Heliyon 9 (2023) e15605

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Impact of acute and sub-acute exposure of magnesium oxide nanoparticles on mrigal *Cirrhinus mrigala*

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ARTICLE INFO

Keywords:
Sub-lethal exposure
Biochemical
Enzymatical
Antioxidant changes
DPPH
HRSA

ABSTRACT

This experiment was conducted to investigate the impact of acute and sub-acute exposure of magnesium oxide nanoparticles on Mrigal *Cirrhinus mrigala*. For sub-acute tests 1/100,1/50,1/10 were selected based on the LC_{50} at 96 h s. Protein, carbohydrate, and lipid, Aspartate aminotransferase, alanine transaminase, lactate dehydrogenase and DPPH, HRSA assays were analyzed in the gill, muscle, and liver of Mrigal. Protein and lipid levels increased on the 7th,14th day compared to control. Carbohydrate levels decreased on the 7th,14th day of exposure, and the enzymatical changes increased on the 7th,14th day. Antioxidant levels highly increased in DPPH assay compared to the HRSA assay. This study provides the biochemical, antioxidant, and behavioral changes in relation to the exposure of MgO NPs.

1. Introduction

The development of nanomaterials expanded opportunities in industries, agriculture, pharmaceutical and chemical industries [1]. The chemical synthesis and stabilization of nanoparticles results in toxic residues in the environment [2,3,4,5,6,7,8]. Heavy metals' acute toxicity to fish appears to depend on their soluble fraction [9,10,11,12,13,14] Different factors can affect the toxicity of metallic nanoparticles such as shape, high surface area, nanoscale size, free ions and structure [15,16]. However, at high concentration it accumulates in different organs, damage tissues and interferes with the normal growth of organisms including fish [17]. Some of leading to sublethal effects or death of fish populations accumulated along aquatic food chains [18]. As a supplemental nutrient, metal nanoparticles, such as iron, iron oxide, selenium, zinc, copper, silver, and magnesium oxide, play a vital role in aquaculture development [19]. [20] MgO has a role in toxic waste remediation and functional semiconductor; optoelectronics has many applications in medicine including drug delivery, catalysis, powerful antimicrobial and antioxidant agents for the most provocative and challenging antibiotic-resistant diseases, cell signalling and imaging [21,22]. to study aquatic toxicity, fish species have been widely used as morphology, surface functional groups, indicator the size, and dose-dependent also responsible for normal tissues, and organs, healthy human cells and normal cells [23]. Acute toxicity tests are typically done to determine mortality and exposure time with acute lethality and LC_{50} representing the level of lethality in a test species. Exposure to chemicals in the environment affects the physiological and biochemical changes in species, tissue distribution and elimination profiles [24,25,26,27] In sublethal toxicant, the effect of Physiological action helps and predict the effects of biochemical, hematological, and histopathological analysis and the mode of action of the toxicant can also ascertained by analysis [28,29,30,31,32,14,33,34] The present study is related to the impact of MgO nanoparticles on the hematological, enzymatic, antioxidant, and biochemical parameters of Mrigal.

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https://doi.org/10.1016/j.heliyon.2023.e15605
Received 19 January 2023; Received in revised form 12 April 2023; Accepted 17 April 2023
Available online 19 April 2023

2. Materials and methods

2.1. Experimentation and laboratory conditions

The fingerlings of Mrigal *Cirrhinus mrigala* (1.5 ± 0.3 g) were collected from K·V·K Fish Farm, Palani, Dindigul, Tamil Nadu, India and acclimated for 15 days under laboratory condition. During acclimation, pH, temperature, dissolved oxygen, and hardness level were maintained. The fishes were fed with basal 0 feed for 15 days before the experiment. The water was changed (one-third), and the excretory waste was removed frequently before the experiment.

2.2. Acute toxicity test

This test was conducted and accordance with OECD (Organization for economic, cooperation and Development guidelines) [35,36]. Healthy fishes (1.5 ± 0.3 g) were selected under static conditions. The median lethal concentration (LC_{50}) of MgO nanoparticles was established in five different concentrations viz. (control as a) 0, 2,4,8 and 16 mg/l. Each treatment has been carried out in triplicate using the factorial design method (Triplicate). Seven groups of fish were exposed to various concentrations of MgO nanoparticles for 96 h s. Behavioral and mortality were measured in 24, 48,72, and 96 h. The death fishes were taken out of the water right away to prevent any possible water quality degradation.

