and CLIO-NPs significantly reduced the platelets (PLT) count and PCT in mice groups. These particles have not shown any effect on the Hb level. The PEGylated AuNPs and Au-Dye NPs reduce RBC, MCV and MCH compared to CLIO-NPs, which does not affect the previous parameters. Remarkably, PEGylated AuNPs and CLIO-NPs reduce WBCs compared to Au-Dye NPs. **Conclusion:** The three nanoparticles used in this study have induced significant modifications in blood indices indicating potential organ toxicity.

Key words: Hematological alternation, thrombocytopenia, platelets, nanoparticles toxicity, magnetic CLIO nanoparticle

Citation: Almarshad, H., A. Elderdery, F. Alenazy, K. Shalaby, I. Hamad and O. Hendawy, 2023. Thrombocytopenia Induced in mice by intraperitoneal administration of gold and iron oxide nanoparticles. Int. J. Pharmacol., 19: 801-809.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heavy metals have been shown to cause damage to cells and their components including nucleus, cell membranes, mitochondria and lysosome. It has become obvious that metal ions interact with proteins and DNA, resulting in DNA damage and cell alterations[1].

Nanotechnology research has been exponentially developed with numerous applications in various areas, like molecular imaging, drug delivery, engineering technology, improvement of materials and clinical gadgets for diagnosis and treatment[2]. Moreover, the biological effects of nanoparticles are highly variable depending on their electronic, magnetic, optical and mechanical properties and their size, composition, shape and surface chemistry[2-4]. The interaction of nanoparticles with different cells and tissues and the subsequent metabolic and immunological responses elicited are not entirely understood. Therefore, it is necessary to investigate their potential systemic toxic effects.

The most significant component of nanotoxicity, both *in vitro* and *in vivo*, is recognized as oxidative stress induced by formation of free radical[5]. Size of nanomaterials affects their cellular uptake, reaction, distribution and ease of removal from the human body. Nanomaterials of smaller size have an exponential increase in surface area relative to volume, which would thereby increase their reactivity and intracellular routing[6] and this has indeed been reported. Moreover, those organs with a high blood supply, such as kidneys and lungs, have shown greater accumulation of injected nanoparticles[7]. The liver and the spleen are among the most exposed organs to nanoparticles, mainly because of the predominance of phagocytic cells in their reticuloendothelial framework[8].

Gold nanoparticles (Au-Dye NPs) have a wide range of applications in diagnostic and therapeutic medical fields due to their small size, high surface area-to-volume ratio and high biocompatibility[9]. Significantly, Au-Dye NPs efficiently penetrate biological barriers and cell membranes[10]. Also, Au-Dye NPs have different shapes of one, two or three dimensions that provide multiple usage varieties[11].

They are widely used in cancer cell imaging, drug delivery photothermal and biosensors due to their powerful and size-adaptive surface plasmon resonance, fluorescence and accessible surface functionalization[12,13]. Recently, because of their high photoelectric absorption, gold nanoparticles have been used as a new radiosensitizer for radiation therapy[14]. The Au-Dye NPs can accumulate in cellular vesicles and induce cell necrosis or increase the synthesis of proapoptotic proteins leading to programmed cell death[15]. However, the cytotoxicity of Au-Dye NPs relies upon a few parameters such as size, tissue distribution, penetration capacity, tissue absorption and cell types[16]. The diameter of Au-Dye NPs influenced the cellular uptake in HELA cells; maximum cellular uptake occurs at 50 nm and there is a gradual decrease in internalization from 70 to 100 nm[17]. Moreover, numerous reports highlighted an enhancement in the cellular uptake of gold nanoparticles utilizing biopolymer coatings[18].

On the other hand, Iron Oxide Nanoparticles (IONPs) are also used comprehensively in the areas of drug delivery, diagnostic image, drug targeting, cell sorting and isolation, magnetic resonance imaging, cell separating molecules, hyperthermia and reinforcement for some composites[19-21]. The presence of both Fe (II) and Fe (III) in their structure provides unique physicochemical characteristics to the Superparamagnetic Iron Oxide Nanoparticle (SPIONs), which makes them useful in a wide range of applications such as chlorinated solvents and metals treatment, groundwater and soil and remediation etc.[22].

