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Antioxidant & Anti Inflammatory Activity 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 evaluate the antioxidant and anti inflammatory activity of magnesium oxide nanoparticles (MgONps) using Phyllanthus emblica fruit extract.

Materials and Methods: MgONPs were synthesised using Phyllanthus emblica fruit seed extract, and antioxidant & anti inflammatory activity of synthesized MgONPs were assessed using DPPH radical assay & by Albumin denaturation 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 peak was at a wavelength of 385.0 nm. DPPH assay was used to evaluate the antioxidant activity of newly synthesized nanoparticles and it showed good antioxidant activity. Albumin Denaturation assay was used to evaluate the anti inflammatory activity of newly synthesized nanoparticles and the results showed excellent anti inflammatory activity.

Conclusion: Biosynthesised MgONPs showed promising results for biomedical applications. Hence, it may be employed in large scale production and may be used in many medicinal applications.

Keywords: Antioxidant activity, Anti inflammatory, Magnesium oxide, Nanoparticles, Phyllanthus emblica.

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 economical precursor and solvents and therefore it is considered as potent

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solid bactericidal material under simple condition .Magnesium Oxide Nanoparticles have unique properties when compared to bulk materials and have excellent properties like high chemical stability, high photocatalytic activity, high electrical properties, non-toxic nature etc. [6]. In the present 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 is principally mediated by polyphenols, especially tannins and flavonoids. The Phyllanthus emblica can incorporate both cancer- prevention and anticancer properties [7]. Magnesium oxide Nps are highly ionic nanoparticle metal oxide with extremely high surface areas and crystal morphologies. Nanotechnology has developed in such a way that it has a huge horizon of applications such as drug delivery, gene transfection, tissue regeneration, and antibacterial as well as antiinflammatory applications. The high biocompatibility of noble metals made them attain more importance among researchers on nanotechnology. One of the major challenges for the production of nanoparticles is the use of environmental-friendly materials that come under green chemistry, to avoid the production of hazardous substances. Magnesium oxide Nps are highly ionic nanoparticle metal oxide with extremely high surface areas and crystal morphologies [8, 9].

Previously our team has a rich experience in working on various research projects across multiple disciplines [10-24] Now the growing trend in this area motivated us to pursue this project.

In the present study, we have used Phyllanthus emblica. fruit extract for the green synthesis of MgONPs and the synthesized nanoparticles were characterized using ultraviolet visible (UV-Vis) spectroscopy. Hence, the aim of the study was to evaluate the antioxidant and anti inflammatory activity of magnesium oxide nanoparticles (MgONps) using Phyllanthus emblica fruit extract.

Materials And Methods

Preparation of extract

To prepare the Phyllanthus emblica (amla) extract; 5 g of amla fruit were washed thoroughly with distilled water and dried for 15-20 minutes at room temperature. The extract solution was prepared by boiling dried fruit in a 500ml beaker consisting 200 ml of distilled water for one hour at 100 degree celsius. Freshly prepared amla 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 4 hours. 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

The 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.

Antioxidant Activity by DPPH assay

Hydrogen donating capacity or free radical scavenging by the nanoparticles was evaluated by the DPPH measure, which depends on the reduction of the methanolic coloured radical type of the DPPH to the non-coloured solution. Different concentrations (10-50 μ g/ml) of the nanoparticle was taken in the ELISA plate. To each ELISA plate a measured quantity of 0.2 mM of DPPH (2,2- diphenyl-1-picryl hydrazyl) was added. 1 ml of DPPH was added to 2 ml of methanol solution to act as a control group. Ascorbic acid was used as the standard, which was employed to compare with test nanoparticles. The ELISA plate was incubated in a dark condition for 30 minutes. After incubation, reduction in the number of DPPH free radicals measured by UV Spectrophotometer at 517 nm absorbance.

