

Research Article

# Zinc oxide nanoparticles attenuate the oxidative damage and disturbance in antioxidant defense system induced by cyclophosphamide in male albino rats

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## Abstract

**Background:** Cyclophosphamide is used for the treatment of malignant and non-malignant diseases, but, it induces oxidative damage and disturbance in the antioxidant defense system. Zinc oxide nanoparticles (ZnO NPs) are used in biomedical applications and consumer products. ZnO-NPs are protected cell membranes against oxidative damage, decrease free radicals and malondialdehyde (MDA) levels, and increase the antioxidant enzyme levels.

**Objectives:** The present aimed to evaluate the ameliorative effect of Zn-O nano-particles on oxidative damage and disturbance in the antioxidant defense system induced by cyclophosphamide in male albino rats.

**Materials and methods:** 24 adult male albino rats were randomly divided into 4 groups (6 rats of each). Group I (Control group): Received 0.2 ml saline /day i.p. injection for 14 days (day by day), group II, (nZnO group): Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days, Group III (CP group): Received CP (20 mg/kg/day) b.w, day by day for 14 days by intraperitoneal injection, 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) b.w., day by day for 14 days by intraperitoneal injection. After 24-hr from the last treatment, all animals were anesthetized using light ether. Blood, lungs, and liver samples were taken and prepared for biochemical measurements.

**Results:** Individual treatment of zinc oxide nanoparticles and CP induced liver cytochrome b5, cytochrome C reductase, and glutathione S-transferase (GST) compared to the control group, while CP increased P450. The combination of nZnO and CP prevents the elevation of cytochrome b5, P450, cytochrome C reductase, and GST compared with the CP treated group. Zinc oxide nanoparticles and CP increased liver thiobarbituric acid reactive substances (TBARS). The combination of nZnO and CP prevents the changes in TBARS concentrations compared with the CP. Injection of CP to rats reduced the activities of serum glutathione reductase (GR) and catalase (CAT) as compared with the control group. However, combination treatment of rats with nZnO and CP increased the activities of these enzymes compared with those treated with CP alone. Zinc oxide nanoparticles and CP increased serum and lung TBARS, while decreased glutathione (GSH) concentration compared to the control group, with more pronounced changes by CP. The combination of nZnO and CP prevents the changes in TBARS and GSH concentrations compared with the CP.

**Conclusion:** It can be concluded that CP induced oxidative stress and disturbance in the antioxidant defense system. Treatment of rats with zinc oxide nano-particles and CP together attenuated the oxidative damage and disturbance in the antioxidant defense system induced by CP. So, Patients treated with CP advised to take nZnO to prevent the side effects of chemotherapy. Further studies are necessary to evaluate the amelioration effect nZnO and other nano-particles against oxidative stress induced by CP in different doses and experimental models.

## More Information

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Keywords: Cyclophosphamide; Zinc oxide nanoparticles; Attenuation; Ameliorative effect; Oxidative damage; Antioxidant defense system



## Introduction

Cyclophosphamide is used for the treatment of multiple myeloma, leukemia, lymphoma, vasculitis, and rheumatoid arthritis has been well-established [1]. Clinical use of CP is limited because of its toxicity associated with increased inflammations and oxidative stress [2]. CP is metabolized by the Cytochrome P-450 pathway. The toxicity of CP is required for bioactivation by hepatic microsomal cytochrome P-450 mixed functional oxidase yields, 4-hydroxycyclophosphamide which is existed in equilibrium with aldophosphamide, and is degraded by  $\beta$ -elimination to form an equimolar amount of byproduct acrolein and phosphoramidate mustard [3,4]. Oxidative stress has linked to the cause of many diseases (chronic kidney disease, coronary artery disease, cerebrovascular disease, amyotrophic lateral sclerosis, heart failure, and neurodegenerative diseases [5-7]. It arises from an imbalance between reactive nitrogen species, insufficient antioxidant defense, and excessive generation of reactive oxygen species (ROS). It can lead to the progression of atherosclerosis by oxidative damage to cellular components. Reactive nitrogen species and excessive generation of reactive oxygen species are unstable and participate in the degradation of the cell membrane and deoxyribonucleic acid.

