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Investigating the Protective Role of *Cynara cardunculus* Ethanolic Extract Against Zinc Oxide Nanoparticle-Induced Toxicity

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Abstract | The swift growth of nanotechnology has resulted in the extensive application of zinc oxide nanoparticles (ZnO-NPs) across various sectors, including cosmetics, sunscreen, medical delivery, and textiles. Nonetheless, the tasks were linked to oxidative stress, blood-related issues, and organ harm. *Cynara cardunculus*, also known as cardoon is a Mediterranean herb, is high antioxidants components and contains significant levels of phenolic compounds, flavonoids, and cynarin, which demonstrate antioxidant, hepatoprotective, and anti-inflammatory effects. However, there are few studies processing the effective role of *Cynara cardunculus* ethanolic extract in reducing nanoparticle toxicity. This research aimed to inspect the protective effect of *Cynara cardunculus* (CC) ethanolic extract against zinc oxide nanoparticle (ZnO-NP) induced toxicity-stress in male mice. A total of 32 Swiss albino male mice were split into four equal groups (no =8 for each group): control, ZnO-NPs (50 mg/kg), CC alone, and a combination of ZnO-NPs + CC (200 mg/kg). Biochemical, hematological, and oxidative stress factors were estimated. Findings showed a remarkable reduction in ZnO-NP-induced toxicity in combining group due to *Cynara cardunculus* ethanolic extract, as pretended by balanced liver enzymes, promoted blood indices, and altered oxidative stress biomarkers. This research confirms the potential role of *Cynara cardunculus* as a natural antioxidant in combating nanotoxicity. In summary, *Cynara cardunculus* effectively mitigate biochemical, hematological, and oxidative stress disruptions induced by ZnO-NPs in mice. The findings support for additional investigation into its bioactive substances and possible uses in reducing nanomaterial toxicity in humans, highlighting the indications of natural antioxidants in modern toxicology.

Keywords | Cynara cardunculus, ZnO-NPs, Oxidative stress, Hematology, Biochemistry

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INTRODUCTION

The swift growth of nanotechnology has resulted increase exposition to design nanoparticles, increasing fears regarding bio compatibility. Rapid growth of this field of technology has led to the prevalent use of materials such as zinc oxide nano-particles (ZnO-NPs) across multiple industries, overall cosmetics, sunscreen, medicine delivery, and textiles, due to their UV-blocking and antimicrobial characteristics (Sirelkhatim *et al*., 2015). Though, due to their diminutive size and high reactivity, there are concerns regarding their toxicity, particularly in relation to oxidative stress- induced harm to biological existence (Sharma *et al*., 2012). ZnO-NPs generate reactive oxygen species (ROS), which can cause damage to lipids and DNA also may trigger apoptosis. Studies have shown that rodents exposed to ZnO-NPs have elevated liver enzymes (ALT and AST) and renal dysfunction (Saptarshi *et al*., 2013). Alterations

in hematological indicators such as anemia and leukemia also have been noted (Sharma *et al*., 2012). It is well known that ROS can harm superoxide dismutase (SOD) activity and upset the cellular redox balance. Malondialdehyde (MDA), a lipid peroxidation marker, rises in response to membrane deterioration (Nel *et al*., 2006). According to certain research, ZnO-NPs have the ability to penetrate the blood-brain barrier, resulting in oxidative stress, biochemical disruptions, and enzymatic activity disruption (Zhao *et al*., 2020; Kumari *et al*., 2021). According to other studies, these nanoparticles can also influence biochemical markers such as total antioxidant levels, glutathione (GSH), and (malondialdehyde (MDA). Medical plants e.g. *Cynara cardunculus*, often referred to as cardoon, they contain bioactive substances that may improve hematological and biochemical parameters and lessen oxidative stress. The phenolic compounds, flavonoids, and cynarin found in this Mediterranean plant are abundant in antioxidants and have anti-inflammatory, hepatoprotective, and antioxidant qualities (Speroni *et al*., 2003). Its activity in lowering oxidative stress and enhancing liver function has been demonstrated by studies. These effects have been documented in a number of studies. Antioxidants derived from plants have been investigated in numerous studies as a means of lowering nanoparticle toxicity. For example, research conducted by Al-Rasheed *et al*. (2022) showed that antioxidants from plants can mitigate oxidative damage caused by ZnO-NPs and enhance the levels of antioxidant enzymes.