Magnetic iron oxide nanoparticles (MNPs) have exponential ability to selectively attach to specific receptors on target cells or tissue due to their functionalization with specific biocompatible targeting molecules also their magnetic coating enables them to be used as contrast agents for magnetic resonance imaging (MRI) and magnetic hyperthermia, so that, MNPs showed combined therapeutic and diagnostic properties, so-called theragnostic[23]. These dual properties enable MNPs to have a higher permeability rate and retention effect (EPR effect)[24].

Pretorius *et al.*[25] and Kelley *et al.*[26] reported that H63D mutation causes elevation of serum ferritin level, leading to hereditary hemochromatosis, which in turn causes alteration in RBC morphology. High levels of iron become deposited in the tissue of most vital organs, causing deterioration in its function and may end with end-stage organ failure. This high level also leads to thrombotic diseases, resulting from unliganded iron and in turn elevation in hydroxyl radicals, forming fibrin networks with hyperserotonemia and/or haemochromatosis and thus alterations in RBCs morphology[27].

A study by Huang *et al.*[28] reported that iron overload may damage organs in mice, by lowering tight junction proteins, elevating D-LA (D-lactate) levels and upregulating some pro-inflammatory cytokines while downregulating some anti-inflammatory cytokines. It also causes fibrosis[29] and atherosclerosis[30] besides damage to other organs, for example, liver[30,31] and pancreatic gland[29]. The advantages of the IP (Intraperitoneal Injection) method are documented in previous studies, causing it to have low systematic toxicity in vital organs and increasing drug penetration in peritoneal nodules. It also is characterized by prolonged retention of the

drug within the circulatory system[32,33]. For this reason, this changes in this study.

The objective of the present study was to investigate the effects of Au-Dye NPs, PEGylated AuNPs and CLIO-NPs in blood components; to evaluate the impact of Au-Dye NPs on blood cell count and the morphological changes in RBCs before their clinical application through intraperitoneal injections of two types of gold nanoparticles, i.e. Methoxy Polyethylene Glycol gold nanoparticles (PEGylated AuNPs), Fluorescently Labeled gold nanoparticles (Au-Dye NPs) and Magnetic CLIO Nanoparticle (CLIO-NPs).

MATERIALS AND METHODS

Study area: The experiments were conducted at the Labs of Clinical Laboratory Science (CLS) Department, College of Applied Medical Sciences, Al-Jouf University, Sakaka, Saudi Arabia for two months duration of time (February 2022 to April 2022).

Three nanoparticles were used in this study; Methoxy Polyethylene Glycol gold nanoparticles (PEGylated AuNPs), Fluorescently Labeled gold nanoparticles (Au-Dye NPs) and Magnetic CLIO Nanoparticle (CLIO-NPs) from Luna Nanotech® (Toronto, Ontario, M5G 1Y8).

Physicochemical properties of nanoparticles: The characterizations of gold particles, AuNPs methoxy PEG (45nm) and AuNPs Fluorescently Labeled Dye (45 nm size) that were used in this study (Table 1), while the characterizations of iron CLIO NPs were delineated in Table 2.

Animal groups: Twenty male healthy adult BALB/c mice, obtained from the Experimental Surgery and Animal Laboratory (ESAL), King Saud University weighing about 25-30 g were used for the experimental study and divided randomly into four groups. The animals were handled according to guidelines of The National Committee of Bioethics at King Abdul Aziz City for Science and Technology (NCBE-KACST) and Jouf University Bioethics Committee guidelines (Ethical Approval no. 10-20-3/40 27-3-1440 H), which comply with the Declaration of Helsinki/international laws for use and care of laboratory animals and according to the approval of the Department of Clinical Laboratory Science (CLS), College of Applied Medical Sciences. Animals were kept in standard polypropylene cages at controlled room temperature (26°C) and on a 12 hrs light-dark cycle. Food and water were given *ad libitum*. All animals were examined daily for any clinical symptoms or mortality. The animals were divided randomly into four main groups and each group consisted of four mice:

