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Evaluation of Cytotoxicity of Magnesium Oxide Nanoparticles - An In vitro Study

Research Article

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Abstract

Introduction: Magnesium oxide (MgO) is an important inorganic material & has shown a promising role for applications in medicine. MgONPs have unique properties like high chemical stability, high photo catalytic activity and non-toxic nature.
Aim: The aim of the study was to study the cytotoxic effects of magnesium oxide nanoparticles (MgONps) synthesized using Phyllanthus emblica (amla) fruit seed.
Materials and Methods: MgONPs were synthesised using Phyllanthus emblica fruit seed extract, and cytotoxic effects of synthesized MgONPs were assessed using brine shrimp lethality assay.
Result and Discussion: The plant extract color was green and when it reacted with magnesium nitrate solution it changed to brown color which indicated the synthesis of MgONPs. The MgONPs have a characteristic band in the ultraviolet visible (UV) region due to their surface plasmon resonance. The graph reached its peak at a wavelength of 385.0 nm. Brine shrimp lethality assay was used to evaluate the cytotoxic effect of newly synthesised nanoparticles.At lower concentrations no cytotoxic effects were observed and at higher concentrations mild cytotoxic effects were observed.
Conclusion: Bio synthesization of MgONPs showed promising results for biomedical applications. Further studies must be conducted at embryonic level, animal studies & in vitro studies to study the cytotoxicity of Magnesium oxide nanoparticles in detail.

Keywords: Cytotoxicity; Magnesiumoxide; Nanoparticles; Spectrophotometer; Brineshrimp Lethality Assay.

Introduction

Magnesium oxide is an important inorganic material and has shown a promising role for application in tumor treatment in medicine. MgO nanoparticles are promising antibacterial agents due to their high resistance to harsh processing conditions [1]. Three main antibacterial mechanisms have been proposed, such as the formation of ROS, the interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell and an alkaline effect. An important aspect of nanoscience is mainly the synthesis of nanoparticles (NPs) of different chemical composition, size, shape and properties [2]. Recently, researchers have found the biological methods for the synthesis of nanoparticles which is an alternative to chemical or physical methods. Biological methods for the production of nanoparticles are considered safe and environmentally friendly, cost- effective and it ensures the complete elimination of toxic chemicals [3]. The synthesis of NPs using biological means, especially plants, is biocompatible, as they secrete functional biomolecules which actively reduce metal ions [4]. Nanoscale magnesium oxide possess unique optical, electronic, magnetic, thermal, mechanical and chemical properties due to its unique properties [5]. Nano magnesium oxide has an advantage of being prepared from readily available and economi-

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cal precursor and solvents and therefore it is considered as potent solid bactericidal material under simple condition .Magnesium Oxide Nanoparticles considered as important because they have unique properties when compared to bulk materials and it has excellent properties like high chemical stability, high photocatalytic activity, high electrical properties, non-toxic nature makes MgO nanoparticles to be very unique [6]. In this work, the synthesis of magnesium oxide was performed using an extract of Phyllanthus emblica. The fruit extract of the Phyllanthus emblica, commonly referred to as indian gooseberries (amla), has potent anticancer properties. The bioactivity in this extract principally mediated by polyphenols, especially tannins and flavonoids.The Phyllanthus emblica can incorporate both cancer- prevention and anticancer properties [7]. This study presents a biological method for the synthesis of MgONPs using the extract Phyllanthus emblica. Magnesium oxide Nps are highly ionic nanoparticle metal oxide with extremely high surface areas and crystal morphologies. Cytotoxicity test is used to measure the degree of toxicity on certain cells. The brine shrimps were used because they are cost effective, extremely sensitive to toxicity, easily available & involves easy laboratory operation methods to determine the acute toxicity.Compounds that have cytotoxic effect often compromise cell membrane integrity [8]. Artemia is one of the most valuable test organisms available for ecotoxicity testing, and the available research suggests that several applications of artemia to toxicology and ecotoxicology will continue to be used widely because of the rapidity, convenience, and low cost of artemia - based assays. Previously our team has a rich experience in working on various research projects across multiple disciplines [9-23]. Now the growing trend in this area motivated us to pursue this project.