% Inhibition was calculated using the following formula [%Radical scavenging activity]:-

% of inhibition = (Control Absorbance - Sample Absorbance/ Control Absorbance) × 100

Anti inflammatory Activity by Albumin Denaturation Assay:

Albumin denaturation assay was done by using Bovine Serum albumin. 2 ml of 1% Bovine albumin fraction was mixed with 400 ml of plant extract in different concentrations. The pH of the mixture was adjusted to 6.8 by adding hydrochloric acid. The reaction mixture was incubated at room temperature for 20 mins in a water bath. The mixture was cooled to room temperature & the absorbance value was recorded at 660 nm. DMSO (Dimethyl Sulfoxide) was used as control. Diclofenac sodium in different concentrations was used as standard.

% Inhibition was calculated using the following formula:

% of inhibition = (Control OD – Sample OD)/ Control OD × 100

Results And Discussion

Visual Observation

Magnesium oxide was synthesized by a green synthesis method from magnesium nitrate, NaOH using amla extract. The influence

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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 1].

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 [25]. In this study, the prepared MgONPs were confirmed by UV-Vis spectroscopy. The absorption spectra response of MgONPs was observed at 385.0nm. [Figure 2]. This proves the reduction of magnesium nitrate and the emergence of MgO.

Antioxidant activity

The mechanism behind the antioxidant property is attributed to the inhibition of chain reaction, decomposition of peroxides, binding of transition metal ion catalysts, radical scavenging activity and inhibition of continued hydrogen abstraction. The free radicals present are unstable which cause cellular damage due to the generation of ROS that interact with other molecules in the biochemical reactions. The properties of absorbing, neutralizing these free radicals or quenching singlet and triplet oxygen are few crucial factors that are responsible for the antioxidant activity. The highest antioxidant activity is attributed due to the presence of various bio-reductive groups of the phytochemicals present on the surface of the MgONPs. According to Figure 3, the radical scavenging property of the MgONPs, when compared with the standard ascorbic acid shows that the plant mediated MgONPs have the potential to used as an antioxidant as it shows mild comparable effect to that of the standard antioxidants.During the experiment the presence of MgONPs dissolved in DPPH, the color changed from deep violet to pale yellow solution, which indicated the scavenging of free radicals is complete.

Anti inflammatory activity

Bovine serum albumin (BSA) makes up around 60% of all the proteins in animal serum. It is commonly used in cell culture, particularly when protein supplementation is necessary and the other components of the serum are unwanted. BSA undergoes denaturation upon heating and starts expressing antigens associated with Type III hypersensitivity reaction which are related to diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness and systemic lupus erythematosus. Denaturation of tissue proteins is one of the causes of inflammatory and arthritis. Formation of auto antigens in certain diseases may be due to denaturation of proteins [18, 19]. Agents that can prevent protein denaturation, would be useful for anti-inflammatory drug development. The ability of a substance to inhibit the denaturation of protein signifies apparent potential for anti-inflammatory activity. The Phyllanthus emblica mediated magnesium oxide nanoparticles showed a dose-dependent anti-inflammatory activity in this study. When compared with the standard diclofenac sodium, the Phyllanthus emblica mediated MgONPs gave almost equal anti inflammatory activity at 50 µl concentration [Figure 4]. From the present study it can be concluded that Phyllanthus emblica mediated MgONPs have excellent anti inflammatory effect.

Our institution is passionate about high quality evidence based research and has excelled in various fields [15, 26-35].

Figure 1. Demonstrating Visual Observation of MgONPs reduced by Phyllanthus emblica.



Figure 2. 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 4. Demonstrating Anti inflammatory property of Phyllanthus emblica mediated Magnesium oxide nanoparticles.



Conclusion

Within the limitations of the study, it can be seen that *Phyllanthus emblica* mediated MgONPs have a potential to be used as an antioxidant and anti-inflammatory agent in dentistry and can be used as an alternative to commercially available products. Hence, MgONPs can be a potent therapeutic agent against many biomedical applications, which could be a potential area that can be explored in future.

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