Schacter, et al. [8] found that the elevation of reactive oxygen species in sickle cells reduced the activity of superoxide dismutase (SOD). Also, Scott, et al. [9] concluded that H<sub>2</sub>O<sub>2</sub> mediates cellular damage through .O<sub>2</sub>-generating systems and lead to a reduction in superoxide dismutase activity. Oxidative stress and inflammation are lead to tissue damage [10]. So, there is a need to reduce oxidative stress for the treatment of diseases. ZnO is widely used metal oxides in several numerous products like coatings, paints, and cosmetics products like sunscreens thanks to their antioxidant, anti-inflammatory, antimicrobial, and UV ray protecting properties [11-13]. ZnONPs are used in biomedical applications and consumer products. Human exposure to ZnONPs is a highly frequent and they have become an issue of concern to public health.

The protective effect of ZnONPs on oxidative stress-induced diabetes in male rats was studied by Hussein, et al. [14] who found that a significant increase in the serum concentrations of MDA and nitric oxide in the diabetic rats, and MDA and nitric oxide were significantly reduced post-treatment with ZnONPs. Also, Khalaf, et al. [13] recorded that ZnONPs cream was protected rats against oxidative damage and skin allergic dermatitis induced by lead oxide which may be due to the antioxidant properties and anti-inflammatory of Zinc oxide nanoparticles [13,15], and Zinc concentration was increased from the dissociation of Zinc oxide nanoparticles. Zinc is the core constituent of antioxidant enzymes (as superoxide dismutase) and it is a protector for sulfhydryl groups and impair lipid peroxidation by displacing transition metals (as copper and iron) from catalytic sites [13,16]. El-Maddawy and Abd El Naby, [17] reported that rats

treated male with doxorubicin showed significant decreases in catalase and glutathione activities and an increase in the testicular malondialdehyde concentration, which indicator of the increase in oxidative damage and lipid peroxidation caused by reactive oxygen species. Co-administration of zinc oxide nanoparticles (3 mg/kg b.w) plus doxorubicin induced a significant increased catalase and glutathione activities and significantly decrease in the testicular malondialdehyde concentration as compared with the doxorubicin-treated group. Zinc oxide nanoparticles possess antioxidant activity concerning mechanisms of both free radical scavenging and reducing activities [18]. Also, zinc oxide nanoparticles are protected cell membranes against oxidative damage, decrease free radicals and malondialdehyde (MDA) levels, and increase the antioxidant enzyme levels [13,17,19].

## Objective

The present aimed to evaluate the ameliorative effect of Zn-O nano-particles on oxidative damage and disturbance in the antioxidant defense system induced by cyclophosphamide in male albino rats.

## Material and methods

### Chemicals

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

### Animals

24 male rats [Sprague Dawley, BW (150 ± 30 g)] were obtained from the faculty of agriculture, Alexandria University, and acclimatized for 2 weeks. Animals were maintained under temperature (25°C), fed with standard food, and had free access to water. Animals were divided into 4 groups and housed in galvanized wire cages. All animal procedures were performed in accordance with the Ethics Committee of Alexandria University and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 2007).

### Experimental design

Adult male rats were divided into 4 groups (6 rats of each) as follows:

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

**Group II. (nZnO group):** Received zinc oxide nanoparticles (nZnO) (5 mg/kg/day) b.w., intraperitoneally for 14 days [20].

**Group III. 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 [21].

**Group IV. CP + nZnO group:** Received nZnO (5 mg/kg/



day) b.w., i.p. injection for 14 days, plus CP (20 mg/kg/day) b.w., day by day for 14 days by intraperitoneal injection.

