REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 01s (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

Anti-Inflammatory Potential Of Green Synthesized Magnesium Oxide From Tamarindus Indica

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Abstract
The development of biocompatible and eco-friendly nanomaterials is a primary objective in contemporary nanomedicine. This study reports the successful green synthesis of magnesium oxide nanoparticles (MgONPs) using the aqueous leaf extract of *Tamarindus indica* as a potent bioreducing and stabilizing agent. The biosynthesized MgONPs were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), and Field Emission Scanning Electron Microscopy (FESEM) coupled with Energy Dispersive X-ray (EDX) analysis. UV-Vis spectra confirmed the formation of MgONPs with a characteristic surface plasmon resonance peak at 284 nm. XRD analysis revealed a highly crystalline, face-centered cubic structure with an average crystallite size of 22.4 nm. FTIR results confirmed the presence of polyphenolic and flavonoid functional groups on the nanoparticle surface, facilitating their stability. The in vitro anti-inflammatory potential was evaluated using the bovine serum albumin (BSA) denaturation assay. The green-synthesized MgONPs exhibited significant dose-dependent anti-inflammatory activity, achieving a maximum inhibition of 83.2% at 100 µg/mL (IC_{50} = 49.6 µg/mL), which was comparable to the standard drug Ibuprofen (IC_{50} = 38.2 µg/mL). These findings suggest that *T. indica*-mediated MgONPs serve as a promising, sustainable, and bioactive alternative for the management of inflammatory conditions.

Introduction
Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. This response involves a coordinated cascade of cellular and molecular events designed to eliminate the initial cause of injury, clear necrotic cells and damaged tissues, and initiate tissue repair. While inflammation is a critical protective mechanism essential for survival, dysregulated and chronic inflammation represents a fundamental driver in the pathogenesis of numerous severe conditions, including rheumatoid arthritis, asthma, and cardiovascular diseases [1, 2]. The transition from acute, self-limiting inflammation to persistent, uncontrolled inflammation underlies the progression of these debilitating disorders and contributes significantly to global morbidity and mortality.
Conventional pharmacological interventions for managing inflammatory conditions predominantly rely on non-steroidal anti-inflammatory drugs and corticosteroids. These agents act through well-characterized mechanisms: non-steroidal anti-inflammatory drugs inhibit cyclooxygenase enzymes, thereby reducing the synthesis of pro-inflammatory prostaglandins, while corticosteroids exert broad anti-inflammatory effects through modulation of gene expression and suppression of multiple inflammatory mediators. However, the prolonged administration of these agents is frequently accompanied by severe adverse effects that limit their long-term clinical utility. The adverse effect profile ranges from gastrointestinal ulceration and hepatotoxicity to cardiovascular complications and renal dysfunction, with the risk increasing substantially with the duration of therapy [3, 4]. These limitations underscore a critical and unmet need to develop alternative, highly efficacious anti-inflammatory therapeutics with significantly improved safety profiles, particularly for patients requiring long-term management of chronic inflammatory conditions.
In recent years, nanomedicine has emerged as a transformative frontier in biomedical research, offering novel modalities for therapeutic intervention that leverage the unique properties of materials at the nanoscale. The ability to engineer materials with precisely controlled size, shape, and surface chemistry has opened new avenues for drug delivery, imaging, and targeted therapy. Metal oxide nanoparticles, in particular, have garnered substantial clinical interest due to their unique physicochemical properties, high surface-area-to-volume ratios, and enhanced cellular penetration compared to their bulk counterparts [5]. These properties enable more efficient interaction with biological systems, potentially translating to improved therapeutic efficacy at lower doses.
Among the diverse array of metal oxide nanoparticles, magnesium oxide nanoparticles are highly regarded for their exceptional biocompatibility and minimal toxicity profile. Recognized as safe by the United States Food and Drug Administration, magnesium is an essential intracellular mineral that participates in numerous physiological processes, including enzymatic reactions, energy metabolism, and maintenance of membrane integrity. Its nanoscale oxide derivatives have been extensively explored for their antibacterial, antioxidant, and tissue-regenerative properties, making them attractive candidates for biomedical applications [6, 7]. The inherent biocompatibility of magnesium oxide nanoparticles offers a distinct advantage over other metal oxide systems that may exhibit cytotoxicity or long-term accumulation concerns.

