RESEARCH ARTICLE
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Evaluation of Toxic Effects of MgO Nanoparticles on *Artemia salina*

ABSTRACT

Background: Nanoparticles (NPs) have a wide range of applications due to their specific physicochemical properties. For instance, MgO NPs are utilized as humidity sensors, catalysts, and reducers. They also exhibit antibacterial properties and show promise in the diagnosis and treatment of cancer. Research has indicated that metal-based NPs can be taken up by various organisms, leading to growth inhibition, severe histological and morphological changes, reduced photosynthesis capacity, oxidative stress, and even death. However, only limited studies have explored the toxicity of MgO NPs. Therefore, the aim was to investigate the toxic effect of MgO NPs on *Artemia salina (A. salina)* using different methods.

Methods: In this study, MgO NPs were characterized by ransmission electron microscopy (TEM). The acute toxicity was assessed after the treatment of MgO NPs with different concentrations (35-500 µg/mL) for different periods (24, 48, and 72 hours) on *A. salina.* Finally, the Malondialdehyde (MDA) assay and catalase assay were used to investigate the possible oxidative damage.

Results: Based on the TEM findings, the mean size of MgO NPs was determined to be 47.2 nm. The acute toxicity assessment reveals that exposure to MgO NPs leads to mortality in the treated larvae. While there was no notable mortality after a 24-hour exposure period, viability of the larvae significantly decreased at 48 and 72 hours. LC^{50} values were estimated at 210.2 and 67.06 µg/mL respectively for these time points. There were no significant alterations observed in MDA or Catalase levels following MgO NP exposure.

Conclusion: MgO NPs pose a danger to nature and aquatic life such as *A. salina* and emphasize the importance of taking necessary precautions against environmental pollution of NPs.

Keywords: *Artemia salina*, Ecotoxicity, MgO, Nanoparticle.

INTRODUCTION

The rapid advancement of nanotechnology in recent times has led to an increased presence of nano-engineered products in various aspects of our daily lives. Fields where nanoparticles are widely applied include medicine, food production, textile manufacturing, the automotive industry, physics, optics, and electronics. Furthermore, ongoing research is focused on crucial areas such as drug development, anti-cancer therapies, and medical biosensors.[1,2] Moreover, the utilization of metal-based nanoparticles is on the rise. Presently they can be found in sunscreen and cosmetic formulations (TiO2, ZnO), detergents and antibacterial products printer ink and textiles (Ag), and dyes (TiO2 and Ag).[3]

MgO nanoparticles are a type of metal-based nanoparticle characterized by a large surface area and crystalline morphological structure. They exhibit strong ionic properties and are recognized for their structural stability.[4] MgO nanoparticles are an affordable substance utilized in diverse sectors including industry, healthcare, and food products. Ongoing research shows the potential application of MgO NPs in cancer therapy such as nano-cryosurgery, hyperthermia, and as biosensors for liver cancer immunoassay. Furthermore, MgO NPs have extensive usage in humidity sensors, production of toxin adsorbents, catalysts, reducing agents, and enhancing light efficiency to enable ultraviolet emission.[5–9]

The impressive surface reactivity and ability to adsorb substances of MgO NPs

Mahmoud Abudayyak[1]
Hatice Nur İnal[2]

[1] *Department of Pharmaceutical Toxicology, İstanbul University, Faculty of Pharmacy, İstanbul, Türkiye*
[2] *Department of Pharmaceutical Toxicology, Karadeniz Technical University, Faculty of Pharmacy, Trabzon, Türkiye*

Corresponding author:
Mahmoud Abudayyak
Address: Department of Pharmaceutical Toxicology, İstanbul University, Faculty of Pharmacy, İstanbul, Türkiye
Email address: abudiak1987@yahoo.com

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have sparked interest in their potential use as adsorbents.[10] Due to the effective bactericidal properties displayed by MgO NPs in water-based settings, they are applied as antibacterial agents for cleaning fuel oil-pipeline systems, preventing sludge buildup in tanks, and safeguarding boilers from corrosion. Furthermore, the strong oil-dispersing capability of MgO NPs makes them a desirable additive for heavy fuel oil.[4]

Two primary origins of NPs in ecosystems exist: natural and anthropogenic. Naturally occurring NPs include both organic and inorganic particles derived from soil and vegetation, such as marine colloids, metal oxides and hydroxides, and imogolites. Anthropogenic sources result from human activities, whether intentional or unintentional. Examples of unintentional NP sources include internal combustion engines, electric motors, and metal fumes, while intentional sources encompass engineered NPs designed for specific purposes within sectors like pharmaceuticals and food production.[11]