After 24-hr from the last treatments all the animals were anesthetized using light ether. Blood samples were taken from the heart within 1 min. 3 ml of blood was collected in glass tubes for coagulation and serum formation, blood was allowed to sit for 30 minutes at 40°C to clot, then centrifuged for 5 minutes at 1000 x g. The serum samples were stored into capped sterile polyethylene tubes at -20°C until used (within 24 hours). The abdominal cavity of each rat was opened where the liver and lung were excised.

### Biochemical analysis

Reduced glutathione was determined according to Moron, et al. [22]. Glutathione reductase was determined spectrophotometrically according to the method of Goldberg and Spooner [23]. Catalase was determined according to Goth [24]. TBARS are expressed in terms of malondialdehyde (MDA) equivalents using the molar absorptivity of 149000 M<sup>-1</sup> cm<sup>-1</sup> [25]. After animals were fasted for 24 hours of the end of the experiment, they were sacrificed. The abdominal cavity opened and lungs and liver were removed, washed with cold phosphate buffer (0.1 M, pH 7.4), weighed, and chilled on ice. A 33% crude homogenate (W/V) was prepared in phosphate buffer (0.1 M & pH 7.4) by homogenization with a Teflon pestle, using 5 strokes. The homogenate was centrifuged at 11,000 x g (4 °C) for 20 minutes to remove the nuclei, mitochondria, and intact cells. The supernatant solution was subsequently centrifuged at 105,000 x g (4 °C) for 60 min to sediment the microsomal pellet. The pellet was re-suspended in phosphate buffer (0.1 M, pH 7.4), and kept in an ice bath and used as the enzyme source. Liver microsomal cytochrome b5 and P450 were estimated according to Omura and Sato [26]. The activity of liver microsomal reduced nicotinamide adenine dinucleotide phosphate - cytochrome-C reductase was assayed according to the method of Williams and Kamin [27]. GST activity was estimated according to Habig, et al. [28].

### Statistical analysis

The data are expressed as mean ± SE. The results were computed statistically using the 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 [29].

## Results and discussion

### Effect of zinc oxide nanoparticles, cyclophosphamide and their combination rat liver microsomal b5, P450 and cytochrome C-reductase

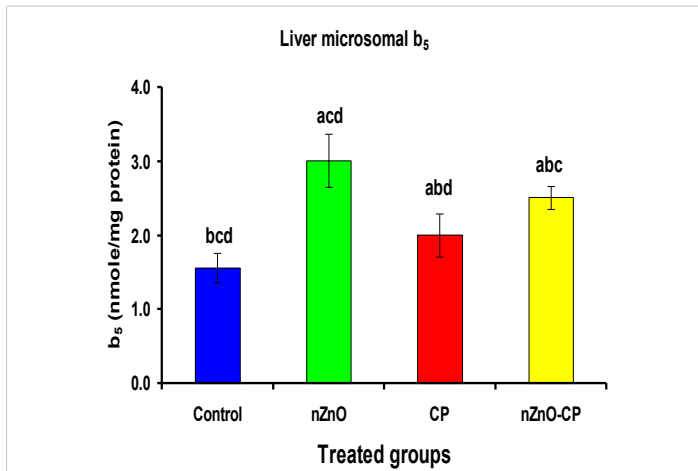
Monoxygenases are hepatic drug-metabolizing enzymes, which contain including phase I enzymes (cytochrome b5, cytochrome p450, and reduced nicotinamide adenine dinucleotide phosphate-cytochrome C reductase) which metabolize most xenobiotics and carcinogens and in phase II, GST, GSH and TBARS [30]. Individual treatment of zinc oxide nanoparticles and CP induced cytochrome b5, cytochrome C reductase, and GST compared to the control group, while CP increased P450. The combination of nZnO and CP prevents the elevation of cytochrome P450, b5, GST, and cytochrome C reductase as compared with the CP treated group (Tables 1 and Figures 1-4). Cytochrome P450 enzyme system is necessary for the bio-activation of insecticides [31,32]. Oxidative stress due to ZnNPs and CP may be ascribed to induction of Cytochrome P450, inhibition of AChE, and disturbance in contents of GSH causing lipid peroxidation [33,34].

