SYNTHESIS OF MAGNESIUM OXIDE NANOPARTICLES FROM *OCIMUM TENUIFLORUM* AND ITS TOXICITY ANALYSIS AND ANTIMICROBIAL ACTIVITY

Keerthana B [1], Dr. Nidhita Suresh [2], Dr. Karthikeyan M [3]

1.Undergraduate Student, Department of Periodontics,
Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences(SIMATS)
Saveetha University, Chennai, Tamil Nadu, India.Email Id: 151901090.sdc@saveetha.com

2. Senior lecturer,Department of Periodontics,Saveetha Dental College,
Saveetha Institute of Medical and Technical Sciences(SIMATS) Saveetha University,
Chennai, Tamil Nadu, India.Email Id: nidhitas.sdc@saveetha.com

3. Senior Lecturer,Department of Periodontics, Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences (SIMATS) Saveetha University,
Chennai-600077, Tamil Nadu,India.

Corresponding Author:Dr. Nidhita Suresh

Senior lecturer, Department of Periodontics,Saveetha Dental College,
Saveetha Institute of Medical and Technical Sciences(SIMATS) Saveetha University,
Chennai, Tamil Nadu, India.Email Id: nidhitas.sdc@saveetha.com

ABSTRACT:

Introduction: Ocimum tenuiflorum aka O.sanctum, known as holy basil, belongs to the laminaceae family. It contains oleanolic acid, Ursolic acid, eugenol and various compounds also has various properties such as anti-diabetic, antioxidant, antimicrobial, anticancer, antihelminthic, cardioprotective. Nanotechnology nowadays is expanding greatly in the field of medicine and pharmacy. An important issue that arises is the drug resistance; to overcome this nanotechnology plays a major role wherein nanoparticles reduce the bacterial growth without creating resistance like antibiotics

Materials and method: Green extract of the plant was prepared and to which nanoparticle synthesis was done with the help of magnesium nitrate hexahydrate. The anti microbial activity was checked for 2 gram positive bacteria and 2 gram negative bacteria using kirby bauer well diffusion assay. The toxicity was checked with the help of zebra fish by adding the prepared nanoparticles and with a control group, and their mortality rate was checked.

Results and discussion: It was seen that the higher the concentration of the nanoparticles synthesized from the plant, the more the zone of inhibition. The toxicity analysis reveals that the nanoparticles showed reduced toxicity.

Conclusion: It is concluded that MgO NPs synthesized with O.tenuiflorum shows greater anti microbial activity and its non-toxic.

1. INTRODUCTION

Nanotechnology has a greater impact in the economics of many countries worldwide and in recent years it has greatly provided a new scope for various human needs. Nanotechnology nowadays is expanding greatly in the field of medicine and pharmacy. An important issue that arises is the drug resistance; to overcome this nanotechnology plays a major role wherein nanoparticles reduce the bacterial growth without creating resistance like antibiotics[(1)]. Many nanoparticles are present like zinc oxide, silver nanoparticles, gold nanoparticles, titanium oxide nanoparticles showing antimicrobial properties, high surface area for interaction with cells, stability in unfavorable conditions. But the limitation is the associated toxicity due to the heavy metal that accumulates in the body[(2)] .Magnesium oxide (MgO) nanoparticles have garnered significant attention due to their diverse application in areas such as catalysis, medicine and environmental remediation. They have a strong antimicrobial activity along with high thermal stability and are inexpensive. MgO is being used as a mineral supplement for magnesium which is an essential nutrient in the body. MgO nanoparticles (MgO NPs) have been reported to have decreased toxicity comparatively [(3)]

Ocimum tenuiflorum, commonly known as holy basil or Tulsi, is a revered herb in Indian culture and holds significant importance in traditional medicine systems like Ayurveda. This aromatic plant belongs to the Lamiaceae family and is native to the Indian subcontinent. Tulsi is a perennial plant that typically grows up to 60-90 cm in height. It has slender stems, delicate green leaves, and small, purple or reddish flowers arranged in spikes. The leaves are the most prized part of the plant, possessing a strong, sweet aroma and a slightly peppery taste. This plant has extensive properties such as anti-diabetic, antioxidant, antimicrobial, anticancer, antihelminthic, cardioprotective etc. Hence this plant is regarded as a “elixir of life” and called holy basil [(4)].