Metal-based engineered nanoparticles have been identified to disperse into the atmosphere, water, and soil. This dispersion can occur through emissions resulting from waste material incineration, which then leads to precipitation in soil and water from different pathways. The primary cause of NP buildup in soil is their presence in wastewater discharged by factories or consumer goods entering the environment. For instance, varying quantities of Ag NPs are released when textile products are washed, while TiO_2 NPs are emitted from dyes.[3] Numerous research findings indicate that about 99% of TiO_2 NPs introduced into wastewater treatment are retained in the sludge phase and can be detected in the soil instead of the water environment. Furthermore, deliberate NP exposure in soil and water due to fertilizer use in agriculture has been documented by Soil and Water Remediation Technologies.[3] Beyond the human implications of NPs in natural settings, it is vital to consider their toxic effects.

One of the most preferred model organisms for ecotoxicological studies is *A. salina* (brine shrimp). *A. salina* has been accepted as one of the test species by the US Environmental Protection Agency (EPA 2002) for acute toxicity tests.[12] Features such as the short life cycle of *A. salina*, its easy reproduction and culture, its small size, and its ability to easily adapt to extreme environmental conditions are among the reasons why *A. salina* is preferred for ecotoxicology tests.[13] Many researchers have used *A. salina* as a test organism to study the toxic effect of NPs.[13–19] There are very few studies evaluating the toxic effects of MgO NPs, which are widely used in different areas, on the ecosystem. For this reason, it was planned to investigate the toxic effect of MgO NPs on *A. salina* larvae using different methods. For this purpose, physicochemical (dispersion, volume) properties were previously examined by transmission electron microscopy (TEM). Then, the potential of MgO NPs to cause larval death and oxidative damage was investigated.

MATERIAL AND METHODS

MgO NPs, Sodium chloride (NaCl), Potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$), Magnesium chloride hexahydrate ($MgCl_2$. $6H_2O$), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA) and 1,1,3,3-tetraethoxypropane (TEP) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Nanoparticle Characterization

MgO nanoparticles obtained from Sigma-Aldrich were utilized in the research. The NPs' size and distribution were assessed through TEM analysis. In this analysis, the NPs were dissolved in sterile water and subjected to ultrasonic treatment for approximately 15 minutes to minimize agglomeration/aggregation. A single drop of NP suspension was applied onto specific carbon-coated copper grids and left to evaporate. Subsequently, images capturing, NPs of various sizes on the grid surface were captured, allowing the determination of the average size and distribution of the nanoparticles from these recorded images.[20]

Preparation of Larvae

A. salina cysts used in this research were sourced from Prof. Dr. İlhan Altınok at the Department of Fisheries Technology, Institute of Science and Technology, Karadeniz Technical University. To initiate reproduction, 0.5 g *A. salina* cysts were incubated with 500 mL distilled water at a temperature ranging between 4-8 °C for 12 hours. The experiment involved creating an artificial seawater environment to support the growth and development of *A. salina*, which was achieved by combining specific quantities of NaCl, KCl, $NaHCO_3$, and $MgCl_2{\cdot}6H_2O$ in distilled water. The larvae, soaked for 12 hours, were transferred to the seawater environment that had been prepared and then incubated for 32 hours at a temperature of 28 C with continuous ventilation and lighting (1500 lux daylight). The hatched larvae were gathered using a Buchner funnel and subsequently counted before being used in further experiments.

Preparation of Nanoparticles

To ensure the exposure of larvae to NPs, an aqueous suspension of NPs was prepared by adding 1 mg of NPs to 1 mL of seawater. The mixture was vortexed for 30 seconds and then subjected to a cold ultrasonic bath for 15 minutes. Various concentrations were made using seawater and applied immediately.

Acute Toxicity Test

The larvae were evenly distributed into 24-well plates; an equal number of larvae were added to each well along with 2 mL of nanoparticle suspension. According to previous studies results, concentrations of 500 µg, 300 µg, 150 µg, 75 µg and 35 µg were utilized in this study. The unexposed group served as the negative control. Each group underwent triplicate testing, and the experiment was repeated on three separate days (n=9).