Reduced nicotinamide adenine dinucleotide phosphate-cytochrome C reductase activity is a component of the microsomal monoxygenase which important in the metabolism of drugs, lipids, and other foreign compounds [35]. The limiting step in the detoxification and activation of toxic substances is dependent on the rate of reduction of cytochrome P450 substrate complex, which in turn is dependant on the activation and turnover rates of reduced nicotinamide adenine dinucleotide cytochrome C-reductase, cytochrome P450 and cytochrome b5 [36]. The changes produced by nanoparticles and chemotherapy treatments to reduced nicotinamide adenine dinucleotide cytochrome C-reductase activity is one of the defense mechanisms to increase the rate of reduction of cytochrome P450 substrate complex [37]. In the present study, the treatment of rats with nZnO and CP revealed a significant liver damage as observed from the elevation of hepatospecific enzyme activities. These findings demonstrate that in vivo sub-acute administration of nZnO with CP antagonize the effects of CP and modulates the activities of drug-metabolizing enzymes in rat liver, suggesting that reactive oxygen species may be involved in the toxic effects of the CP through modulation of cytochrome P450 and b5.

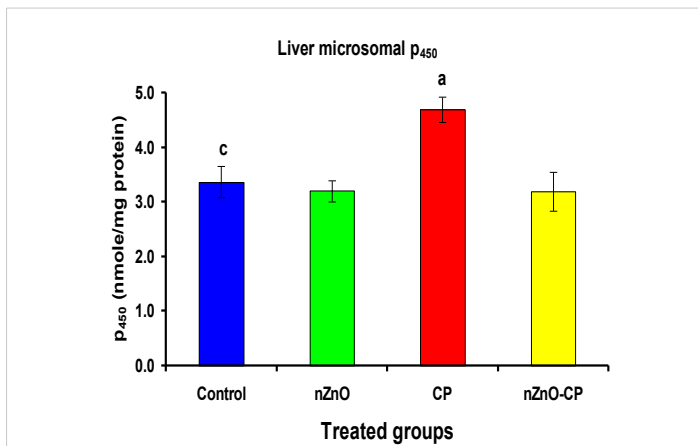
**Table 1:** Effects of treatment of rats with zinc oxide and/or cyclophosphamide on liver microsomal b<sub>5</sub>, P<sub>450</sub> and cytochrome C-reductase.

Groups	Control	nZnO	CP	nZnO + CP
Parameters	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Liver microsomal b <sub>5</sub> (nmole/mg protein)	1.56 ± 0.20 <sup>bcd</sup>	3.01 ± 0.36 <sup>acd</sup>	2.00 ± 0.29 <sup>abd</sup>	2.51 ± 0.15 <sup>abc</sup>
Liver microsomal P <sub>450</sub> (nmole/mg protein)	3.37 ± 0.29 <sup>c</sup>	3.20 ± 0.19	4.69 ± 0.23 <sup>a</sup>	3.19 ± 0.36
Liver microsomal cytochrome C-reductase (nmole cytochrome C-reductase/mg protein/min)	1.71 ± 0.13 <sup>bcd</sup>	2.55 ± 0.12 <sup>acd</sup>	3.41 ± 0.24 <sup>abd</sup>	2.02 ± 0.17 <sup>abc</sup>
Liver GST (U/mg protein)	25.79 ± 0.9 <sup>bcd</sup>	33.85 ± 1.03 <sup>acd</sup>	46.33 ± 1.69 <sup>abd</sup>	21.30 ± 1.3 <sup>abc</sup>