Previous studies revealed that Ocimum tenuiflorum leaf extract contains alkaloids, terpenoids, phlobatannins, and glycosides whereas saponins were absent. Alkaloids in plants are used as anesthetic agents [(5)]. MgO NPs along with its non toxic activity, it also seemed to be biodegradable and it inhibits the growth of biofilm. They are also being used in removal of industrial pollutants, anti-arthritic and anti-cancer activities [(6)]. The metal nanoparticles synthesized from plants were found to be more stable and it was found to be effective against 3 bacterias which were E.coli, C. bacterium, B.subtilis. [(7)]

The synthesis of MgO NPs from Ocimum tenuiflorum holds great promise for several reasons. Firstly, the use of plant extracts not only aligns with sustainable practices but also minimizes the need for hazardous chemicals, making it environmentally benign. Secondly, the phytochemicals present in Ocimum tenuiflorum may serve as reducing and stabilizing agents during the synthesis process, influencing the size, shape, and properties of the resulting MgO NPs.[(8)] This unique interplay between the plant's bioactive compounds and the nanoparticle synthesis process offers an exciting area of investigation[(8)].This study aims to synthesize Magnesium oxide nanoparticles from Ocimum tenuiflorum extracts and evaluate their toxicity profile as well as investigate their antimicrobial potential.

2. MATERIALS AND METHOD

2.1. SAMPLE COLLECTION

Fresh leaves of O. tenuiflorum were collected, followed by dense cleaning of the leaves that were collected using distilled water in order to remove the dust particles. The leaves were dried for a week in shade and grinded to produce fine powder which was stored for further use.

2.2. PREPARATION OF GREEN EXTRACT

Wet chemical method was done for the green synthesis of the plant. 10 g of the powdered leaves were dissolved in 100ml of distilled water and the mixture obtained was maintained at a boiling boiling temperature of 80°C. Whatman grade 1 filter paper was used to filter the extract. The resulting solution was treated to nano-particles as a stabilizing agent.

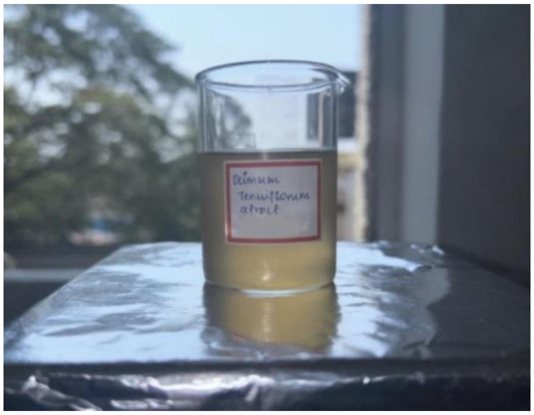

Figure 1: Colloidal suspension or formulation of Ocimum tenuiflorum nanoparticles, displayed in a laboratory setting with a reflective surface in the background.

2.3. SYNTHESIS OF NANOPARTICLES

1 million molars of magnesium nitrate hexahydrate was added to 100ml of prepared O.tenuiflorum extract which was kept in a magnetic stirrer for 2 hours. Clear

white precipitate was obtained which was dried for 24 hours followed by which calcination was done for 2 hours.

Figure 2: Colloidal suspension of magnesium oxide (MgO) -based nanoparticles prepared in formulation, displayed in a laboratory setting with a reflective surface background.

2.4. IN VITRO TOXICITY ASSESSMENT

The zebrafish embryos were used to test the toxicity of the NPs. The assessment of mortality rate of the zebrafish over a predetermined period of time was done by taking the NPs with varying concentrations which were treated with plant extract and compared with the control (untreated embryos). The OECD-203 guidelines were followed for this assessment. 25 marked fish eggs were taken which were treated with Hank’s solution at a different concentration (25,50, 75,100 and 200 μg/mL); after which the eggs were moved to a separate well for the development of head, tail and eyes. The evaluation was done every 24 hours in which the number of dead and live fish were recorded. The water was maintained at the same temperature throughout the evaluation.