Plates were incubated in a controlled environment with temperatures maintained at 23-25°C, and 16 hours of light/ 8 hours of darkness. After 24, 48, and 72 hours of

exposure, larvae were collected for counting and evaluation. Live larvae were determined based on their movement, and mortality percentages were calculated by comparing the numbers of die larvae to those in negative control wells using formula (1);

% Lethality = 100- [(Test) / (Control)] × 100] ..... Formula (1)

Oxidative Damage

Malondialdehyde (MDA) determination

For MDA determination, larvae exposed to 35, 75, and 150 µg/mL concentrations of MgO NPs were collected, washed with cold artificial seawater, and 3 mL phosphate buffer was added to the larvae and homogenized using a homogenizer. A volume of 0.4 mL from the uniform mixture was combined with 0.4 mL of a solution containing 15% Trichloroacetic acid and mixed using a vortex mixer. The resulting mixture was then centrifuged at 2000 rpm for 15 minutes. Following this, 0.6 mL of the supernatant was transferred to a new tube, into which 0.3 mL of a solution comprising 37% Thiobarbituric acid was added and thoroughly mixed in. The mixture was incubated in a boiling water bath for 15 minutes. After cooling, the absorbance at a wavelength of 532 nm was measured using spectrophotometry against the blank. A concentration-absorbance curve was prepared using 1,1,1,3,3-tetraethoxypropane (TEP) as the standard. The amount of MDA in the samples was then calculated as nmol/g larvae using this standard curve. Each group underwent two replicates, and the experiment was repeated on three different days (n=6).

Catalase determination

For catalase determination, 10 µL of the supernatant prepared for MDA analysis was taken and mixed with 1990 µL phosphate buffer. 1 mL of 30 mM H_2O_2 solution was added to the mixture. The resulting mixture was monitored at 240 nm for 3 min and the absorbance values of 30-second intervals were recorded. The absorbances obtained were used to calculate changes in enzyme activity using the Formula (2).[21] Groups were performed in two replicates, and the experiment was repeated on three different days (n=6).

U = (2.3/ Δx) x log (A1/ A2) ..... Formula (2)

Statistical analysis

Data were expressed as means. Statistical significance of differences between the unexposed (control) and exposed groups was calculated by one-way analysis of variance (one-way ANOVA) of the Dunnett t-test using the Statistical Package for Social Sciences, version 17.0 software (SPSS Inc.; Chicago, IL, USA). *P* values less than .05 were chosen as the significance level.

RESULTS

Nanoparticle Characterization

TEM images of MgO NPs are depicted in Figure 1. The individual diameters of the particles were measured, and their average diameter was found to be 47.2 nm. As per the findings, 11.3% of the NPs had a diameter between 20-30 nm, while 23.1% fell within the range of 30-40 nm. Additionally, 41.7% ranged from 40-50 nm, with another group constituting 15.0% falling in the range of 50-60 nm; only a small percentage (9.3%) exceeded a size larger than 60 nm.