Group 1: Mice received a standard diet and were injected intraperitoneally 0.5 mL of distilled water (D.W.) with the vehicle (20 mL kg^{-1})

Group 2: Mice were injected intraperitoneally with Methoxy Polyethylene Glycol gold nanoparticles (PEGylated AuNPs) at 10 mg kg^{-1} b.wt., with an equivalent volume of vehicle (D.W.)[34]

Group 3: Mice were injected intraperitoneally with Fluorescently Labeled gold nanoparticles (Au-Dye NPs) at 10 mg kg^{-1} b.wt., with an equivalent volume of vehicle (D.W.)[34]

Group 4: Mice were injected intraperitoneally with CLIO-NPs Nanoparticle at 10 mg kg^{-1} b.wt., with an equivalent volume of vehicle (D.W.)[35]

Hematological analysis: After 48 hrs of injection of the vehicle or test nanoparticles, mice in all four groups were anesthetized with chloroform. About 0.5 mL of blood was collected from the medial epicanthus of the animal's eyes in tubes containing tubes EDTA. A complete blood count (CBC) was performed on an automated blood cell analyzer (MINDRY-BC3200, Nanshan, Shenzhen, China), hemoglobin concentration test was validated on the HemoCue Hb system (HemoCue Corporate, Kuvettgatan, Ängelholm, Sweden). The OLYMPUS digital microscope (Olympus BX microscope and DP72 camera, Melville, Florida, USA) used manual slide counting for validation and blood smear morphology assessment. Blood samples of all groups were prepared as blood films and stained by the GIEMSA method. Hematological Analyzer was used to determine different hematological parameters, such as

Table 1: Shows characteristics of gold particles, methoxy PEG AuNPs/fluorescently labelled AuNPs

Concentration (M)	Molar extinction (M^{-1} cm^{-1})	OD	Peak SPR wavelength (nm)	Size dispersity (+/-) nm	Diameter (nm)
4.07E-10	1.23E+10	50	530	5.8	45
Particles per mL (#)	Weight concentration (mg mL^{-1})	MW (g mol^{-1})	Atoms/particle	Particle volume (nm^3)	Surface area (nm^2)
2.45 E+12	2.26	5.57E+8	2.83E+6	4.77E+4	6.36E+3

OD: Optical density and MW: Molecular weight

Table 2: Shows characteristics of iron particle-CLIO NPs

Iron % (by weight)	Concentration (mg mL^{-1})	Blocking temperature, Tb (K)	PDI	Hydrodynamic diameter (nm)	Iron oxide core diameter nm	NPs
15-25%	5	35 (±5)	0.25 (±0.5)	50-110	8-10	CLIO

Red Blood Cells (RBC), White Blood Cells (WBCs), Hemoglobin (HB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red blood cell Distribution Width (RDW), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW)%, plateletcrit (PCT)% and Platelets (PLTs)[36].

The mice were euthanized at the end of the experiment and then blood samples and kidney tissue specimens were collected. The relative organ weights of BALB/c mice in grams were approximately 1.09, 0.34, 0.22, 1.09, 3.21 and 0.07 for the kidney, spleen, thymus, liver and bladder respectively. The former constitutes the theme of this work[37]. About 10% neutral buffered formalin was used to fix the tissue samples. Thin tissue-paraffin slices of the kidneys were prepared and H&E stain was applied.

Statistical analysis: Statistical analysis of CBC was using the GraphPad© tool to compare the control group versus treatment, a $p<0.05$ was considered statistically significant.

Ethical approval: All experimental procedures were in compliance with the National Committee of Bioethics at King Abdul Aziz City for Science and Technology guidelines and approved by Jouf University Local Bioethics Committee guidelines under Ethical Approval no. 10-20-3/40 27-3-1440 H.