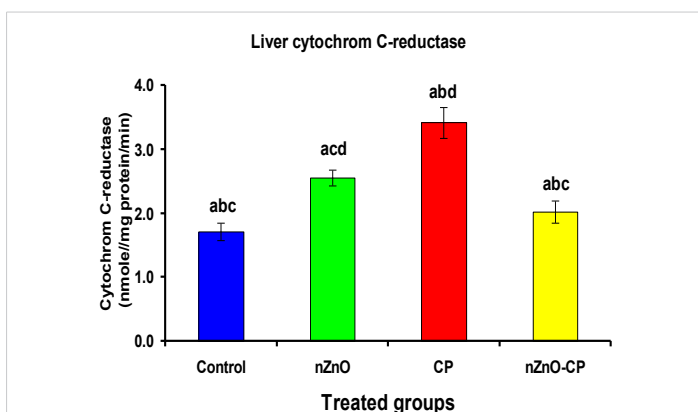
Significance at *p* > 0.05; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). a Comparison of control and other groups, b Comparison of nZnO and other groups, c Comparison of CP and other groups, d Comparison of nZnO + CP and other groups, Means having the same letters are not significantly different.



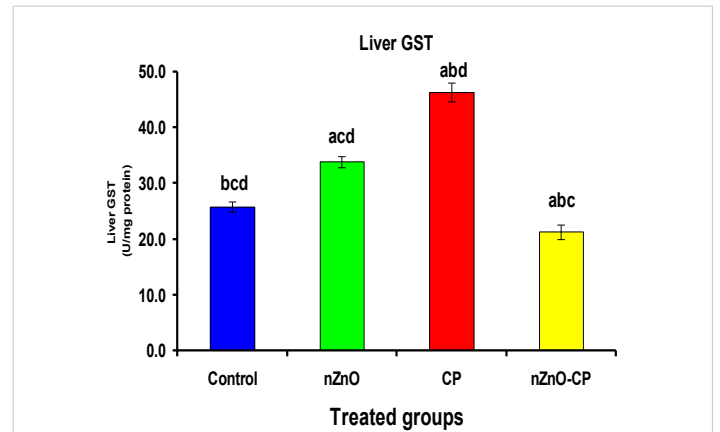
**Figure 1:** Liver microsomal b<sub>5</sub> (nmole/mg protein) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). a Comparison of control and other groups, b Comparison of nZnO and other groups, c Comparison of CP and other groups, d Comparison of nZnO + CP and other groups, Means having the same letters are not significantly different.



**Figure 2:** Liver microsomal P<sub>450</sub> (nmole/mg protein) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.



**Figure 3:** Liver microsomal cytochrome C-reductase (nmole cytochrome C-reductase/mg protein/min) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.



**Figure 4:** Liver GST (U/mg protein) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.

### Effect of zinc oxide nanoparticles, cyclophosphamide and their combination rat liver homogenate thiobarbituric acid reactive substances

Previous studies recorded that the administration of CP leads to an increase in lipid peroxidation and a decrease in the activities of antioxidant enzymes in serum, lung, and liver of rats and mice [38-43]. Individual treatment of zinc oxide nanoparticles and CP increased ( $p < 0.05$ ) liver homogenate TBARS compared to the control group, while the combination of nZnO and CP prevents the elevation of TBARS compared with the CP treated group (Table 2 and Figure 5). Lipid peroxidation (LPO) is used as a biomarker to show the index of oxidative stress, and causes of cell membranes damage, increased permeability to ions, and decreased membrane potential [44]. Induction of lipid peroxidation has been reported in different tissues of experimental animals after CP administration [39,45-47]. CP and its metabolite acrolein cause inactivation of microsomal enzymes and result in increased lipid peroxidation and ROS generation [48,49]. Co-administration of nZnO with CP caused a significant decrease in thiobarbituric acid reactive substances levels, which may be due to the antagonistic effect of this combination and/or the free radical scavenging potential of nZnO.