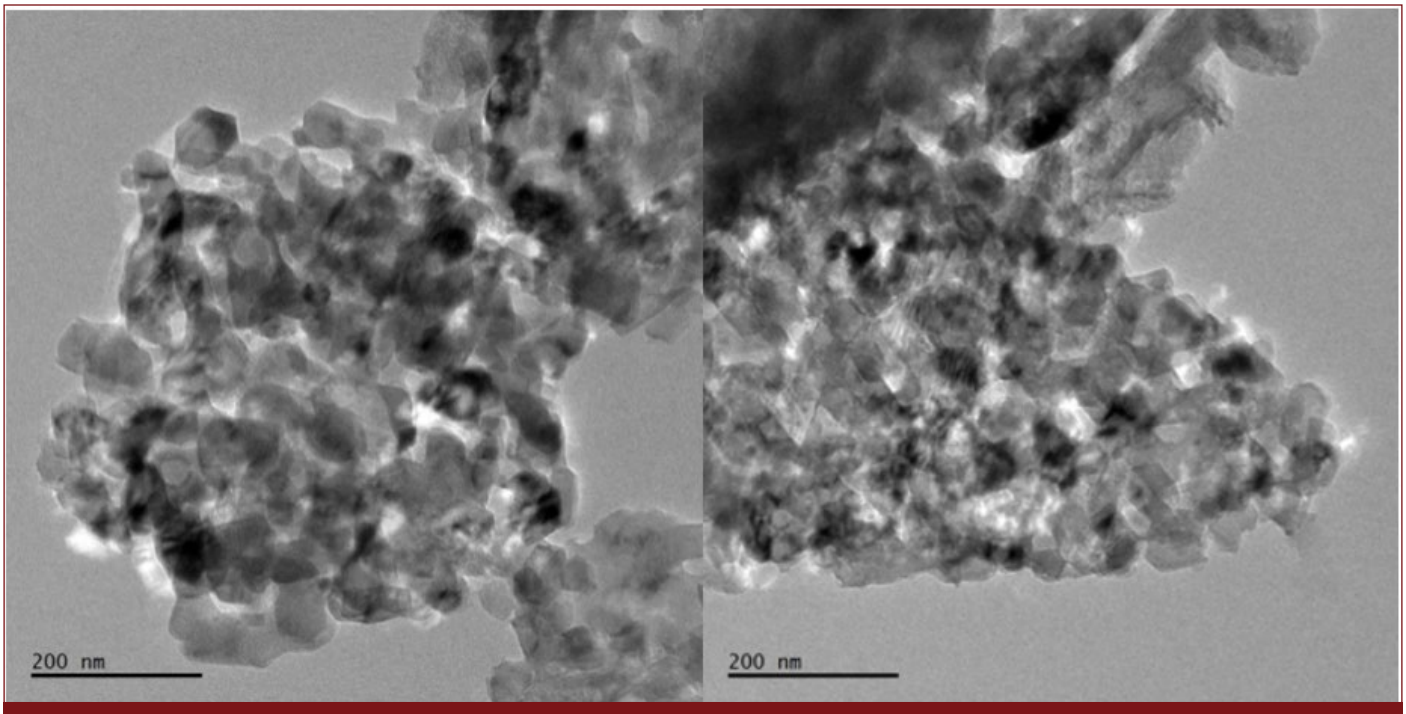

Figure 1. TEM image of MgO NPs

Acute Toxicity Test

In the experiment, we determined the toxic effect of 5 different concentrations of MgO NPs (35-500 µg/mL) on larvae by counting the number of moving larvae after 24, 48, and 72 hours of exposure. The percentage of mortality was calculated at each concentration compared to the control. (Table 1). At the end of the experiment, it was observed that the mortality rate increased proportionally with the concentration (Table 1). As the exposure time increased, the mortality rate in larvae increased, and mortality-concentration curves were generated by averaging the replicates for each exposure time (Figure 2-4). In the 24-hour exposure group, the highest mortality rate was approximately 16% with no calculated LD50 value. For the 48-hour and 72-hour exposure groups, the LD_{50} values were determined to be 210.2 µg/mL and 67.06 µg/mL, respectively.

Table 1. Mortality rates in larvae after 24, 48, 72 hours of exposure

Concentration (µg/mL)	Exposure (Hour)	500	300	150	75	35	LD_{50} (µg/mL)
% Mortality	24	16.33	16.66	10.93	4	0	-
	48	66.54	58.55	46.88	23.59	15.05	210.2
	72	83.87	79.83	70.30	54.54	34.58	67.06

