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Research Article

TOXICITY OF MAGNESIUM OXIDE NANO PARTICLES IN TWO FRESH WATER FISHES TILAPIA (OREOCHROMIS MOSSAMBICUS) AND ZEBRA FISH (DANIO RERIO)

JOHN THOMAS, S. VIJAYAKUMAR, S. THANIGAIVEL, AMITAVA MUKHERJEE, NATARAJAN CHANDRASEKARAN*

Centre for Nanobiotechnology,VIT University, Vellore, Tamil Nadu. Email: nchandrasekaran@vit.ac.in, nchandra40@hotmail.com

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ABSTRACT

Objective: The objective of the present study is to study the toxicity of Magnesium oxide nanoparticle and Magnesium oxide bulk against two fishes (Tilapia (*Oreochromis mossambicus*) and Zebra fish (*Danio rerio*) for toxicity. The Biochemical and antioxidant activity were also studied in both control and the injected fish. Bioaccumulation was also studied.

Methods: Magnesium oxide bulk and Nano particles were prepared by dissolving it in 1µg/mL concentration and sonicated for half an hour. Different concentrations of Magnesium oxide both bulk and nano particles from 10 to 200 ppm were determined.

Results: There was a significant decrease in protein. In the Catalase activity there was a gradual and sporadic increase. The specific activity of GST enzyme increased significantly with an increase in the concentration of Magnesium nanoparticles. There was an increase in the concentration of Magnesium nanoparticles and more accumulation was seen.

Conclusion: Heavy metals are common pollutants and restricted to frequent inhalation in aquatic organism due to their larger size. The results revealed that magnesium oxide bulk particle was found to be more toxic when compared to nano particle.

Key words: *Oreochromis mossambicus,* Magnesium oxide, Histopathology, Biochemical parameters, Fishes.

INTRODUCTION

Growing exploration of nanotechnology has resulted in the identification of many unique properties of nano materials such as enhanced magnetic, catalytic, optical, electrical, and mechanical properties when compared to conventional formulations of the same material [1-4] These materials are increasingly being used for commercial purposes such as fillers, opacifiers, catalysts, water filtration, semiconductors, cosmetics, microelectronics etc. leading to direct and indirect exposure in humans [5]. In studies on Mg requirements in fish, Mg sulphate is commonly used [6-8] although in diets with high levels of Mg supplementation. The acute toxicity of heavy metals to fishes appears to be a function of the soluble fraction. The solubility of heavy metals, in turn, appears to be dependent upon the alkalinity of water

Nanomaterials (NMs), with at least one dimension of 100 nm or less, are being increasingly used for commercial purposes in various areas, such as textiles, electronics, pharmaceutics, cosmetics, and environmental remediation. Nanoparticles (NPs) have at least one dimension of 100 nm or less. Nanotechnology industry is a rapidly growing science producing nano-sized particles [9, 10]. The NPs have always existed in our environment, from both natural and anthropogenic sources.

MATERIALS AND METHODS

Magnesium oxide

Magnesium oxide nano-powders and bulk were procured from sigma Aldrich (St. Louis, Missouri). The supplier's data states that the particle size of the gamma phase nanopowder, is <50 nm, (BET). These particles will be designated as MgO-n and MgO for nano and bulk respectively. Dispersion of 1mg/mL was prepared in Millipore filtered water using ultrasonic processor (20 kHz) 101 (Sonics, USA).

Collection and maintenance of experimental animals

Healthy fish Tilapia (*Oreochromis mossambicus)* were collected for experimental purpose from a fish farm located at Walajapet, Vellore dist, Tamil Nadu. Zebra fish (*Danio rerio*) were collected from a farm near Chennai. The fish were transported in live condition and maintained in 100-l fiber glass tanks with continuous aeration at room temperature (27-30 ºC) in fresh water. The animals were fed twice a day with commercial fish feed.

Preparation of Magnesium oxide bulk and nano powder

Magnesium oxide bulk and Nano were prepared by dissolving in 1µg/mL concentration. The nano particle was then sonicated for half an hour using high energy sonication in a sonicator (Sonics Vibracell Ultrasonicator, 130 W, and 20 kHz). The prepared nanoparticle was used in the experiment.