### Effects of zinc oxide nanoparticles, cyclophosphamide and their combination on rat serum catalase and glutathione reductase

Hydroxyl radical anion, hydrogen peroxide, and superoxide are important mediators of tissue injury and DNA damage [50]. Glutathione reductase and catalase are the antioxidant enzymes that play an important role in cell defense against free radical damage. Glutathione reductase also, is an important enzyme regenerates GSH by converting GSSG. Catalase is important in the detoxification of H<sub>2</sub>O<sub>2</sub> concentrations [51]. Injection of CP to rats reduced serum GR and CAT activities compared to control. However, combination treatment of rats with nZnO and CP increased the activities of these enzymes compared with those treated with CP alone (Table 3, Figures 6,7). These data showed that the antioxidant defense

**Table 2:** Effects of treatment of rats with zinc oxide and/or cyclophosphamide on liver Liver TBARS ( $\mu\text{mole/g}$  tissue).

Groups	Control	nZnO	CP	nZnO + CP
Parameters	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Liver TBARS ( $\mu\text{mole/g}$ tissue)	1.33 $\pm$ 0.03 <sup>bcd</sup>	1.73 $\pm$ 0.11 <sup>ac</sup>	2.07 $\pm$ 0.18 <sup>abd</sup>	1.73 $\pm$ 0.11 <sup>ac</sup>

Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.

**Table 3:** Effects of treatment of rats with zinc oxide and/or cyclophosphamide on serum glutathione reductase ( $\mu\text{M/ml}$ ) and cytochrome C-reductase.

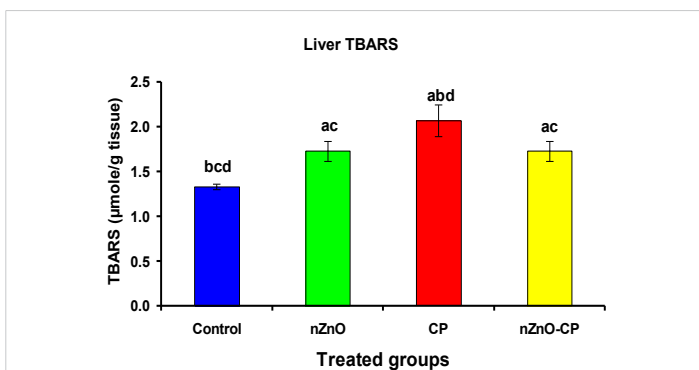
Groups	Control	nZnO	CP	nZnO + CP
Parameters	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Serum glutathione reductase ( $\mu\text{M/ml}$ )	0.31 $\pm$ 0.01 <sup>bcd</sup>	0.34 $\pm$ 0.01 <sup>acd</sup>	0.22 $\pm$ 0.02 <sup>abd</sup>	0.27 $\pm$ 0.01 <sup>abc</sup>
Serum catalase (ng/ml)	5.43 $\pm$ 0.33 <sup>bcd</sup>	4.51 $\pm$ 0.18 <sup>ac</sup>	3.38 $\pm$ 0.33 <sup>abd</sup>	4.60 $\pm$ 0.25 <sup>ac</sup>

Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.

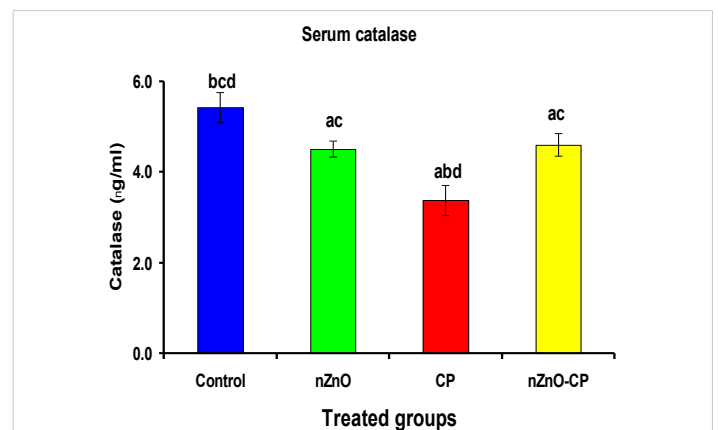
**Table 4:** Effects of treatment of rats with zinc oxide and/or cyclophosphamide on Lung TBARS (nM/g tissue) and Lung GSH (nM/g tissue).