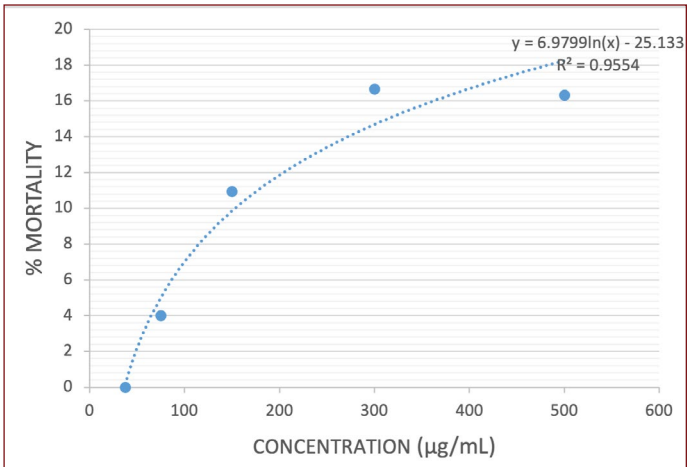

Figure 2. Mortality of larvae after 24 hours of exposure

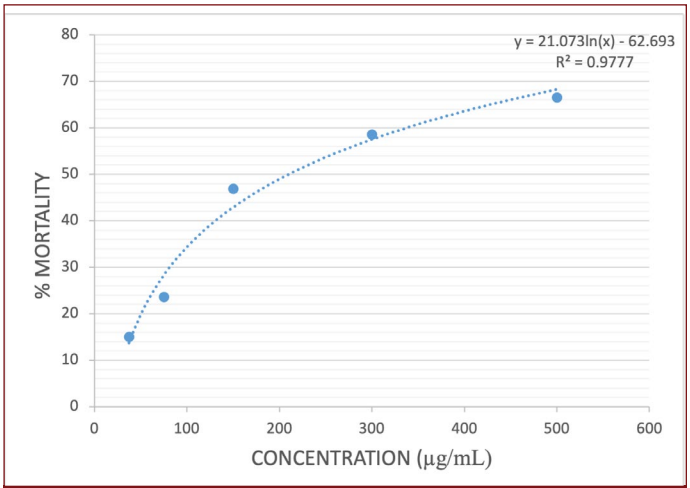

Figure 3. Mortality of larvae after 48 hours of exposure

Oxidative Damage

Malondialdehyde and catalase determination

The potential of MgO NPs to cause oxidative damage in larvae was examined at three different concentrations and negative control groups through the MDA and Catalase determination methods. The results obtained by both the MDA determination and Catalase determination methods did not reveal any statistically significant differences between the experimental and negative control groups.

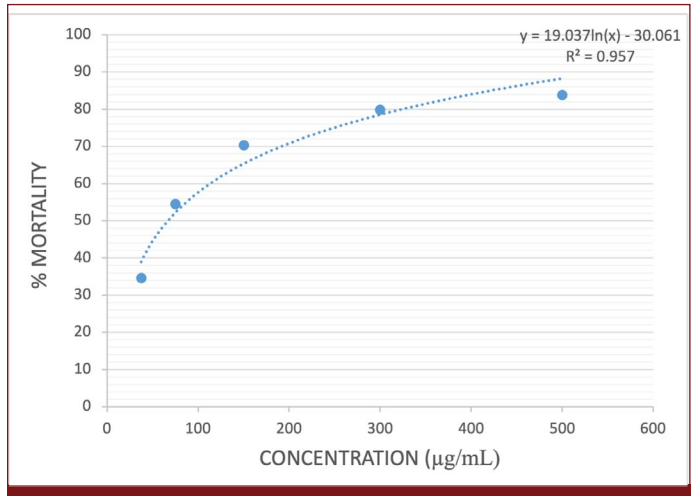

Figure 4. Mortality of larvae after 72 hours of exposure

DISCUSSION

The growing use of metal-based NPs has raised concerns about their impact on human and environmental health. To assess this toxicity, studies have been carried out on various aquatic invertebrates and fish (Table 2).[22] In this study, *A. salina* larvae were chosen for their quick reproduction, easy availability, low cost, and simple application. MgO NPs are an economical and highly effective molecule that is increasingly utilized in diverse fields including industry, medicine, and food production.[23] In addition to that, there is no requirement for ethical approval while applying this protocol and could be accepted as an alternative method compatible with to 3R principles. MgO NPs are widely used as antibacterial agents, moisture sensors, and catalysts.[6-9] MgO nanoparticles, like other nanomaterials, have the potential to be absorbed through various routes such as the skin, lungs, and digestive system. Therefore, it is crucial to comprehensively study their toxic effects on

cells and organs.[4] Despite the widespread use of MgO NPs, there is insufficient information on their impact on human and environmental health.[8, 20] The widespread use of MgO NPs suggests the need to investigate their effects on human and environmental health. In this study, an acute toxicity test was conducted on *A. salina* larvae at five different concentrations (35 µg, 75 µg, 150 µg, 300 µg, and 500 µg) over durations of 24, 48, and 72 hours. Table 1 shows that the mortality rate in the larvae increases with longer exposure times Ghobadian et al.,[4] investigated the acute toxicity of MgO NPs with a size of 20 nm on *Danio rerio* (zebrafish) at four different concentrations of 50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L. It was observed that the applied NP concentrations caused 20%, 23%, 30%, and 56% mortality on larvae, respectively. Thomas et al.[24] investigated the biochemical activities, oxidative damage potential, and bioaccumulation of MgO NPs in *Tilapia and Danio rerio* (zebrafish). According to the results obtained, MgO bulk particles were found to be more toxic than MgO NPs. While no mortality was observed in zebrafish after exposure to MgO NPs, 100% mortality was recorded as a result of 10 ppm exposure to bulk particles. According to biochemical and oxidative damage parameters, an increase in NP concentration led to increased activities of catalase, glutathione S-transferase, and superoxide dismutase.[24]

Table 2. Previous studies evaluated the ecotoxicity of MgO NPs.