This research examines the protective effect of Cynara cardunculus CC extract against toxicity induced by zinc oxide nanoparticles (ZnO NP) in male mice.

MATERIALS AND METHODS

Experimental animals, chemicals and extraction

Thirty-two male Swiss albino mice (20 ± 4 gr) were kept in an animal facility at a regulated temperature (22-26˚C) with a 12 h light/dark cycle and given unrestricted access to food and water for one week prior to the experiment. The Institutional Ethical Committee at the College of Veterinary Medicine, Department of Physiology at Shatrah University in Thiqar, Iraq, approved the study protocol, and all experimental procedures conformed to the Guidelines for the Care and Use of Laboratory Animals. The ZnO nanoparticles (20–30 nm according to the specification sheet) were sourced from Skyspring Nanomaterials Inc. USA, appearing as a white to light-yellow Nano powder with a purity of 99.8%, a density of 5.606 g cm3, and a spherical shape.

Extraction preparation: Dried plant material was obtained from a local market in Iraq. Dried CC leaves underwent ethanol extraction (70%, Soxhlet), were concentrated, and then lyophilized. Phytochemical analysis indicated the presence of 120 mg GAE/g polyphenols (Folin-Ciocalteu) and 45 mg QE/g flavonoids.

Experimental design

Animals: 32 male mice were randomly divided into four equal groups (n=8 per group):

- G1: Control group (no treatment, distill water 0.5 ml/animal)
- G2: ZnO-NP group (50 mg/kg ZnO NPs orally)
- G3: Cynara cardunculus extract group (received 200 mg/kg Cynara aqueous extract orally)
- G4: ZnO-NP + Cynara group (received both ZnO-NPs and Cynara extract at the same doses, orally)

At the end of the experimental treatment period, which lasted 4 weeks, the mice from each group were subjected to overnight fasting and then euthanized through cervical decapitation to gather blood samples. Plasma samples were obtained by centrifuging the blood for 15 minutes at 3000 rpm with EDTA as the anti-coagulant. Additional blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum samples. The plasma and serum samples were stored at -80˚C for future biochemical analysis and oxidative stress evaluation.

- Biochemical assays: Serum ALT, AST, and urea were measured by spectrophotometer.
- Hematological analysis: RBC, WBC and hemoglobin were assessed by using CBC-apparatus.
- Antioxidant activity: SOD (Serum superoxide dismutase), GSH (reduced glutathione), CAT (catalase levels) and MDA (malondialdehyde) were determined by the technique (HPLC high-performance liquid chromatography) as the way that explained by Karatas *et al*. (2002) and Omidi *et al*. (2016).

Statistical analysis

Data analysis was done by one-way ANOVA, followed by the Tukey post hoc test, via SPSS 26.0. The results are expressed as mean ± SD. Significance was considered at ≤ 0.05.

RESULTS

Biochemical results showed a significant ($P \leq 0.05$) ameliorative effects of *Cynara cardunculus* extract on liver enzymes in combination group -G4/ ZnO-NP + Cynara group- as compared with the same parameters in the second group (ZnO-NP) (Table 1).

Hematological results revealed a significant ($P \leq 0.05$) ameliorative effects of *Cynara cardunculus* extract on RBC,

WBC and Hemoglobin concentrations in combination group -G4/ ZnO-NP + Cynara group- as compared with those in the second group (ZnO-NP) (Table 2).

Table 1: Levels of liver enzymes in groups of male mice treated with ZnO-NPs and Cynara extract.