Groups	Control	nZnO	CP	nZnO + CP
Parameters	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Lung TBARS (nM/g tissue)	304 $\pm$ 45.3 <sup>bcd</sup>	416 $\pm$ 55.9 <sup>acd</sup>	2245 $\pm$ 127 <sup>abd</sup>	1431 $\pm$ 102 <sup>abc</sup>
Lung GSH (nM/g tissue)	2.08 $\pm$ 0.01 <sup>bcd</sup>	1.27 $\pm$ 0.05 <sup>acd</sup>	1.15 $\pm$ 0.06 <sup>abd</sup>	1.55 $\pm$ 0.28 <sup>abc</sup>

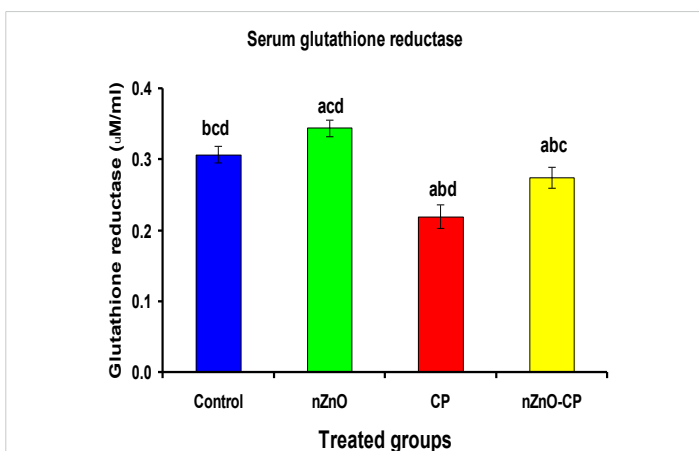
Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). a Comparison of control and other groups, b Comparison of nZnO and other groups, c Comparison of CP and other groups, d Comparison of nZnO + CP and other groups, Means having the same letters are not significantly different.



**Figure 5:** Liver TBARS ( $\mu\text{mole/g}$  tissue) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.



**Figure 7:** Serum catalase (ng/ml) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.



**Figure 6:** Serum glutathione reductase ( $\mu\text{M/ml}$ ) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.

mechanisms take part in the toxicity of cyclophosphamide [52]. Durken, et al. [53] have shown that patients receiving high-dose cyclophosphamide displayed significant reductions of antioxidant parameters in plasma. In the present study many changes in rat blood serum anti-oxidative systems also have been observed after CP administration. The CP injection leads to a decrease in the activity of CAT and GR, which may be due to protein structure modification by acrolein and/or ROS generated during CP metabolism. In the present study, a significant increase in the activities of catalase and glutathione reductase and a decrease in CP-treated rats as reported earlier [47,51], which may be due to an inactivation of cellular antioxidants by the reactive oxygen species and lipid peroxides due to CP intoxication. Co-administration of nZnO with CP decreased the formation of lipid peroxidation and restored the enzyme levels, i.e. Zinc oxide nanoparticles improved the antioxidant activity, and enhanced the activities