Properties of MgO NPs	Animal used	Results	References
Synthesized using Cassia Oleoresin (70 nm) (5-25 µL)	*Brine shrimp nauplii*	Concentration-related death in the 15, 20 and 25 µL groups	25
CaMgO2 (24 & 48 ppm)	*Cyprinus carpio*	LC50: 212,409 mg/L.	26
(37.5 -150 g/L)	*Lyngbya majuscula* (Green algae)	The proliferation of algae and chlorophyll a and increased carotenoid levels. The effect seen depends on the concentration.	27
Synthesized with Cystoseira crinita (Brown alga) (2-10 µg/mL)	*Musca domistica* (housefly)	It caused the death of the larvae in all their stages, depending on the concentration.	28
MgO (30 – 40 nm) (93.8 – 281.3 mg/L)	Blue-green algae *Wollea salina*	The proliferation of algae and chlorophyll a and increased carotenoid levels. Morphological changes at the cellular level and an increase in lipid content	29
(1 – 4 g/L) 20 nm	Bombyx mori Silkworm	806 gene expression was altered. Vitality and quality of silk are not affected.	30
2- 10 mM	Drosophila *melanogaster*	It caused behavioral disorders.	2
25 – 400 µg/ml)	*Eisenia hortensis*	LC50: 70 µg/ml caused DNA damage.	31
Synthesized with Calotropis gigantea (55±10 nm) 50 – 1000 µg/mL)	*Danio rerio*	Toxicity due to oxidative damage and apoptosis. LC50: 520 µg/mL. (LC50 for control MgO NPs: 410 µg/mL)	32
Synthesized with Moringa oleifera (21-65 nm) (0.1-500 µg/mL)	*Danio rerio*	No oxidative damage parametry was increased and no death was detected.	33
1) 10 mM) (11 nm)	*Drosophila melanogaster*	The expression of some genes has been altered, but no genotoxicity has been detected.	2
(50 -70 nm) (25 – 400 mg/ Kg soil)	*Eisenia andrei, Folsomia candida, and Enchytraeus crypticus.*	No significant toxic effects other than a decrease in the viability of F. candida.	34

Another study demonstrated that MgO NPs resulted in cumulative mortality in zebrafish.[4] The researchers investigated the potential ecotoxicology of MgO NPs on zebrafish embryos and larvae using the ELS test, a widely utilized method for detecting teratogenic effects. They observed a dose-dependent decrease in the hatching rate of larvae and the survival rate of embryos. The study involved evaluating various factors including hatching rate, survival rate, malformation, cellular apoptosis, and intracellular reactive oxygen species in zebrafish embryos from 4 to 96 hours after fertilization with MgO NP exposure. In conclusion, the incubation rate significantly decreased in treated groups compared to the control group, the survival rate of embryos decreased proportionally with the dose, malformations were observed at a certain rate in the exposed embryos compared to the control group, and cellular apoptosis occurred.

In another study, evaluated the histopathological changes in zebrafish larvae following exposure to MgO NPs.[23] The results revealed various changes including psoriasis-like epithelial hyperproliferation, muscle cell degeneration, neurodegeneration in the spinal cord, swelling and edematous changes in the pericardium, yolk sac enlargement and

edematous changes, severe eye edema, retina shrinkage, and deterioration in retinal lamination.

In our study, we determined oxidative damage caused by MgO NPs in larvae using the MDA method and catalase determination. Three different concentrations (150 µg, 75 µg, and 35 µg) were used for assessing oxidative damage. Our findings revealed that despite the acute toxicity of MgO NPs and the death after exposure to these NPs, no significant changes in MDA levels or catalase enzyme activity in larvae exposed to MgO NPs after 24, 48, and 72 hours of exposure. Ateş et al.,[16] utilized *A. salina* larvae and examined the potential oxidative damage caused by α-Al2O3 and γ- Al2O3 NPs using the MDA method. The levels of MDA detected in the larvae showed no statistically significant difference from the control group after 24 hours of exposure to both NPs. A notable increase in MDA amount was observed after 96 hours of exposure. *(P*< .05).

CONCLUSION

The present study showed that MgO NPs can negatively affect *A. salina* larvae, causing death and acute toxicity in dose and time dependent manner. However, this effect could be by mechanisms other than the oxidative stress as the oxidative lipid peroxidation and catalse depleastion results reviled no effects at the tested concentrations. the research on the toxic effects of MgO NPs in the environment is limited. More studies are necessary to comprehend the toxic effects and mechanisms of MgO NPs utilized across various domains.

Ethics Committee Approval: There is no requirement for ethical approval while applying this protocol and could be accepted as an alternative method compatible with to 3R principles.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A, H.N.I.; Design – M.A, H.N.I.; Supervision – M.A, H.N.I.; Resources – M.A, H.N.I.; Materials – M.A, H.N.I.; Data Collection and/or Processing – M.A, H.N.I.; Analysis and/or Interpretation – M.A, H.N.I.; Literature Search – M.A, H.N.I.; Writing Manuscript – M.A, H.N.I.; Critical Review – M.A, H.N.I.

