Research Article

Advances in Hematology and Oncology Research

Ameliorating Effect of Zinc Oxide Nanoparticles against Hematotoxicity Induced by Cyclophosphamide in Male Albino Rats

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Abstract

Background: *Cyclophosphamide (CP) is a drug with a wide spectrum of clinical uses. Zinc oxide is the most widely used nanoparticles. Nanoparticles could induce oxidative stress that eventually leads to cell toxicity, inflammation and hemolysis.*

Objectives: *The objective of this study was to evaluate the hematological changes induced by Zn-O nano-particles and/or Cyclophosphamide in male rats.*

Materials and Methods: *Twenty four adult male rats (Sprague Dawley) were grouped randomly into four groups of six rats each. Group I. Control group: Received 0.2 ml saline /day i.p. injection for 14 days (day by day), group II (CP group): Received CP 20 mg/kg/day body weight (b.w.) day by day for 14 days by intraperitoneal injection, Group III (nZnO group): Received nZnO (5 mg/kg)/day b.w., intraperitoneally for 14 days. Group IV (CP + ZnO NPs group): Received nZnO group: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days, plus CP 20 mg/kg/day body weight (b.w.) day by day for 14 days by intraperitoneal injection. At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken for hematological evaluation.*

Results: *Red blood cells count, hemoglobin concentration, and white blood cells count were significantly decline in rats treated with CP in comparison to control group, while combination of nZnO with CP reduced changes in red bood cells and hemoglobin values. Neutrophils, lymphocytes, eosinophils, and monocytes count were significantly decreased in CP-immunosuppressed group when compared with the control group. In CP-immunosuppressed animals treated with nZnO, these parameters were improved when compared with CP treated groups.*

Conclusion: *It can be concluded that CP induced changes in the hematological parameters. Treatment of rats with zinc oxide nano-particles and CP together ameliorated the toxicity induced by CP. These results may provide further visions into proper treatment of patients by improving side effects of chemotheraby. However further studies are necessary to establish optimal doses of nZnO and receive the best safety profile.*

Keywords: Zinc Oxide Nanoparticles, Cyclophosphamide, Hematotoxicity, Hematological changes, RBCs, WBCs, White blood cells differential count

Introduction

Cyclophosphamide (CP) is a drug with a wide spectrum of clinical uses. Its effectiveness in the treatment of cancer (lymphoma, acute and chronic leukemias, multiple myeloma) and non-malignant diseases such as rheumatoid arthritis and vasculitis has been well established [1]. However, this drug also induces acute inflammation of the urinary bladder, renal damage and liver damage [2-5]. CP use is limited due to myelotoxicity, and its marked organ toxicity associated with increased oxidative stress and inflammation [6, 7]. CP is a prodrug with its two active metabolites, phosphoramide mustard (PM) and acrolein. PM is an anticancer metabolite whereas acrolein is a toxic metabolite, responsible for hematological and myelotoxicity [6, 8].

Biosafety issues of manufactured nanomaterials have recently attracted significant public attention and research interest. Zinc oxide (ZnO) nanoparticles (NPs) are mass-produced nanomaterials and have been widely used in the industrial production of sunscreen, antibacterial reagents, rubber additives and pigments [9]. ZnO NPs have also found applications in the production of solar cells, photocatalysts, transparent conductive films and ultraviolet photodetectors [10-13]. Previous studies have demonstrated that ZnO NPs are toxic to microorganisms, cells, plants, aquatic biota and rodents [14-19]. The red blood cells (RBCs) are susceptible to oxidative stress damage. Nanoparticles could induce oxidative stress that eventually leads to cell toxicity and hemolysis of RBCs at certain doses [20, 21]. Smaller nanosized particles caused granulocyte activation and hemolysis and induced inflammation and hemolysis in human blood samples [21-23]. Zinc oxide is the most widely used nanoparticles among various nanomaterials. Recently, these nanoparticles have been shown to specifically kill cancerous cells;

therefore, it is believed that these nanoparticles may be used as an alternative antitumor agent. However, it is also known that these nanoparticles pose several deleterious effects to living beings [24]. Many previous studies have suggested that the high solubility of ZnO NPs (i.e. the ability to release a large number of free zinc ions) might play an important role in the cytotoxicity. ZnO NPs are one of the most abundantly used nanomaterials in consumer products and biomedical applications. As a result, human exposure to these NPs is highly frequent and they have become an issue of concern to public health. Although toxicity of ZnO NPs has been extensively studied and they have been shown to affect many different cell types and animal systems, there is a significant lack of toxicological data for ZnO NPs.

Objectives

The objective of this study was to evaluate the hematological changes induced by Zn-O nano-particles and/or Cyclophosphamide in male rats.