2.5. ANTIMICROBIAL ACTIVITY

The antibacterial property was assessed towards the disease causing microorganisms. The gram positive bacteria (S. aureus, S. mutans) and the gram negative bacteria (E.coli, K. pneumoniae) were considered for this study. For the assay, the nutrient broth was freshly prepared and sterilized. The bacteria samples were inoculated separately and allowed to incubate at 37°C for 8 hours. According to Kirby and Bauer, the well diffusion assay was done and the inoculums were spread on the outer surface of Muller Hinton Agar plates. The plates include the discs of 5mm which were added with the standard antibiotic to A, the NPs at low concentration to B and NPs with higher concentration were added to C. The plates were then allowed to incubate for 8 hours at 37°C and the results were obtained as zones. (9)

3. RESULTS

	Control (Mortality %)	Sample (Mortality %)
24h	0.03	0.12
48h	0.12	0.32
72h	0.32	0.45
96 h	0.54	0.65
120 h	0.65	0.95

Table 3.1: Toxicity analysis table comparing mortality rates of the control and synthesized magnesium oxide nanoparticles over 120 hours in a model organism. This table presents a comparative analysis of mortality rates (%) over time for the synthesized nanoparticles sample versus the control. Higher mortality is observed for the sample, increasing consistently over 120 hours, indicating dose-dependent toxicity.

Bacteria	Concentration with inhibition
Staphylococcus aureus	Abs-14mm
	LC- 17mm
	HC- 21mm
Streptococcus mutans	Abs-15mm
	LC- 16mm
	HC- 19mm
Escherichia coli	Abs- 15mm
	LC-16mm
	HC-21mm
Klebsiella pneumoniae	Abs- 16mm
	LC- 18mm
	HC- 21mm

Table 3.2: Table summarizing the zone of inhibition (in mm) against bacterial strains at different concentrations of synthesized magnesium oxide nanoparticles (ABS: Absolute, LC: Low Concentration, HC: High Concentration). This table quantifies the antimicrobial activity of the nanoparticles against four bacterial strains. The inhibition zones are measured in millimeters at three nanoparticle concentrations

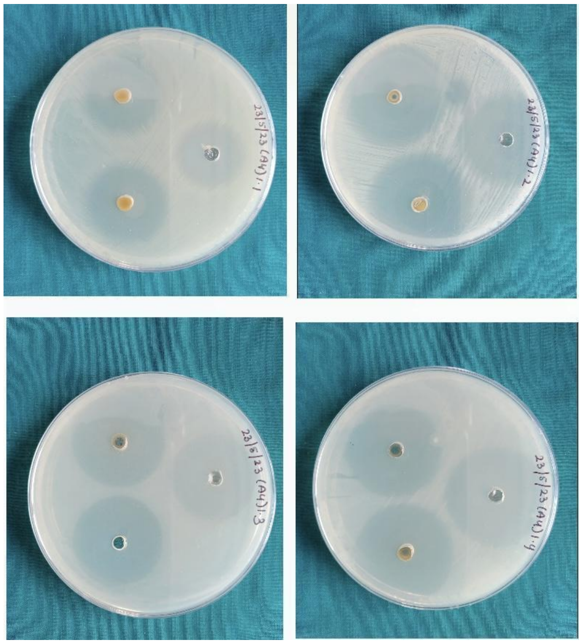

Figure 3.3: Zone of Inhibition assay demonstrating antibacterial activity of magnesium oxide nanoparticles against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Klebsiella pneumoniae* at various concentrations (absolute, low, and high). The image displays Petri dishes showing zones of inhibition against bacterial strains. These zones are formed around discs treated with synthesized magnesium oxide nanoparticles. Clear areas indicate antibacterial activity, where microbial growth has been inhibited.