In the present study, we have used Phyllanthus emblica fruit seed for the green synthesis of MgONPs and the synthesized nanoparticles were characterized using ultraviolet visible (UV-Vis) spectroscopy. Finally, cytotoxicity analysis of the prepared MgONPs was done using brine shrimp lethality assay [BSLA] [24, 25].

Materials And Methods

Preparation of plant extract

To prepare the Phyllanthus emblica (amla) extract; 5 g of amla leaves were washed thoroughly with distilled water and dried for 15-20 minutes at room temperature. The extract solution was prepared by boiling dried leaves in a 500ml beaker consisting 200 ml of distilled water for one hour at 100 degree celsius. Freshly prepared amla leaf extract was used for the synthesis of MgONPs .

Synthesis of magnesium oxide nanoparticles

5 ml of fresh Phyllanthus emblica extract and 20ml of distilled water was added to a 250ml beaker and heated at 600 C. 5 gram of Magnesium Nitrate is added to the solution and heated at 800 C with continuous stirring for 4hours. The Magnesium nitrate ions were reduced to Magnesia or Magnesium Oxide nanoparticles by using Phyllanthus emblica (amla) extract. The formation of Magnesium oxide nanoparticles (MgONPs) have been observed by color change of the solution from yellow to yellowish-brown color.

Optimization of synthesis of magnesium oxide nanoparticles

The green synthesis of MgONPs was mediated by using different concentrations of Phyllanthus emblica (amla) extracts. Different concentration viz. 5ml, 10ml, 25ml, 50ml of freshly prepared aqueous extracts have been used as a reducing agent. Among 5ml, 10ml, 25ml, 50ml concentrations used 5ml of extract was found to be good for the synthesis of MgONPs.

Effect of stirrer temperature T

he influence of stirring temperature for the green synthesis of MgONPs were studied by exposing the precursors in range of temperature from 350 C to 1500 C. Among 350 C, 500 C, 800C, 1000 C and 1500 C Stirring temperatures used 800 C of Stirring temperature was found to be good for the synthesis of MgONPs.

Brine Shrimp Lethality Assay [BSLA]

Brine shrimp eggs were obtained from the Aquatic Remedies, Chennai. Filtered, artificial seawater was prepared by dissolving 36 g of sea salt in 1 litre of distilled water for hatching the shrimp eggs. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) attracts the hatched shrimps. Two days were allowed for the shrimps to hatch and mature as nauplii (larva). After 2 days, when the shrimp larvae were ready, 5 mL of the artificial seawater and 5 mL of nanoparticles solution were added to each test tube, and 10 brine shrimps were introduced into each tube [Figure 1 and 2].The negative control wells contained 10 Nauplii and artificial sea water only without magnesium oxide nanoparticles. In a well of 12 ELISA plates, 6-8 ml of salt water was added followed by adding 10 nauplii to each well at different concentrations of magnesium oxide nanoparticles (5ul,10ul,20ul,40ul & 80ul) and incubated for 24 hours. After 24 hours, the total number of live and dead nauplii was counted and the mortality rate was checked.The percentage mortality (%M) was calculated by dividing the number of dead nauplii by the total number and then multiplied by 100.This is to ensure that the dead (Mortality) of the nauplii is attributed to the compounds present in the nanoparticles.

% of death = (Number of dead Nauplii/ Number of dead Nauplii + Number of Live Nauplii) × 100

The test tubes were left uncovered under the lamp. The number of surviving shrimps observed under compound light microscope were counted and recorded after 24 hours. Using the lethality concentration [LC 50] determined by evaluating the percentage % mortality.

Results And Discussion

Visual Observation

Magnesium oxide was synthesized by a green synthesis method from magnesium nitrate, NaOH using amla extract. The influence of various parameters viz., Stirring temperature, Concentration of Amla extract, Color change of MgONPs were also checked and conditions were optimized for the synthesis of MgONPs [

Figure 3].

UV- Vis Spectroscopy

UV-Vis absorption spectroscopy is the most widely used method for characterizing the optical properties and electronic structure of nanoparticles, as the absorption bands are related to the diameter and aspect ratio of metal nanoparticles [26]. In this study, the prepared MgONPs were confirmed by UV-Vis spectroscopy. The absorption spectra response of MgONPs was observed at 385.0nm. [Figure 4]. This proves the reduction of magnesium nitrate and the emergence of MgO.