2.3. Sub-acute toxicity test

Sub-acute toxicity test was conducted by taking 1/100,1/50, and 1/10 of LC_{50} values along with control for 14 days. The behavioral changes and mortality were observed twice per day [37]. blood samples were collected at 7th and 14th day of MgO nanoparticle exposure for enzymatical parameters. The gill, muscle, and liver were dissected for biochemical parameters.

2.4. Macronutrient parameters

After the treatment, proteins, carbohydrates, and lipids were estimated by Lowry's [38], Anthrone [38] and Folch method [39] respectively.

2.5. Behavioral changes

In this experiment, behavioral changes such as swimming, non-specific interactions, chafing opercular movement, unconscious behavior, fin movement were observed twice a day [40,41,42].

2.6. Enzymatical changes

Aspartate aminotransferase, alanine transaminase, and lactate dehydrogenase was analyzed by estimating oxaloacetate and pyruvate, respectively. The AST is the indicating liver disease, ALT indicating the liver disease and tissue damage and the LD H indicating the identification of damaged tissues in the located body. The blood serum was used for enzyme assay [43].

2.7. Antioxidant

2.7.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH assay)

$$(\text{DPPH}) + (\text{H-A}) = \text{DPPH-H} + (\text{A}) \tag{1}$$

2.96 ml of DPPH (0.1 mM) solution was added by different quantities of organs (gill, muscle, liver) (2–20 μl) [44] made up to 40 μl with ethanol solution, the solution mixture was incubated at room temperature in the dark for 20 min, after incubation the absorbance was read at 517 nm. DPPH as control. The % radical scavenging activity of the MgO was calculated by using the following formula, (Tangkanakul et al., 2009).

$$\text{RSA}\% = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \text{X } 100 \tag{2}$$

2.7.2. Absorbance control

Eq. (A.1). RSA: Radical Scavenging Assay, Absorbance control: Control, Absorbance Sample: Fish Samples.

2.7.3. Hydroxyl Radical Scavenging Assay

1 ml of $C_{10}H_{12}FeN_2O_8$-2 and 05 ml of $C_{10}H_{16}N_2O_8$, 1 ml of CH_3OH, 0.5 ml of $C_6H_8O_6$ and 1 ml of different quantities of organs (gill, muscle, liver) mixture solution incubated at boiling water bath for 15 min at 90 °C, at room temperature, 1 ml of ice-cold TCA was added to 3 ml of NaSH reagent, and the mixture was incubated for 15 min. The % HRSA activity was calculated in accordance with the formula below [45,46].

$$RSA\% = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} X\ 100 \quad (3)$$

Eq. (A.2). HRSA: Hydroxyl Scavenging assay, Absorbance control: Control, Absorbance Sample: Fish samples.

2.8. Statistical analysis

In this study, data was processed using SPSS (16.0) to determine mean ± SD when 156 comparing groups. ANOVA was used to determine the level of variation among groups. Statistical 157 significance was set at $P < 0.05$.

3. Results and discussion

3.1. Behavioral changes

Abnormal behavioral changes were observed in MgO nanoparticles treated fish. The intensity of abnormal changes increases as the quantity of nanoparticles increases (Table 1A) where *Oreochromis mossambicus* treated with ZnO showed vertically hanging in water, abnormal jerking locomotion and constant opercular movement. *Channa punctatus* showed abnormal swimming with jerking movements, hectic excitability and convulsions [47]. [48] nickel, copper, and zinc exposure in *Poecilia reticulata* caused higher excretion, excessive secretion, anorexia, and swollen fin movement.

Table 1A
Behavioral changes of Mrigal exposed to MgO nanoparticles.

S·NO	Behavioral changes	Treatment			
		T_1 (Control)	T_2	T_3	T_4
1.	Respiration	*	*	++	+
2.	Swimming	*	*	+++	+++
3.	Opercular movement	*	+	++	+++
4.	Hyper Active	–	++	+++	+++
5.	Mucues Secretion	–	+	++	++
6.	Chafing	–	+++	+++	+++
7.	Unconscious Behavior	–	–	–	–

Subacute exposure note: * = normal, - = no, + = low activity, ++ = medium activity, +++ = high activity.