RESULTS

Particles characterization: The mean size of PEGylated AuNPs, Au-Dye NPs and CLIO-NPs was 78.98, 94.28 and 88.65 nm, respectively. The polydispersity index (PDI) was 0.153, 0.040 and 0.368 for PEGylated AuNPs, Au-Dye NPs and CLIO-NPs, respectively. The PDI is an indicator of the aggregation/agglomeration of particles. When the PDI value is closer to zero, it indicates the monodispersity conduct of the system. Therefore, AuNPs are considered to be the most stable nanoparticles (Table 1 and 2).

Hematological parameters: Results of the hematological parameters in the four groups showed significant variations in the CBC and blood films. Thrombocytopenia was observed in subjects after IP injection with AuNPs and CLIO NP nanoparticles.

Blood count: PEGylated AuNPs and CLIO-NPs induced a statistically significant decrease in WBC count (4.925±0.39 and 4.05±0.39, respectively) compared to the control group (7.950±0.53) while the Au-Dye NPs injected mice have not shown any significant change in WBCs count (Table 3).

Both the PEGylated AuNPs and Au-Dye NPs led to a slight non-significant decrease in RBC count and a non-significant reduction in hemoglobin concentration compared to normal mice. Similarly, CLIO-NPs have not shown any effect on both parameters. Concerning this, all the three nanoparticles, i.e., PEGylated AuNPs, Au-Dye NPs and CLIO-NPs have no significant effect on the HGB levels (Table 3).

This study concluded that injection of PEGylated AuNPs and Au-Dye NPs caused a significant decrease in the Mean Corpuscular Volume (MCV) of RBCs (43.175±3.76 and 42.50±1.51, respectively). The CLIO-NPs have not shown any effect in MCV compared to the control group (52.150±1.03). Both the PEGylated AuNPs and Au-Dye NPs have shown a statistically significant increase in Mean Corpuscular Hemoglobin (MCH) (22.575±1.87 and 21.30±1.12, respectively) and Mean Corpuscular Hemoglobin Concentration (MCHC) (48.525±1.11 and 50.35±1.14, respectively), while CLIO-NPs have not shown any effect in both parameters when compared to the control group (17.4±0.53 for MCH and 30.725±0.88 for MCHC) (Table 3).

Additionally, the Red Blood Cells Distribution Width Coefficient Variation (RDW-CV), which indicates width variation between red blood cells, was significantly increased in mice of both PEGylated AuNPs and Au-Dye NPs groups (14.125±0.79 and 14.50±0.36, respectively), while Red Blood Cells Standard Deviation (RDW-SD) showed a significant decrease in both the treatment groups (21.200±2.08 and 20.15±1.13, respectively). In contrast, the CLIO-NPs injected group has shown a more significant increase in both parameters (17.3±0.88 and 25±3.74, respectively) in comparison to the control group, which showed 12.850±0.58 and 24.150±0.9 for RDW-CV and RDW-SD, respectively (Table 3).

All three nanoparticles, i.e. PEGylated AuNPs, Au-Dye NPs and CLIO-NPs have led to statistically significant suppression of platelets count (PLTs) (458.50±41.24, 340±19.69 and 352±18.55, respectively) compared with the normal control group (828.50±114.64).

The values of Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) were significantly changed due to the injection of any of the three nanoparticles in comparison to the control group (5.700±0.22 and 14.950±0.47 for MPV and PDW, respectively).