Determination of magnesium toxicity

Tilapia (*O. mossambicus) a*nd zebra fish (*D. rerio)* were reared in aquarium tanks of 50 l capacity containing sterilized freshwater with continuous aeration. Air stones and air tubes were sterilized by immersing them in 2.6% sodium hypochlorite and washing them thoroughly with sterilized tap water before use. The tanks were covered to prevent contamination. Aseptic techniques were used throughout the experiment. Fish were fed with commercial fish feed. For the experiments the fishes were exposed to different concentrations of Magnesium oxide both bulk and nano from 10 to 200 ppm. 100 mg of Magnesium oxide both bulk and nano were dispersed in 500ml of water. It was then sonicated for 20min in a sonicator (Sonics Vibracell Ultrasonicator, 130 W, and 20 kHz). This was further diluted to obtain different concentrations from 10 to 200ppm. This was confirmed by ICP-OES. The control consisted of fish exposed to freshwater only. They were observed daily for 120 hrs. Three replicates in each concentration and control were carried out. Animals were checked twice daily for any signs and mortality. Dead animals were removed. The experiments were conducted in triplicates to avoid pseudo replication [11].

Estimation of biochemical and antioxidant parameters

Total protein, superoxide dismutase, glutathione S transferase and catalase were determined in experimental and control fishes. Biochemical parameters were analysis routine laboratory by procedures [12]

Total Protein

Total protein was determined spectrophotometrically at 640 nm based on the method of [13]. Solution A (1% Copper sulphate), solution B (2% Sodium potassium tartarate) and Solution C (2% Sodium carbonate in 0.1 N NaOH) were prepared. The solutions were mixed in the ratio of C: B: A (98:1:1). 4.5ml of this mix was pipetted into clean, marked glass tubes. The standard BSA was

added to the tubes at concentrations of 10 µg, 20 µg, 40 µg, 80 µg and 100 µg. 10 µl of homogenised samples were added to each marked tube and the tubes were incubated at room temperature for 10 minutes. 0.5 ml of Folin's reagent (1:1 solution of Folin's Ciocalteau reagent in distilled water) was added to the tubes. The tubes were incubated in the dark for 30 minutes and the optical density was read at 640 nm. The readings from protein standards were used to plot a standard graph and to estimate value of the test samples.

Assay of Superoxide Dismutase

SOD activity was determined according to the method of Beauchamp and Fridovich (1995) Phosphate buffer: (50 mM, pH 7.8), Riboflavin: 20 µM dissolved in phosphate buffer 50 mM, pH 7.8 and Nitro blue tetrazolium (NBT) mixture: (0.1 mM EDTA, 13 µM methionine, 0.75 mM NBT). Nitro blue tetrazolium NBT, and 20 µM of reaction mixture (0.1 mM EDTA, 13 µM methionine, 0.75 mM NBT and 20 µM riboflavin in phosphate buffer 50 mM, pH 7.8),with 0-100 µl of the homogenized sample were placed under fluorescent light for 2 min or until A_{560} in control tubes reached 0.2-0.25 OD. The ratio of the specific activity of the stimulated cells to the specific activity of control cells was used as an index for comparing the effects of different treatments on the SOD activity. The results were expressed as relative enzyme activity U/mg protein.

Assay of Glutathione-S-Transferase

Glutathione-S-transferase activity was determined by the method of [14]. Phosphate buffer: 0.3 M, pH 6.5. , 1-chloro-2, 4-dinitrobenzene (CDNB):30 mM and reduced glutathione (GSH): 30 mM were prepared.

The reaction mixture containing 1.0 ml of buffer, 0.1 ml of CDNB and 0.1 ml of tissue homogenate was made upto 2.5 ml with water. This was pre incubated at 37°C for 5 minutes. 0.1 ml of GSH was added and the change in absorbance was measured at 340 nm for 3 minutes at 30 seconds intervals. The enzyme activity was expressed as mili Moles of CDNB conjugate formed/min/mg protein.

Assay of Catalase

Catalase was assayed according to the method of [15]. The reagent used includes Phosphate buffer 0.01 M pH 7.0, hydrogen peroxide 0.2 M solution in the above buffer and Dichromate acetic acid reagent (Potassium dichromate with glacial acetic acid in 1:3 ratios).

The tissue was homogenised in phosphate buffer 0.01 M (pH 7.0). To 0.1 ml of the homogenate 1 ml of phosphate buffer was added. To this 0.4 ml of hydrogen peroxide was added and the reaction was arrested after 30 and 60 seconds by the addition of 2.0 ml dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling water bath for exactly 10 minutes, cooled and absorbance read at 620 nm. The enzyme activity was expressed as units/min/mg/protein.