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References

1. Roco MC. International perspective on government nanotechnology funding in 2005. *Journal of Nanoparticle Research.* 2005;7(6):707-712. **[Crossref]** https://doi.org/10.1007/s11051-005-3141-5
2. Yalçın B, Güneş M, Kurşun AY, Kaya N, Marcos R, Kaya B. Genotoxic hazard assessment of cerium oxide and magnesium oxide nanoparticles in Drosophila. *Nanotoxicology.* 2022;16(3):393-407. **[Crossref]**
3. Tourinho PS, van Gestel CAM, Lofts S, Svendsen C, Soares AMVM, Loureiro S. Metal-based nanoparticles in soil: Fate, behavior, and effects on soil invertebrates. *Environ Toxicol Chem.* 2012;31(8):1679-1692. **[Crossref]**
4. Ghobadian M, Nabiuni M, Parivar K, Fathi M, Pazooki J. Toxic effects of magnesium oxide nanoparticles on early developmental and larval stages of zebrafish (Danio rerio). *Ecotoxicol Environ Saf.* 2015;122:260-267. **[Crossref]**
5. Bakardjieva S, Šubrt J, Štengl V, Opluštil F, Olšanska M. Magnesium oxide nanoparticles as destructive sorbent for toxic agents. *Microscopy and Microanalysis.* 2004;10(S02):476-477. **[Crossref]**
6. Di DR, He ZZ, Sun ZQ, Liu J. A new nano-cryosurgical modality for tumor treatment using biodegradable MgO nanoparticles. *Nanomedicine.* 2012;8(8):1233-1241. **[Crossref]**
7. Kiranmai G, Reddy ARN. Antioxidant status in MgO nanoparticle-exposed rats. *Toxicol Ind Health.* 2013;29(10):897-903 **[Crossref]**
8. Krishnamoorthy K, Moon JY, Hyun HB, Cho SK, Kim SJ. Mechanistic investigation on the toxicity of MgO nanoparticles toward cancer cells. *J Mater Chem.* 2012;22(47):24610-24617 **[Crossref]**
9. Lei ZQ, Li L, Li GJ, et al. Liver cancer immunoassay with magnetic nanoparticles and MgO-based magnetic tunnel junction sensors. *J Appl Phys.* 2012;111(7):07E505. **[Crossref]**
10. Moussavi G, Mahmoudi M. Removal of azo and anthraquinone reactive dyes from industrial wastewaters using MgO nanoparticles. *J Hazard Mater.* 2009;168(2-3):806-812. **[Crossref]**
11. Dhawan A, Sharma V. Toxicity assessment of nanomaterials: Methods and challenges. *Anal Bioanal Chem.* 2010;398(2):589-605. **[Crossref]**
12. Weber C. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.* Fifth Edition.; U.S. Environmental Protection Agency; 2002.
13. Gambardella C, Mesarič T, Milivojević T, et al. Effects of selected metal oxide nanoparticles on A. salina larvae: Evaluation of mortality and behavioural and biochemical responses. *Environ Monit Assess.* 2014;186(7):4249-4259. **[Crossref]**
14. Ates M, Daniels J, Arslan Z, Farah IO. Effects of aqueous suspensions of titanium dioxide nanoparticles on Artemia salina: assessment of nanoparticle aggregation, accumulation, and toxicity. *Environ Monit Assess.* 2013;185(4):3339-3348. **[Crossref]**
15. Ates M, Daniels J, Arslan Z, Farah IO, Rivera HF. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (Artemia salina) larvae: effects of particle size and solubility on toxicity. *Environ Sci Process Impacts.* 2012;15(1):225-233. **[Crossref]**
16. Ates M, Demir V, Arslan Z, Daniels J, Farah IO, Bogatu C. Evaluation of alpha and gamma aluminum oxide nanoparticle accumulation, toxicity and depuration in A. salina Larvae. *Environ Toxicol.* 2015;30(1):109. **[Crossref]**
17. Ates M, Demir V, Arslan Z, Camas M, Celik F. Toxicity of engineered nickel oxide and cobalt oxide nanoparticles to A. salina in seawater. *Water Air Soil Pollut.* 2016;227(3). **[Crossref]**
18. Madhav MR, David SEM, Kumar RSS, et al. Toxicity and accumulation of Copper oxide (CuO) nanoparticles in different life stages of Artemia salina. *Environ Toxicol Pharmacol.* 2017;52:227-238. **[Crossref]**
19. Rajabi S, Ramazani A, Hamidi M, Naji T. A. salina as a model organism in toxicity assessment of nanoparticles. *DARU Journal of Pharmaceutical Sciences.* 2015;23(1). **[Crossref]**