Parameters	G1: Control	G2: ZnO-NPs	G3: Cynara	G4: ZnO-NPs + Cynara
ALT (U/L)	35.41±2.2	78.6±4.2**	36.2±2.4	49.1 ± 3.3 *
AST (U/L)	42.8±3.2	91.3±5.5**	43.4±3.2	58.5 ± 3.9 *
Urea (mg/dl)	29.1±1.7	52.2±3.3**	29.8±1.7	38.3 ± 2.9 *

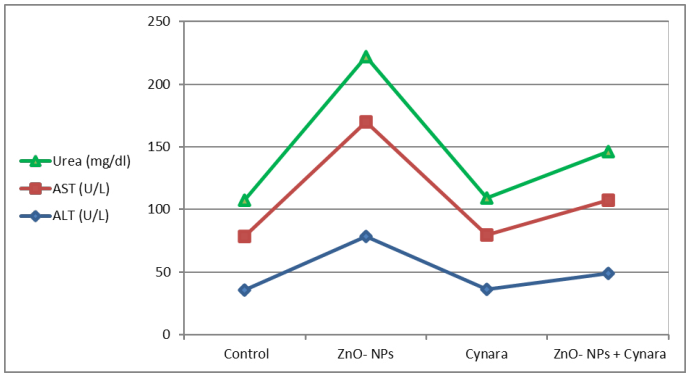

Figure 1: Levels of liver enzymes in groups of male mice treated with ZnO- NPs and Cynara extract.

Table 2: Levels of hematological parameters in groups of male mice treated with ZnO- NPs and Cynara extract.

Parameters	G1: Control	G2: ZnO-NPs	G3: Cynara	G4: ZnO-NPs + Cynara
RBC (10^6/µL)	7.2±0.2	5.6±0.2**	7.0±0.4	6.1 ± 0.3 *
WBC (10^3/µL)	8.3±0.5	6.1±0.5**	8.4±0.6	7.5 ± 0.2 *
Hemoglobin (g/dl)	14.1±0.9	10.5±0.7**	14.3±0.9	12.2 ± 0.7 *

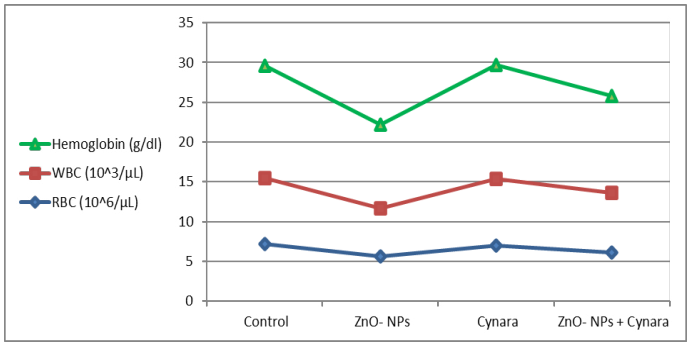

Figure 2: Levels of hematological parameters in groups of male mice treated with ZnO- NPs and Cynara extract.

Results of oxidative biomarkers showed a significant ($P \leq 0.05$) ameliorative effects of *Cynara cardunculus* extract on MDA, SOD and CAT in combination group -G4/ ZnO-NP + Cynara group- as compared with the same parameters in the second group (ZnO-NP) (Table 3).

Table 3: Levels of oxidative biomarkers in groups of male mice treated with ZnO- NPs and Cynara extract.