Bioaccumulation

Bioaccumulation is an important process to be borne in mind when evaluating hazards and risks from MgO. Risk assessment requires consideration of both exposure and effects because exposure and subsequent bioaccumulation of a xenobiotic is usually a precursor to toxicity i.e. the chemical must be retained by the organism before it can cause toxicity [16]. Nanoparticles containing magnesium are unlikely to be an exception to this generalization. Bioaccumulation is also a direct way to evaluate the processes that influence bioavailability, where bioavailability is defined as the pollutant concentration that an organism takes up from environmental media, summed across all possible pathways of uptake, including water and food [17]. Dynamic Light Scattering measurements were done for the particle size distribution (90Plus Particle Size Analyzer, Brookhaven Instruments Corporation, Holtsville, NY, USA). [18]

RESULT

Determination of magnesium toxicity

The results revealed that in Tilapia no mortality was observed in MgO-n where as in the bulk at 50,100, 150 and 200ppm there was 33%, 35%, 66% and 66% of mortality respectively at 120hrs. No mortality was observed in MgO bulk at concentrations of 10, 20 and 25 ppm.

In zebra fish (*D. rerio*) no mortality was observed in MgO-n, where as in bulk 100% mortality was observed only at 10 ppm.

Estimation of biochemical and antioxidant parameters

The biochemical and antioxidant parameters were determined in the control and magnesium oxide treated fish. Total protein, superoxide dismutase, glutathione S transferase and catalase were determined in experimental and control Tilapia and zebra fishes. There was a significant decrease in protein showed in figure1. In the Catalase activity there was a gradual and sporadic increase. Catalse activity increased with increasing concentration. The specific activity of GST enzyme increased significantly with increasing concentration as showed in figure 3