Plateletcrit (PCT) % was significantly reduced in all the treatment groups. In contrast to the control group (0.4400±0.02), the PEGylated AuNPs, Au-Dye NPs and CLIO-NPs have shown 0.2275±0.02, 0.22±0.02 and 0.175±0.04,

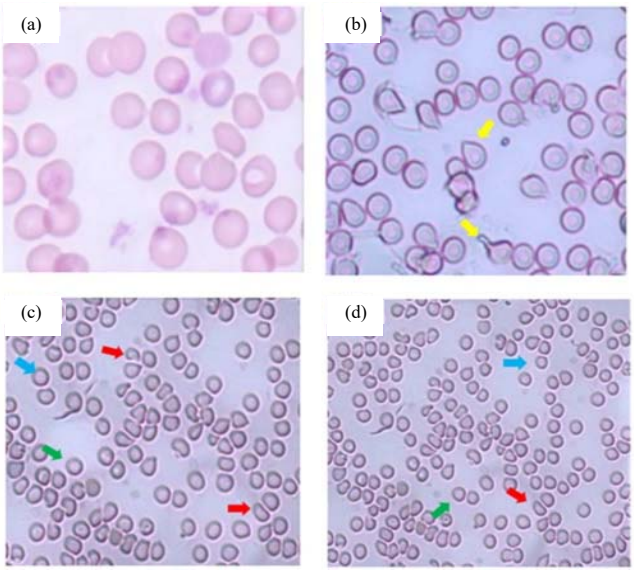

Fig. 1(a-d): Blood cells of mice injected with PEGylated AuNPs, Au-Dye NPs and CLIO-NPs, (a) Control specimen showing normal cells, normocytic and normochromic RBCs (i.e. indicating normal iron stores), (b) Blood smear, yellow arrows demonstrate tear drops and (c-d) Showing abnormal cell morphology, green arrows indicate microcytic cells, blue arrows indicate anisocytosis (variation in RBCs size) and red arrows indicate poikilocytosis (variation in RBCs shapes), using GIEMSA stain

Table 3: Effect of intraperitoneal injection of PEGylated AuNPs, Au-Dye NPs and CLIO-NPs nanoparticles on mice hematological parameters

Parameter	PEGylated AuNPs	Au-Dye NPs	CLIO-NPs	Control	Reference range*
WBCs 10^9 L^{-1}	4.925±0.39*	8.90±0.58	4.05±0.39*	7.950±0.53	3.4-9.8 (6.0±1.8)
RBCs 10^{12} L^{-1}	6.085±0.17*	5.90±0.22*	7.325±0.58	7.313±0.53	7.2-10.3 (8.4±0.9)
HGB g dL^{-1}	12.300±0.40	12.23±0.58*	12.925±0.25	13.650±0.56	13.9±1.0 (11.5-15.6)
MCV fL	43.175±3.76*	42.50±1.51*	50.13±1.88*	52.150±1.03	49.0-53.0 (51.0±1.3)
MCH pg	22.575±1.87*	21.30±1.12*	17.55±0.85	17.4±0.53	13.6-18.6 (16.8±1.4)
MCHC g dL^{-1}	48.525±1.11*	50.35±1.14*	37.3±2.32*	30.725±0.88	30.4-36.4 (33.1±1.8)
RDW-CV %	14.125±0.79*	14.50±0.36	17.3±0.88*	12.850±0.58	
RDW-SD fL	21.200±2.08*	20.15±1.13*	25±3.74	24.150±0.9	
PLT 10^9 L^{-1}	458.50±41.24*	340.0±19.69*	352.0±18.55*	828.50±114.64	899.4±99.1(635.0-1118.0)
MPV fL	5.025±0.25*	6.35±0.35*	6.1±0.54	5.700±0.22	
PDW	13.725±0.22*	14.925±0.66	13.025±2.54	14.950±0.47	
PCT %	0.2275±0.02*	0.22±0.02*	0.175±0.04*	0.4400±0.02	

Values indicate mean±SD, Statistics: Independent t-test and *p>0.05

respectively (Table 3). Figure 1 shows alterations in the blood morphology, tear drops; microcytic RBCs, anisopoikilocytosis (variation in RBCs, size and shape), using GIEMSA stain.