Materials and Methods

Experiment was carried out at Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University laboratories

Chemicals

All reagents were of the highest quality. Zinc oxide as nanoparticles with an average size 67 nm is a gift from Dr. Eman El-Trass. Cyclophosphamide (200 mg/ampoule) was purchased from Sigma-Aldrich.

Animals

24 male rats with average body weight of 150±30 g were obtained from faculty of agriculture, Alexandria University, and acclimatized for two weeks before starting the experiment. The rats were maintained under temperature (25°C). They were fed with standard food and had free access to water. Animals were randomly divided into 4 groups and housed in Universal galvanized wire cages.

Experimental design

Adult male rats (Sprague Dawley) were randomly divided into four groups (six rats each) as follows:

Group I. Control group: Received 0.2 ml/day saline i.p. injection for 14 days (day by day).

Group II. CP group: Received CP 20 mg/kg/day body weight (b.w.) (i.p. injection of saline) day by day for 14 days by intraperitoneal injection [25].

Group III. nZnO group: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days [26].

Group IV. CP + nZnO group: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days, plus CP 20 mg/kg/day b.w., day by day for 14 days by intraperitoneal injection.

After 24-hr from last treatments all the animals were anesthetized using light ether. Blood samples were taken from the heart within 1 min. Tubes was used to compile blood drawn from the heart directly; 1 ml were collected on sodium heparin for hematological studies.

Hematological Mesurements

One milliliters of blood collected into heparinized sample bottles were analyzed for hematological parameters; was used for complete blood picture analysis by Coulter (Hemat 8 analyser; SEAC).

Statistical Analysis

The values are expressed as mean±SE. The results were computed statistically using statistical Package for Social Sciences (SPSS software package, version 25) using one- way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparison on using the LSD. $P<0.05$ was considered as significant [27].

Results and Discussion

Effects of zinc oxide nanoparticles, cyclophosphamide and their combination on blood RBCs count, hemoglobin concentration, WBCs count

In the present experimental study, it has been observed that red blood cell counts, hemoglobin and white blood cells count were significantly decline in rats treated with CP in comparison to control group (Tables 1, 2 and Figures 1-3). Our results may suggest that CP suppresses bone marrow ability to produce new ones, resulting in lowering of blood cells counts which results in decrease of Hb percentage in the blood. In connection to this, cyclophosphamide altered liver and kidney functions by modulating liver enzymes [28, 29]. However, the activity of this enzyme is not limited only to the liver as it is also present in the brain, muscle and red blood cells [30]. The cyclophosamide-induced changes in the level of blood cells in this study were similar to those observed in humans during treatment with this drug. The most common abnormalities found in humans are leukopenia and anemia. Similar results were obtained in rats after a single intraperitoneal dose of CP (50 mg/kg): Significant decreases in WBC count and Hb concentration were noted [31]. Chemotherapy-induced anemia is one of the most common side effects experienced by cancer patients, occurring in approximately 70-90% of those undergoing treatment for the disease [32].

The response to stress is characterized by hormone change (catecholamine and corticosteroid) that induced alteration on the haematological parameter. Increase on the volume of the plasma, loss of water in the RBCs and haemolysis of RBCs in the blood stream. It has already been proved that the organs where the Zn levels are of prime importance, the deficiency caused disturbance in the redox balance and oxidative stress leading to the cellular damages [33]. Initial experiment on Zn provides the evidence that at appropriate concentration Zn can protect CP-induced damage in the urinary bladder and blood of rat [34]. It has also been suggested that further experiments can explore the underlying mechanisms of Zn protection against CP-induced toxicity. Pharmacological modulation of metalothianin was used as one of the strategies to overcome the toxic issues of several drugs, especially with anticancer agents [35, 36]. Previous studies have reported that pharmacological increase in metallothionein levels during anticancer drug regime protects the normal cells from the toxic insults of the drug [35].

Table 1: Effects of treatment of rats with zinc oxide nanoparticles or cyclophosphamide, and their mixture on RBCs count and hemoglobin concentration

Groups	Groups			
	Control	nZnO	CP	nZnO + CP
Parameters	Mean±SE	Mean± SE	Mean± SE	Mean± SE
RBCs Count (x10^6/µl)	7.57 ± 0.22 ^{bcd}	6.62 ± 0.18 ^{acd}	4.82 ± 0.10 ^{abd}	5.96 ± 0.22 ^{abc}
Hb (g/dl)	14.42 ± 0.66 ^{bcd}	13.44 ± 0.33 ^{acd}	8.94 ± 0.63 ^{abd}	11.68 ± 0.716 ^{abc}

Significance at P > 0.05. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; d Comparison of nZnO + CP and other groups.