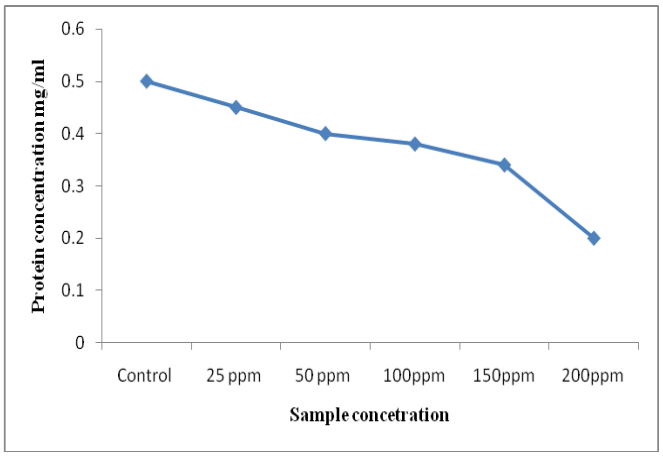

Fig. 1: Total protein

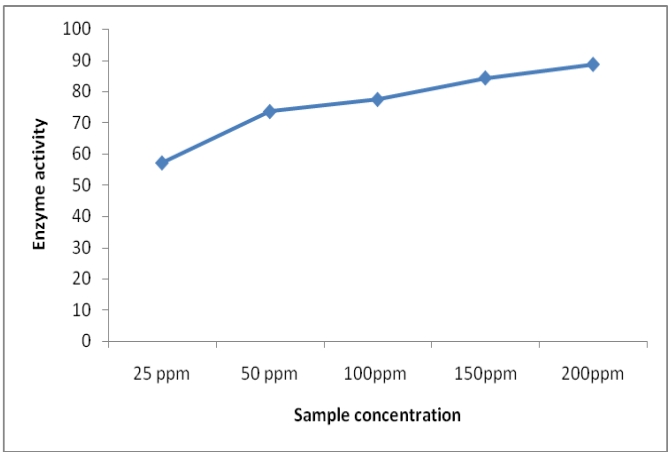

Fig. 2: Catalase activity

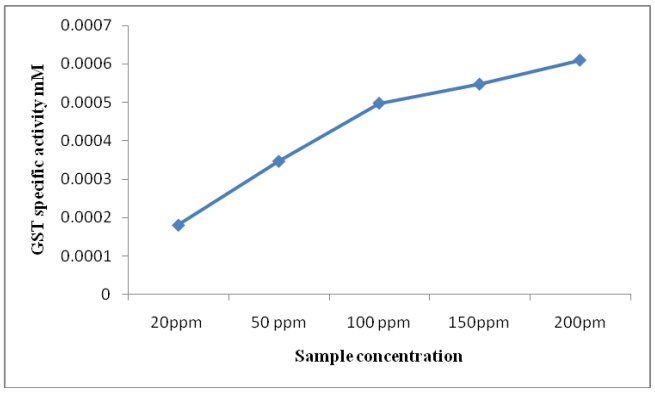

Fig. 3: Glutathione S-transferase

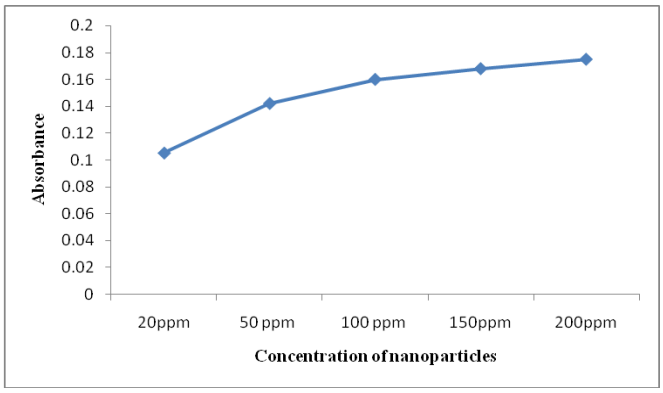

Fig. 4: Super oxide dismutase

Activity of glutathione S-transferase activity increased with increasing concentration increase in the concentration of Magnesium nanoparticles. At 20 ppm, there was almost a two fold increase while at 300 ppm, the specific activity was quite high as compared to the control and the 20 ppm level. Among all the antioxidant enzymes, SOD was found to respond better compared to the others and showed a consistent increase of activity with the increase in the nanoparticle concentration showed in figure 4. Activity of Super oxide dismutase increased with increasing concentration

Bioaccumulation

The analysis done using ICP-OES showed a gradual increase in the bioaccumulation of Magnesium with an increasing concentration of Magnesium nanoparticles. This proved that with an increase in the concentration of Magnesium nanoparticles, more accumulation was seen. The DLS data revealed that the size of the nanoparticle was found to be less than 50nm.

DISCUSSION

Magnesium (Mg) is the main intracellular earth metal cation with a free concentration in the cytosol around 0.5 mmol/ l. (Doudoroff and Katz 1953) prepared a review of toxicity studies on many metals including iron to various fish species Nanomaterials (NMs), with at least one dimension of 100 nm or less, are being increasingly used for commercial purposes in various areas, such as textiles, electronics, pharmaceutics, cosmetics, and environmental remediation. Most of the available nano exotoxicological data concern aquatic species, as the effects of nanomaterials on the aquatic environment are of great importance since it ultimately receives run-off and wastewater from domestic and industrial sources and has been targeted for some nano-scale environmental remediation. In studies of Mg requirements in fish, Mg sulphate is commonly used [6-8] although only in diets with high levels of Mg supplementation used acetate instead of sulphate in order to avoid the possible detrimental effect of sulphate. The pollution of the aquatic environment with heavy metals has become a worldwide problem in recent times because they are indestructible and most of them have toxic effects on organisms. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulation in aquatic ecosystems [18]. In the present study no mortality was observed in Tilapia treated with MgO-n where as in the bulk at 50,100, 150 and 200ppm there was 33%, 35%, 66% and 66% of mortality respectively at 120hrs. Similarly no mortality was observed in MgO bulk at concentration of 10, 20 and 25 ppm. At a lower concentration no mortality was observed. Likewise in zebra fish (*D. rerio*) no mortality was observed in MgO-n, where as in bulk 100% mortality was observed only at 10 ppm.

Heavy metals are common pollutants and restricted to frequent inhalation in aquatic organism due to their larger size. However, nano size of these heavy metals may cause inhalation and bioaccumulation very frequently as research is now showing that when normally harmless bulk materials are made into nano particles they tend to become toxic [19, 20]. Penetration of nanoparticles into the aquatic environment is fraught with numerous consequences, as yet unpredictable because of insufficient information.

Several workers have proposed that the size effect seems more important to nanoparticle toxicity than the actual composition of the material [21, 22]. No studies have yet demonstrated that NP accumulation in cells causes cytotoxicity. However, NPs have been reported to induce toxicity in cell membranes [23].

Knowledge on the physiological action of the toxicant helps to predict important sub lethal effects and Biochemical, haematological, and histopathological analyses may be used to determine the mode of action of the toxicant. In general, the presence of toxicants in the aquatic media exerts its effect at cellular or molecular level which results in significant changes in biochemical parameters. Our results revealed that there was a significant decrease in the protein.

In the Catalase activity there was a gradual and sporadic increase. The specific activity of GST enzyme increased significantly with an increase in the concentration of Magnesium nanoparticles. Due to metal complex formation, normal functioning of cell is disturbed and that in turn may result in variation in the physiological and biochemical mechanisms of animals [24]. Gradual and sporadic increase in Catalase activity and significant increase in the specific activity of GST seen in the present study may be due to metal complex formation as reported by [25]. The influence of stressors on carbohydrate metabolism of fish includes alterations in glucose, glycogen, and lactic acid content. Changes in the enzyme activities are widely used to detect tissue damage and biomarkers of animals exposed to chronic concentrations of a toxicant [26].

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