Cytotoxicity Analysis

Results of brine shrimp lethality bioassay were estimated using lethality concentration .The study revealed that Phyllanthus emblica mediated magnesium oxide nanoparticles have mild cytotoxic effects at higher concentrations. After 24 hours of incubation of the brine shrimps in the nanoparticle solution, most of the brine shrimps survived at all concentrations ,only 10% nauplii were dead at 80µl concentration. [Figure 5]. Cytotoxicity test was used to measure the degree of toxicity on certain cells. The brine shrimps lethality bioassay was employed here in order to predict its suitability for pharmaceutical applications [27]. Generally, this brine shrimp cytotoxicity bioassay test may be used as a preliminary test to investigate anticancer and pesticidal activities. Therefore, this cytotoxicity test contributes to a significant impact on this research.

From the current study, it is noted that magnesium oxide nanoparticles prepared from Phyllanthus emblica showed least cytotoxic effect at the lowest concentrations. Hence, if the concentration is below 80 ul, it can be used for biomedical applications.

Our institution is passionate about high quality evidence based research and has excelled in various fields [13, 28-37].

Conclusion

The biosynthesis of MgONPs show promising results for biomedical applications. An absorption peak at 385.0 nm in UV- Vis Spectrum proves the formation of MgONP's from Phyllanthus emblica fruit extract. The overall cytotoxicity of magnesium oxide nanoparticles is less, as only 10% nauplii were dead. Further studies must be conducted in the embryonic level, animal studies & in vitro studies to confirm the cytotoxicity of Magnesium oxide nanoparticles.

Acknowledgement And Declarations

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Figure 1. Demonstrating ELISA plate added with salt water, MgONPs & nauplii.

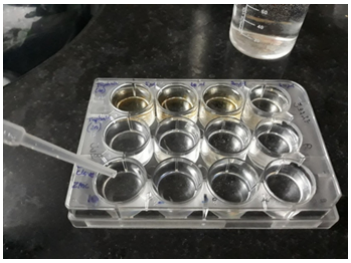


Figure 2. Demonstrating Brine shrimp nauplii under light microscope at 40X magnification.

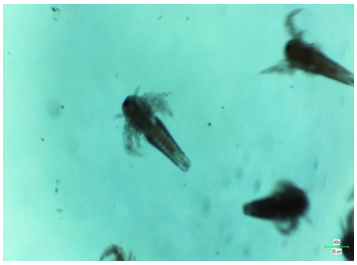


Figure 3. Demonstrating Visual Observation of MgONPs reduced by Phyllanthus emblica.

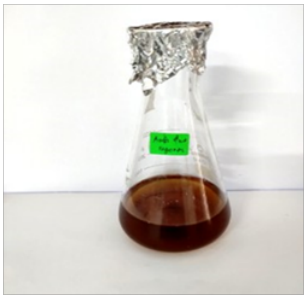


Figure 4. Demonstrating the absorption spectra response of MgONPs synthesized using Phyllanthus emblica extract . The peak was observed at 385.0nm at 48 hour confirmed the presence of MgONPs.

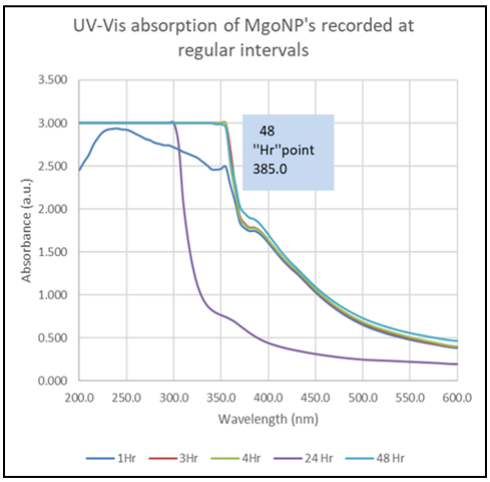
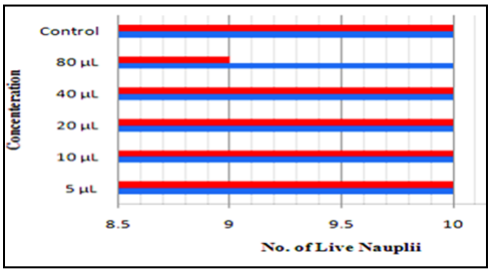


Figure 5. Bar graph representing the Cytotoxicity of magnesium oxide nanoparticles.



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