DISCUSSION

Current findings show that the injection of iron nanoparticles (CLIO-NPs) induce thrombocytopenia that causes bleeding disorders. This was in agreement of Pretorius E and co-workers, high level of iron affects function of vital organ and may end with thrombotic diseases[27], which is the main cause platelets consumption. Other studies also reported that iron overload causes liver damage[30,31], which is the main organ for thrombopoietin synthesis and therefore, thrombocytopenia occurs. The current results reveal that gold particles (PEGylated AuNPs and Au-Dye NPs) can also affect platelet formation, causing thrombocytopenia. This was in agreement with other studies, which reported that PEGylated AuNPs causes liver toxicity and dyshematopoiesis.

The physiochemical properties of nanoparticles play an important role in their interaction with plasma proteins, cellular uptake and toxicological effects. For example, it has been reported that IONPs with a size larger than 100 nm could be trapped rapidly in the spleen and liver through macrophage phagocytosis. In contrast, IONPs smaller than 10 nm in diameter could be cleared through kidneys[38].

Superparamagnetic IONPs smaller than 50 nm are believed to benefit from the slower opsonization and clearance from the reticuloendothelial system[39]. From here, comes the distinctiveness of our study, in which the size of nanoparticles used was in the range of 70 and 100 d.nm.

PEGylated AuNPs and CLIO-NPs injection induced a significant decrease in WBC count, which could be due to the size resemblance of NPs to small proteins or even viruses and the subsequent excitation of the immune system inducing immunotoxicity[40]. Neutrophils, lymphocytes, monocytes, eosinophils and basophils play diverse functions in body protection. Therefore, the numbers of each of these cell types provide an important clue about the situation of our immune system. A significant increase or decrease in the cell numbers may indicate a defect in normal body conditions such as allergy, infection, specific reaction to any drugs or chemicals and many other conditions. In this contest, Au-Dye NPs appear to induce a much less toxic effect as no significant decrease in WBC was monitored in Au-Dye NP-injected mice.

The PEGylated AuNPs, Au-Dye NPs and CLIO-NPs led to a slight non-significant decrease in RBC count. Zhang *et al.*[41] reported that PEGylated AuNPs significantly increased alanine transaminase and aspartate transaminase enzymes, indicating liver toxicity. The small particle size and wide distribution of Au-Dye NPs in dominant organs like the liver, the main organ responsible for erythropoietin hormone secretion, probably affect erythropoietin secretion leading to defective hemopoiesis[42].

Figure 1 shows alterations in blood morphology. The RBCs are derived from hematopoietic cells in the bone marrow; the RBC count and shape variation might be due AuNP induced inflammatory response and alternation of immune system activity[43]. Most of the reported toxicological effects induced by gold nanoparticle injections are either blood clotting or the destruction of red blood cells and the release of hemoglobin in the bloodstream. Mineral injections are known to cause hemolysis and changes in cell physiology. The study also concluded that the presence of abnormal RBC morphology included moderate to marked anisocytosis (different sizes), poikilocytosis (different shapes), rouleaux formation, polychromasia (presence of immature red cells) due to hemolysis, BM-hyperplasia and a variable number of microcytes (small RBCs). The decrease in the total WBC count (leukopenia) can be a sign of an injury, improper cell replenishment, or leukopoiesis due to foreign materials[44].

A meager RBCs count (anemia) indicates an increase in the RBCs destruction or lyses, dietary iron deficiency, folic acid, or vitamin B12. In addition to some chronic diseases, it may also indicate a low number of RBCs produced by the bone marrow, Zhang *et al.*[45] concluded the same about the RBC count and showed that the oral and intraperitoneal routes of administration displayed increased toxicity. The decrease in HGB levels is due to RBC structural deficits or probably due to hemolysis leading to decreased oxygen-carrying capacity, hypoxia and anemia[46]. Reports showed that GNPs induce an inflammatory reaction, trigger an increase or decrease in the immune system activity and change the related hematologic factors such as blood cell counts[46,47].