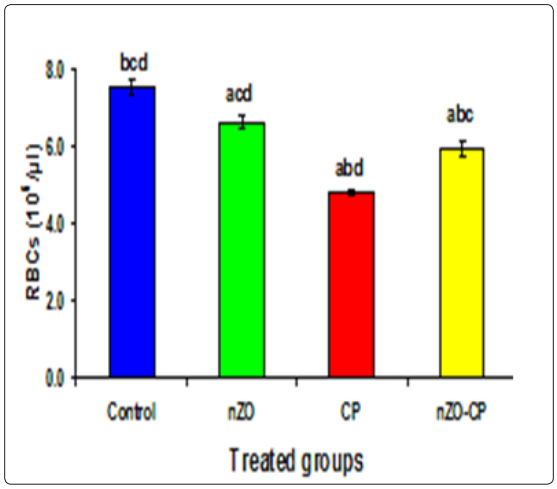

Figure 1: Effect of treatment of rat with zinc oxide nanoparticles (nZnO) and/or cyclophosphamide (CP) on RBCs count (x10^6/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

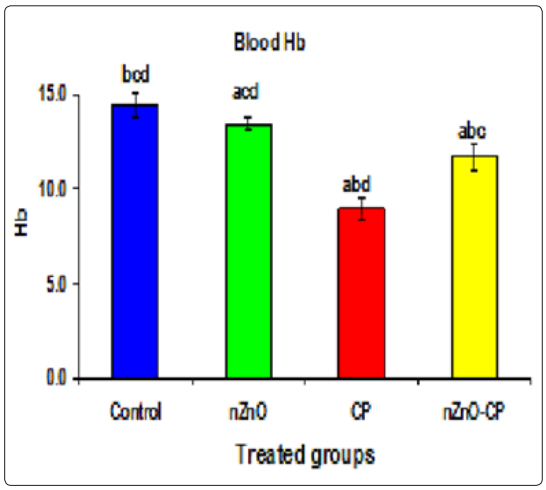

Figure 2: Effect of treatment of rat with zinc oxide nanoparticles (ZnONPs) and/or cyclophosphamide (CP) on Haemoglobin Concentration (Hb, g/dl). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

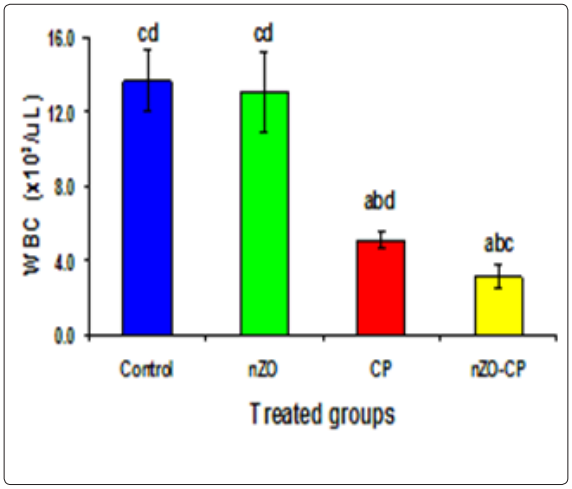

Figure 3: Effect of treatment of rat with zinc oxide nanoparticles (nZnO) and/or cyclophosphamide (CP) on RBCs count (x106/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

Effects of Zinc Oxide Nanoparticles, Cyclophosphamide and Their Combination on Blood Neutrophils, Lymphocytes, Eosinophils, and Monocytes Count

History has shown that CP is one of the most potent immunosuppressive drugs. In the present study, we found that CP induced leukopenia, neutropenia and lymphocytopenia in rats, these results parallel to the result of winkelstein who demonstrated that CP is an effective inhibitor of cell mediated immune response and leads to a depletion of lymphocytes in the peripheral blood and tissue [37].

In the present work, regarding to the result of neutrophils, lymphocytes, eosinophils and monocyte were significantly decreased in CP - immunosuppressed group when compared with the control group. In CP- immunosuppressed animals treated with nZnO, these parameters were improved when compared with CP treated groups (Tables 2 and Figures 4-7).

Chemotherapy-induced anemia is one of the most common side effects experienced by cancer patients, occurring in approximately 70-90 % of those undergoing treatment for the disease [32]. This is because chemotherapeutic drugs kill rapidly dividing cells in the body, including cancer cells and normal cells which include red blood cells, at the same time suppress bone marrow ability to produce new ones, resulting in decrease of blood Hb level.

The greater efficacy shown by the pretreatment group might be the result of the compound providing some added protection to the target cells before exposure to the chemotherapeutic agent. From this observation, it is possible to conclude that ZnO NPs its chemoprotective effect against CP-induced cellular toxicity in part by scavenging the free radicals generated by CP reactive metabolites. Because CP does not act through oxidative stress mechanism, it is expected that the Zn would be beneficial to the host but without reducing the efficiency of the treatment.