3.2. Toxical exposure

During the experiment, the number of mortality fish was recorded in 24 h s immediately removed to contamination of water. pH, Dissolved oxygen, salinity, and hardness of the water were also monitored (2.2321 mg/l) and also the [49] concentration of 2 mg/l no mortality in Mrigal, control, and 16 mg/l of MgO nanoparticles caused 100% mortality in 96 h s of LC_{50} at the 95%confidence is 4.689 mg/l. Fish mortality or survival was recorded after 96 h and the median lethal concentration (LC_{50}) was calculated as 23.89 mg l-1 at which 50% mortality occurred [46] in selenite exposure [45]. reported the acute toxicity level of concentration increased and the number of mortality fish also increased in time 24–96 the highest concentration of CuO nanoparticles were recorded in the highest mortality in 96hr. The 96hr of ZnO NPs and for bulk ZnO materials LC_{50} value was found at 2.19 ± 0.003 mg/l [50]. [49] as particle concentration increased, A concentration of 30 mg/l and LC_{50} values of 4.92 mg/l and 3.31 mg/l calculated for bulk ZnO suspensions after 96 h led to 100% mortality of zebrafish [51,47,52,53,54,49]. statistical analysis showed that G-MgO NP and MgO NP had significant differences at 100, 250, 500 and 1000 g/ml ($P < 0.05$).

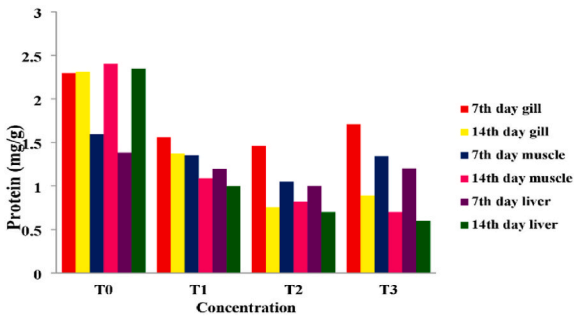

Fig. 1. A.1. Protein level in gill, liver and muscle of *Cirrhinus mrigala* ($P < 0.05$).

3.3. Biochemical parameters

Protein in the gill, muscle, liver of *Cirrhinus mrigala* exposed to different quantities of MgO nanoparticles, when the concentration increased, the protein level gradually decreased on 14th day is presented in. Fig. 1 A.1 ($P < 0.05$). Lipid in concentration level decreased gill, muscle, liver of *Cirrhinus mrigala* is presented in Fig. 2 A.2 ($P < 0.05$). Magnesium is a macronutrient for aquatic animals, co-factor of protein, enzymes and helps to remove the bad cholesterol in the body. Carbohydrate concentration levels decreased in the gill, muscle, and liver Fig. 3 A.3 ($P < 0.05$). And also, the metabolism level of glucose and glycogen suffered in contaminated waters [14]. toxicants in the aquatic media exert their results revealed significant changes in biochemical parameters and effects at the cellular or molecular level, and there was a substantial decrease in the protein [25].sublethal contamination is identified by behavioral endpoints appearing at lower concentrations than those that cause chronic death [29,55,56].

3.4. Enzymatical changes

Enzymes are quickly considered biomarkers of the toxic metal in aquatic organisms. ALT, AST, and LDH are predominantly present in the liver [14] Fig. 4 B.1, B.2, B.3. MgO NPs decreased the serum levels of ALT, AST in Asian Sea Bass. These findings [57] the addition of these nanoparticles results in increased liver activity, improved antioxidant capacity, membrane stabilization, and protection against cell damage [58,59]. In Nano-Magnesium treatment, the trypsin enzyme level reached its maximum, and in Nano-SE treatment, the level was at its lowest ($P < 0.05$) [60]. At the level of AST and ALT serum activities increased in comparison with controls, Zn, Cd, and Zn + Cd concentrations in *O. niloticus* [61] a dose of 1000 mg/kg MgO NPs significantly increased AST and ALT in serum ($P < 0.01$) [58] all doses of NPs, at low and medium doses. The enzyme levels increased dose-dependently, but the effect was non-significant at both concentrations [62].