Parameters	G1: Control	G2: ZnO-NPs	G3: Cynara	G4:ZnO-NPs+Cynara
MDA (nmol/mg)	2.4±0.2	6.5±0.4**	2.7±0.3	4.1±0.3 *
SOD (U/mg)	35.1±2.2	18.3± 1.5**	35.9±2.1	28.5±1.9 *
CAT (U/mg)	29.3±1.7	14.2±1.3**	30.2±1.9	22.8±1.5 *
GSH (µmol/g)	12.5±1.1	5.1±0.8**	12.2±1.3	10.3±1.0 *

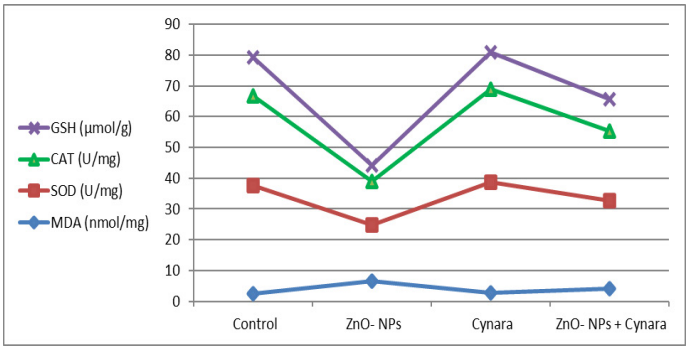

Figure 3: Levels of oxidative biomarkers in groups of male mice treated with ZnO- NPs and Cynara extract.

DISCUSSION

Few studies highlight the significant impact of Cynara cardunculus extract in reducing nanoparticle toxicity. This study addresses the gap by conjecturing its impact on biochemical, hematological, and antioxidant pathways in models exposed to ZnO-NP. Our findings show that ZnO-NPs cause notable biochemical, hematological, and oxidative stress changes in male mice. Nonetheless, the joint administration of Cynara cardunculus improved these effects, showcasing its potential as a safeguard against toxicity induced by nanoparticles.

The noted enhancements in oxidative stress markers indicate the participation of antioxidant processes. In this investigation, the normalization of ALT, AST, and urea levels by Cynara cardunculus (Figure 1) demonstrated the hepatoprotective function of this plant, with findings consistent with those of Kollia *et al.* (2016). Improving the hematological parameters of RBC, WBC, and Hb in this study (Figure 2) indicates that Cynara cardunculus reduces oxidative harm to erythrocyte membranes. The restoration of hematological parameters induced by Cynara cardunculus extract suggests safeguarding against oxidative stress in bone marrow, essential for the production of blood cells.

Findings on oxidative biomarkers in the current study (Figure 3) align with several studies indicating that Cynara cardunculus has chlorogenic acid, luteolin, and inulin, which capture free radicals and boost antioxidant defenses (Lattanzio *et al.*, 2009). The phenolic compounds have demonstrated protective effects against oxidative stress

induced by various toxins by capturing ROS and enhancing the antioxidants within host cells (Subapriya *et al*., 2005). Polyphenols from Cynara cardunculus probably scavenge ROS, decreasing lipid peroxidation while boosting SOD, CAT, and GSH activity, and may also chelate zinc ions. In this framework, the significance of medicinal plants, especially concerning phenolic glycosides like flavonoids and phenolic acids present in (*Cynara cardunculus*), which display antioxidant and free radical scavenging properties alongside metal chelating abilities, has garnered interest regarding their potential as chemopreventive agents (Khalaf *et al*., 2020). These results coincide with research on other plants high in antioxidants, establishing Cynara cardunculus as a promising option for reducing nanomaterial toxicity.

CONCLUSIONS

This research highlights the effectiveness of Cynara cardunculus in mitigating biochemical, hematological, and oxidative stress disturbances caused by ZnO-NP in mice. The findings advocate for additional investigation into its bioactive components and possible uses in mitigating nanomaterial toxicity in humans, highlighting the significance of natural antioxidants in modern toxicology.

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NOVELTY STATEMENT

The novelty of this study was focus on the side effects of using zinc oxide nanoparticles.

Ethical approval statement

This work was accepted by the Medical Ethics Committee, College of Veterinary Medicine in Shatrah University in Nasiriya city in 2025. All animal studies were performed in compliance with the institutional guidelines and ethical standards. Written and oral informed consent were sought from the responsible academic quarters before the outset of the study.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The author has declared no conflict of interest.

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