Table 2: Effects of treatment of rats with zinc oxide nanoparticles or cyclophosphamide, and their mixture on total WBCs, neutrophils, lymphocytes, eosinophils, and monocytes count

Groups	Groups			
	Control	nZnO	CP	nZnO + CP
Parameters	Mean±SE	Mean±SE	Mean±SE	Mean±SE
WBCs Count (x10^3/µl)	13.64 ± 1.65 ^{cd}	13.04 ± 2.20 ^{cd}	5.10 ± 0.50 ^{abd}	3.10 ± 0.66 ^{abc}
Neutrophils Count (x10^3/µl)	2.90 ± 0.22 ^{cd}	3.08 ± 0.18 ^{cd}	1.40 ± 0.15 ^{abd}	2.13 ± 0.19 ^{abc}
Lymphocytes Count (x10^3/µl)	5.14 ± 0.866 ^{bcd}	6.34 ± 0.20^{acd}	1.62 ± 0.15 ^{abd}	3.62 ± 0.31 ^{abc}
Eosinophils Count (x10^3/µl)	0.844 ± 0.03 ^{bcd}	1.04 ± 0.05a ^{cd}	0.67 ± 0.04 ^{abd}	0.73 ± 0.04 ^{abc}

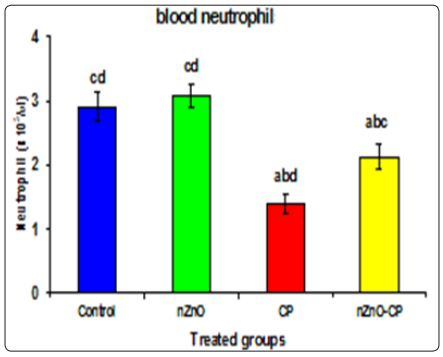

Figure 4: Effect of treatment of rat with zinc oxide nanoparticles (nZnO) and/or cyclophosphamide (CP) on neutrophils count (x10^6/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

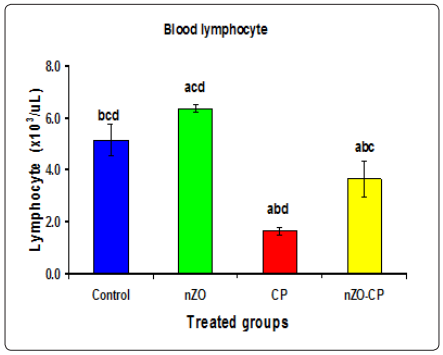

Figure 5: Effect of treatment of rat with zinc oxide nanoparticles (ZnONPs) and/or cyclophosphamide (CP) on lymphocytes count (x10^6/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups

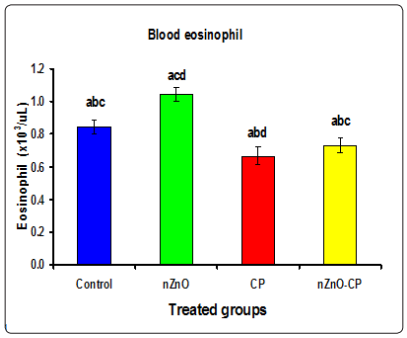

Figure 6: Effect of treatment of rat with zinc oxide nanoparticles (nZnO) and/or cyclophosphamide (CP) on eosinophils count (x10^6/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

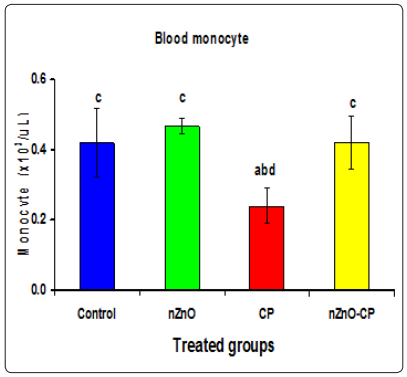

Figure 7: Effect of treatment of rat with zinc oxide nanoparticles (ZnONPs) and/or cyclophosphamide (CP) on monocytes count (x10^6/µL). Significance at $P > 0.05$. [a]Comparison of control and other groups; [b]Comparison of nZnO and other groups; [c]Comparison of CP and other groups; [d]Comparison of nZnO + CP and other groups.

Conclusion

It can be concluded that CP induced changes in the hematological parameters. Treatment of rats with zinc oxide nano-particles and CP together ameliorated the toxicity induced by CP. These results may provide further visions into proper treatment of patients by improving side effects of chemotheraby. However further studies are necessary to establish optimal doses of nZnO and receive the best safety profile.

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