3.5. Antioxidant

3.5.1. DPPH assay

MgO Nanoparticles had excellent antioxidant properties. The DPPH reacts with MgO nanoparticles the red color turns to yellow color. Hydrogen donating ability of antioxidants reacts with DPPH, and the degree of decoloration, the consequence absorbance decreases indicate the ability of antioxidant compounds to scavenge free radicals. The antioxidant level increased compared to the control as given in Fig. 5 C. In this study, MgO nanoparticles quickly decolorized in higher concentration at 550 and 517 nm with an antioxidant proportion of [63] in comparison with the lowest concentration, MgO NPs exhibited antioxidant properties of 0.461 at 517 nm with a proportion of 57.783% [64]. high purity of cubic phase MgO NP samples were correlated by diffraction peaks with crystal planes.

3.5.2. Hydroxyl Radical Scavenging Assay

In different organs (Gill, Muscle, Liver), the free hydroxyl radicals (body cells damages) were found in Hydroxyl scavenging assay. In this method, ascorbic-acid and Iron EDTA are involved in the hydroxyl radical generating system. These two generate hydroxyl radicals assay [65] MgO nanoparticles and phospholipids react with polyunsaturated fatty acid moieties in the cell membrane, causing damage to fish organs and cells (gill, muscle, liver) shown in Fig. 6 D. Due to oxidative damage caused by fish exposure, low levels of antioxidant enzymes may cause this effect [66]. MgO NPs tend to be higher Antioxidant activity than *Moringa olifera* Bark [67]. In vitro, MgO NPs synthesized via biosynthesis have potential antioxidant activity, and in vivo, MgO NPs synthesized chemically have potential antioxidant activity [21]. Antioxidant assays showed that 90% FRSA, 91% SARSA, 78% LPA, 87% HRSA and 83% FTC assay of extracts contributed to the compounds [68].

3.6. Conclusion

Magnesium Oxide is a micronutrient of aquatic animals and when it is it became toxic to the aquatic environment. LC_{50} value of MgO nanoparticles was observed as 4.689 mg/l. The level of sub-lethal exposure was analyzed in behavioral changes, biochemical

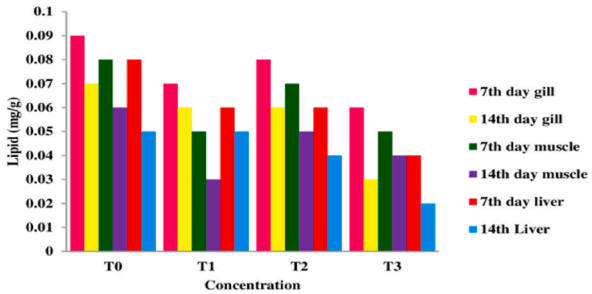

Fig. 2. A. 2. Lipid level in gill, liver and muscle of Cirrhinus mrigala ($P < 0.05$).

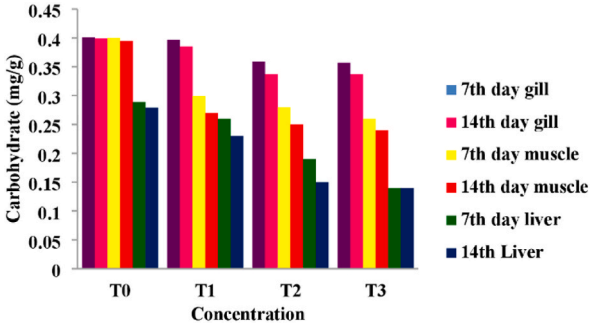

Fig. 3. A. 3. Carbohydrate level in gill, liver and muscle of *Cirrhinus mrigala* (P, 0.05).