The PEGylated AuNPs and Au-Dye NPs caused a significant decrease in MCV. The MCV was relative to the size of RBCs. The decrease in size of RBCs and changes in morphology were reflected in the slight increase in Red Cell Distribution Width (RDW) % by PEGylated AuNPs and Au-Dye NPs and more increase in RDW induced by CLIO-NPs.

The three nanoparticles, i.e., PEGylated AuNPs, Au-Dye NPs and CLIO-NPs have led to statistically significant suppression of platelet count (PLTs). The PLTs play an essential role in blood coagulation, where the platelets aggregate to form a sticky plug to stop the bleeding.

Depression in MPV, PDW and PCT% indicates disturbed platelet production from the bone marrow[46]. The significant decrease in MPV indicates the disturbance induced in bone marrow manufacturing.

Smear blood from the control (Fig. 1) demonstrates normochromic normocytic, reflecting normal iron stores, but with other smears showing striking anisopoikilocytosis, with microcytes and teardrop cells. Some spherocytes are visible in the background. Such changes in the RBCs may be explainable by increased unliganded or free iron in mice subjected to intraperitoneal iron infusion, which would increase the chance of thrombotic events. Such changes were observed in subjects with hereditary hemochromatosis, hyperferritinemia and possible renal damage[25,27,48].

Previous studies have indicated that gold nanoparticles have the capacity to penetrate the nuclear membrane and damage DNA[49]. Another study has shown that GNPs cause proapoptotic induction (increase, Fas, caspase 3 and caspase 9 expressions) and increased ROS generation in the tissues of important organs[50].

The current research findings suggest that the altered hematological parameters upon the administration of the gold and iron nanoparticles need to be considered in public health settings. Therefore, it is necessary to understand the extent and nature of interactions between nanoparticles, blood components and other tissues, especially in situations with a high prevalence of impact of nanoparticles on tissue degeneration and cytotoxicity. In this context, we intend to incorporate anti-inflammatory and antioxidant natural

products to protect and reduce the toxicity and side effects of nanoparticles and benefit from nanoparticles' properties in drug delivery and therapy. Further studies regarding the effects of PEGylated AuNPs, Au-Dye NPs and CLIO-NPs on differential leukocyte count must be done, because of the modifications in blood indices and possible alteration in renal tissue as a result of AuNPs and CLIO nanoparticles.

CONCLUSION

The present study investigated the effect and possible blood cell alterations and toxicity associated with intraperitoneal injections of gold and iron nanoparticles in an animal model. In comparison to the control group, alterations in cell count and other hematological parameters indicate compromised hemopoiesis and immune system homeostasis in response to gold and iron nanoparticles administration. It is suggested that the interaction between nanoparticles and animal cells needs to be considered in further and specific research settings to ensure the safe application of the advancements in the field of medical nanotechnology. This study also suggests that additional *in vitro* experiments are needed using particle localization by Transmission Electron Microscopy (TEM) and FIB-SEM techniques.

SIGNIFICANCE STATEMENT

Gold and iron nanoparticles have a wide range of useful applications in pharmacological and biomedical sciences. However, they can affect the vital organs, leading to nanotoxicology because of using an unappropriated size, dosage and injection route. Our research aimed to investigate the impact of intraperitoneal (IP) injection of two forms of gold and iron nanoparticles on liver toxicity and in turn hematological parameters among a mouse model. This study reveals that intraperitoneal administration of gold and iron oxide nanoparticles induces thrombocytopenia, causing bleeding disorders. This is because these particles can affect the liver as the main organ producing thrombopoietin, the most important factor in thrombopoiesis process. Thus, the interaction between nanoparticles and animal cells needs to be considered in further and specific research settings to ensure the safe application.

ACKNOWLEDGMENTS

The authors acknowledge the financial support received from the Deanship of Scientific Research at Jouf University (827). The authors extend their appreciation to The Deputyship for Research Innovation, Ministry of Education in Saudi Arabia for supporting this research work through project number 375213500. We would also like to thank the Department of Clinical Laboratory Science at Jouf University for its support in conducting this research study.

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