20. Mahmoud A, Ezgi Ö, Merve A, Özhan G. In vitro toxicological assessment of magnesium oxide nanoparticle exposure in several mammalian cell types. *Int J Toxicol.* 2016;35(4):429-437. **[Crossref]**
21. Öztürk R, Maltaş Çağil E, Vinclozolin' in Galleria mellonella L. (Lepidoptera: Pyralidae)' nın glukoz oksidaz, katalaz ve ksantin oksidaz aktiviteleri üzerine etkisi. *Nevşehir Bilim ve Teknoloji Dergisi.* 2019;8(2):174-182. **[Crossref]**
22. Farré M, Gajda-Schrantz K, Kantiani L, Barceló D. Ecotoxicity and analysis of nanomaterials in the aquatic environment. *Anal Bioanal Chem.* 2009;393(1):81-95. **[Crossref]**
23. Ghobadian M, Nabiuni M, Parivar K, Fathi M, Pazooki J. Histopathological evaluation of zebrafish (Danio rerio) larvae following embryonic exposure to MgO nanoparticles. *Iran J Fish Sci.* 2017;16(3):959-969. **[Crossref]**
24. Thomas J, Vijayakumar S, Thanıgaıvel S, TMukherjee A, Chandrasekaran N. Toxicity of magnesium oxide nanoparticles in two fresh water fishes tilapia (oreochromis mossambicus) and zebra fish (danio rerio). *Int J Pharm Pharm Sci.* 2014;6(2):487-490.
25. Anjana G, Roy A, Rajeshkumar S, Ezhilarasan D. Cassia oleoresin mediated synthesis of Magnesium Oxide nanoparticles and brine shrimp lethality assay. *J Pharm Res Int.* Published online August 24, 2020:75-82. **[Crossref]**
26. Bhavya C, Yogendra K, Mahadevan KM. Acute and sublethal toxicity of Calcium Magnesiate nanoparticle to the freshwater fish, Cyprinus carpio. *J Emerg Technol Innov Res.* 2019;6(6):322-329. www.jetir.org
27. Bahel D, Alghanimy G, Alghanmi HA. Effect of different Magnesium Oxide nanoparticles concentration on the growth of the Lyngbya majuscula. Ecology, Environment and Conservation Journal. 2020;27(2):110-115.
28. Fouda A, Eid AM, Abdel-Rahman MA, et al. Enhanced antimicrobial, cytotoxicity, larvicidal, and repellence activities of brown algae, cystoseira crinita-mediated green synthesis of magnesium oxide nanoparticles. *Front Bioeng Biotechnol.* 2022;10. **[Crossref]**
29. Alghanmi HA, Al-Khazali ZK, Author C, Names Haider Alghanmi Zahra KM Al-Khazali AA, Journal AQ. Effect of magnesium oxide nanoparticles on growth and biochemical content of blue-green Alga wollea salina. *Al-Qadisiyah Journal of Pure Science.* 2021;26(4):114-129. **[Crossref]**
30. Ma L, Andoh V, Shen Z, Liu H, Li L, Chen K. Subchronic toxicity of magnesium oxide nanoparticles to Bombyx mori silkworm. *RSC Adv.* 2022;12(27):17276. **[Crossref]**
31. Güneş M, Yalçın B, Ali MM, Ciğerci İH, Kaya B. Genotoxic assessment of cerium and magnesium nanoparticles and their ionic forms in Eisenia hortensis coelomocytes by alkaline comet assay. Microsc Res Tech. 2022;85(9):3095-3103. **[Crossref]**
32. Verma SK, Nisha K, Panda PK, et al. Green synthesized MgO nanoparticles infer biocompatibility by reducing in vivo molecular nanotoxicity in embryonic zebrafish through arginine interaction elicited apoptosis. *Sci Total Environ.* 2020;713. **[Crossref]**
33. Vijayakumar S, Chen J, González Sánchez ZI, et al. Moringa oleifera gum capped MgO nanoparticles: Synthesis, characterization, cyto- and ecotoxicity assessment. *Int J Biol Macromol.* 2023;233. **[Crossref]**
34. Ogliari AJ, Borges WG, Silva LL, et al. Magnesium oxide nanoparticles and their ecotoxicological effect on edaphic organisms in tropical soil. *J Appl Toxicol.* 2022;42(4):553-569. **[Crossref]**