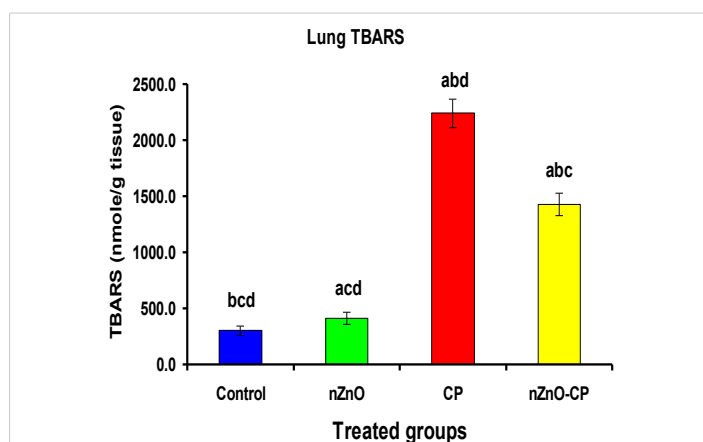
of antioxidant enzymes and decreased the levels of free radicals [54]. These improvements in the antioxidant defense system may be attributed to the antioxidant properties and/or free radical scavenging capacity of Zinc oxide nanoparticles.

### Effects of cyclophosphamide, nZnO, and their combination on rat lung thiobarbituric reactive substances and glutathione (GSH) concentration

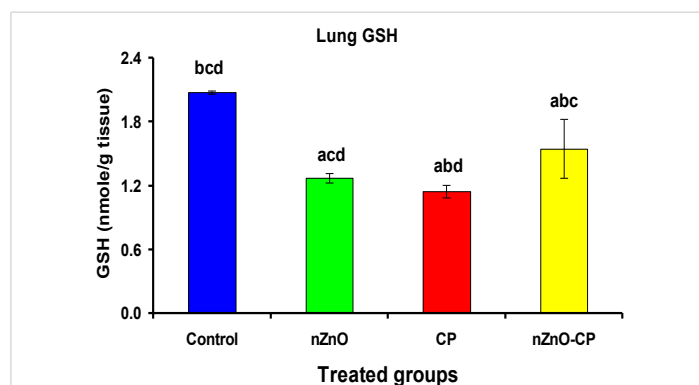
Individual treatment of zinc oxide nanoparticles and CP increased ( $p < 0.05$ ) lung homogenate TBARS compared to the control group, while the combination of nZnO and CP prevents the elevation of TBARS compared with the CP treated group (Table 4 and Figure 8). Co-administration of nZnO with CP caused a significant decrease in thiobarbituric reactive substances levels, which may be due to the antagonistic effect of this combination and/or the free radical scavenging potential of nZnO. Glutathione is an intracellular non-enzymatic antioxidant, detoxify the endogenous and exogenous toxic substances, including xenobiotics and free radicals [55]. Individual treatment of zinc oxide nanoparticles and CP decreased ( $p < 0.05$ ) lung homogenate GSH compared to the control group, while the combination of nZnO and CP prevents the reduction in TBARS compared with the CP treated group (Table 4 and Figure 9). Treatment of patients with CP caused a decrease in a highly reactive electrophiles and glutathione, which may be due to the electrophilic burden on the cells and the formation of acrolein, which leads to a decrease in glutathione content [56]. Treatment of rats with nZnO increased glutathione levels in the lung glutathione catalyzes the conjugation of glutathione with a highly reactive electrophiles, which plays a major role in the detoxification of alkylating agents [57].

## Conclusion

It can be concluded that CP induced oxidative stress and disturbance in the antioxidant defense system. Treatment



**Figure 8:** Lung TBARS (nm/g tissue) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.



**Figure 9:** Lung GSH (nm/g tissue) of rat treated with nZnO, CP, and their combination. Significance at  $p > 0.05$ ; Zinc oxide nanoparticles (nZnO); Cyclophosphamide: (CP). 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, Means having the same letters are not significantly different.

of rats with zinc oxide nano-particles and CP together attenuated the oxidative damage and disturbance in the antioxidant defense system induced by CP. So, Patients treated with CP advised to take nZnO to prevent the side effects of chemotherapy. Further studies are necessary to evaluate the amelioration effect nZnO and other nano-particles against oxidative stress induced by CP in different doses and experimental models.

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