DISCUSSION:

The table 1 shows the toxicity analysis of the nanoparticles that were treated with O. tenuiflorum extract and without the treatment. The assessments were made at 24, 48,72,96 and 120 th hours and it can be seen from the table that the NPs that were added with the plant extract tend to show less toxicity when compared to the control.

The table 2 shows the tabulated result of the antimicrobial assay of gram positive bacteria (S. aureus, S. mutans) and the gram negative bacteria (E.coli, K. pneumoniae) against standard antibiotics, plant extract NPs at low and high concentration respectively. It can be seen that standard antibiotics against staphylococcus aureus show 14mm inhibition, at low concentration of NPs 17mm inhibition and at high concentration 21mm inhibition was observed. Streptococcus mutans show 15mm inhibition with antibiotics, 16mm with NPs at low concentration and 19mm with NPs at high concentration. Escherichia coli shows 15mm inhibition with antibiotics, 16mm with NPs at low concentration and 21mm with NPs high concentration. Klebsiella pneumoniae shows 16mm with antibiotics, 18mm with NPs at low concentration and 21mm with NPs at high concentration. From this it can be observed that increased concentration of nanoparticles increases in the zone of inhibition.

The synthesis of magnesium nanoparticles with natural sources like O. tenuiflorum and their subsequent toxicity and anti-microbial assay shows a widely interested field for research in recent years(10). Many previous studies have also explored similar themes.(11)

Singh et al synthesized silver nanoparticles from various plant derived species. This provides a potential plant derived material as a reducing agent(12). Similarly Shukla et al explored the metal oxide nanoparticles synthesized using plant extracts elevating the importance of botanical sources in the research field(13). Also Khan et al in the year focused on the zinc oxide nanoparticles that were synthesized using plant extracts including O.tenuiflorum highlighting the cost-effectiveness, eco friendly method that provide enhanced stability and biocompatibility

Furthermore the toxicity analysis is one of the critical aspects of evaluating the biocompatibility of the nanoparticles. Jia et al assessed the toxic effects of titanium dioxide nanoparticles in animal models (14) and Hackenberg et al investigated the cytotoxic effect of the zinc oxide nanoparticles on human cells (15) This can be evident in providing a foundation of understanding on the potential risks that are associated with nanoparticles exposed to humans.

The antimicrobial activity of the magnesium nanoparticles is important to find the efficiency against a particular disease causing organism. The study conducted by Siddiqi et al shows the antimicrobial activity of zinc oxide nanoparticles against various strains of microorganism(16) This tells us the metal oxide nanoparticles in combating the disease. The study conducted by Priya et al shows the copper oxide nanoparticles highlight the broad spectrum application of the nanoparticles (17). Priya et al shows the potential activity of magnesium oxide nanoparticles against various gram positive and gram negative bacteria which is similar to this study (17,18).

The biomedical application has also been explored in many studies. Behzadi et al have mentioned the application of magnesium oxide nanoparticles in cancer therapy due their ability to induce apoptosis in the cancer cells (19) . This expresses the versatility of the magnesium nanoparticles and their potential for targeted therapy that could be a future avenue of research. The synthesis of magnesium oxide nanoparticles from Ocimum tenuiflorum provides a promising area for a wide range of applications such as biomedicine, agriculture etc. Future research should focus on harnessing the full potential of these nanoparticles and ensuring their safety.

4. CONCLUSION

The synthesis of magnesium oxide nanoparticles (MgO NPs) using Ocimum tenuiflorum demonstrates an eco-friendly and efficient approach. These nanoparticles exhibit significant antimicrobial activity against various pathogens, highlighting their potential as an alternative to conventional antibiotics. The toxicity analysis reveals a low cytotoxic profile, ensuring biocompatibility for biomedical applications. Overall, the green synthesis of MgO NPs from Ocimum tenuiflorum provides a sustainable pathway for developing advanced antimicrobial agents. Further studies can optimize their usage in healthcare and environmental applications.

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