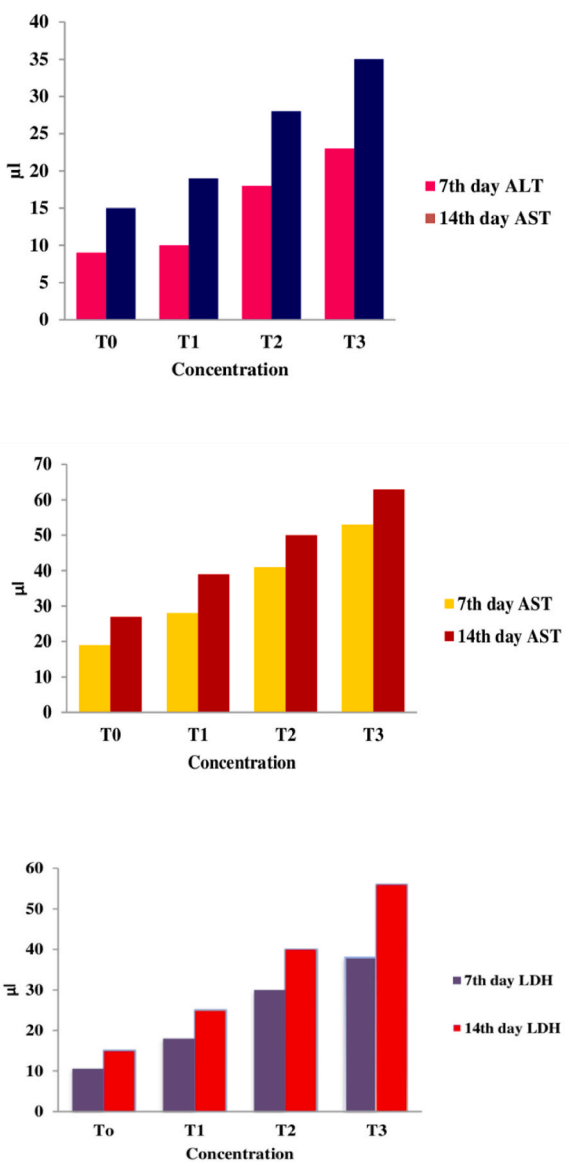

Fig. 4. B.1. ALT, B.2. AST and B.3. LDH activity in serum of *Cirrhinus mrigala* treated with different concentration of MgO nanoparticles.

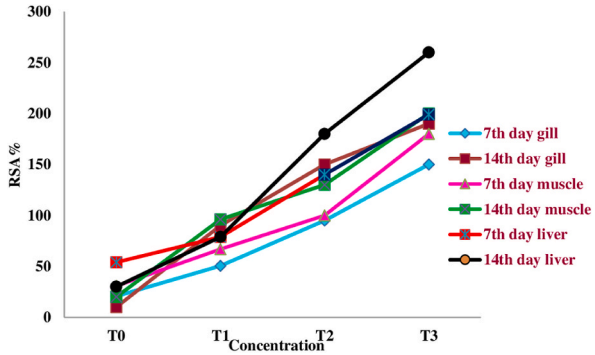

Fig. 5. C. Antioxidant level in gill, liver and muscle of *Cirrhnius mrigala* for DPPH assay.

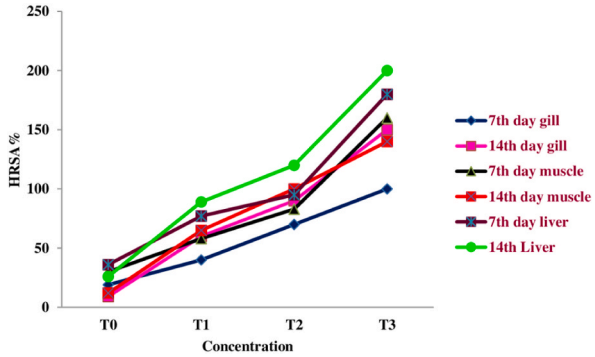

Fig. 6. D. The antioxidant level in gill, liver and muscle of *Cirrohnius mrigala* for HRSA assay.

changes, and antioxidant changes were analyzed. The nanoparticle toxicity is based on the size and the level of concentration exposure. The Toxicity of magnesium oxide is the dose and concentration dependence of the organism. Nowadays, MgO is a crucial role in biomedicine, food industry, cosmetics, electrical, etc., In this aspect knowledge on magnesium oxide toxicity is very limited so it deserves more research to be carried out.

Author contribution statement

Shanmugam Sudhabose: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Balakrishnan Sooryakanth; Muthuswami Ruby Rajan, Ph. D: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

4. Data availability statement

Data will be made available on request.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical clearance

The experimental design and use of animals for research purpose was carried out as per the existing Animal welfare law of India. Treatment and handling of fishes for this study was performed as per the supervision and guidance given by Institutional Ethical Committee for Research on Human and Animal Subject (IECRHAS), The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Dindigul, Tamilnadu, India.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Our sincere gratitude goes out to the Head of the Biology Department, GRI-DTBU, for providing the necessary experimental resources. We are grateful to the GRI General Library for supplying the ithenticate and Grammarly access.

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