The conventional synthesis of nanoparticles typically involves physical and chemical methodologies that necessitate high energy consumption, toxic solvents, and hazardous chemical reducing agents. These protocols, while effective in producing nanoparticles with controlled properties, not only pose significant environmental risks but also leave toxic residues adsorbed on the nanoparticle surface. These residual contaminants severely limit the applicability of conventionally synthesized nanoparticles in biological systems, as they can induce unintended cytotoxic effects or interfere with the intended therapeutic activity [8]. To circumvent these limitations, biological or green synthesis has been rapidly adopted as an eco-friendly, cost-effective, and biocompatible alternative that aligns with the principles of sustainable chemistry.

Plant-mediated synthesis is particularly advantageous among the various biological approaches, as botanical extracts possess a rich milieu of phytochemicals that can serve dual functions during nanoparticle formation. The diverse array of secondary metabolites present in plant tissues, including flavonoids, alkaloids, phenols, and terpenoids, function simultaneously as potent bioreducing agents that convert metal ions to their zero-valent or oxide states and as stabilizing or capping agents that prevent nanoparticle agglomeration and ensure colloidal stability [9, 10]. This integrated approach eliminates the need for external reducing and stabilizing agents, simplifying the synthesis process and producing nanoparticles with surfaces that may retain bioactive phytochemicals, potentially contributing synergistic therapeutic effects.

Tamarindus indica L., belonging to the Fabaceae family and commonly known as tamarind, is a ubiquitous tropical plant with a well-documented history in traditional medicine systems across Asia, Africa, and the Americas. The various parts of this plant, including the fruit pulp, seeds, bark, and leaves, have been employed in traditional remedies for conditions ranging from digestive disorders to infectious diseases. The foliar extracts of T. indica are exceptionally rich in diverse polyphenolic compounds, including orientin, vitexin, and ascorbic acid, which are known to possess potent antioxidant properties. These phytochemical constituents have been empirically shown to exhibit robust antioxidant and immunomodulatory properties in various experimental models [11, 12]. The presence of these bioactive compounds positions T. indica as a promising candidate for the green synthesis of functional nanoparticles.

Despite the extensive phytochemical profiling of T. indica and its historical use in managing inflammatory ailments, its specific utility as a bioreductive factory for the green synthesis of therapeutic magnesium oxide nanoparticles remains inadequately explored in contemporary literature. This gap in knowledge represents a missed opportunity to develop a sustainable, biocompatible nanomaterial that could potentially address the limitations of conventional anti-inflammatory therapies.

Therefore, the present study aims to synthesize magnesium oxide nanoparticles utilizing the aqueous leaf extract of T. indica as a green reducing and capping agent, thereby establishing an eco-friendly and sustainable fabrication protocol. Furthermore, to validate their therapeutic viability, this study rigorously evaluates the in vitro anti-inflammatory potential of the biosynthesized magnesium oxide nanoparticles utilizing the established protein denaturation assay, which serves as a reliable screening tool for anti-inflammatory activity. By elucidating the relationship between the green synthesis approach, the physicochemical properties of the resulting nanoparticles, and their biological activity, this study aims to explore their potential as a novel nanotherapeutic alternative to conventional anti-inflammatory drugs, offering a pathway toward safer and more sustainable treatments for inflammatory conditions.

Materials and Methods

Materials and Reagents

Magnesium nitrate hexahydrate, bovine serum albumin, ibuprofen, sodium hydroxide, and hydrochloric acid were of analytical grade and procured from Sigma-Aldrich. All aqueous solutions were prepared using double-distilled water to ensure the absence of interfering ions that could affect the synthesis process or subsequent biological assays. Fresh, healthy leaves of Tamarindus indica were collected from the local region during the growing season to ensure optimal phytochemical content. The plant material was authenticated by a botanical taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference.

Preparation of Tamarindus indica Leaf Extract

The collected T. indica leaves were thoroughly washed with tap water to remove epiphytes, adhering dust particles, and other surface contaminants, followed by a final rinse with double-distilled water to eliminate any residual ions that might interfere with the synthesis process. The leaves were shade-dried at room temperature for seven days, a method chosen to prevent the degradation of heat-sensitive phytochemicals that could be compromised by exposure to elevated temperatures. The drying process was monitored until constant weight was achieved, indicating complete removal of moisture.

The dried leaves were pulverized into a fine powder using a mechanical blender, ensuring uniform particle size to facilitate consistent extraction efficiency. To prepare the aqueous extract, 10 grams of the leaf powder was added to 100 milliliters of double-distilled water in a 250 milliliter Erlenmeyer flask. The mixture was heated at 60°C for 30 minutes under continuous magnetic stirring, a temperature and duration selected to optimize the extraction of bioactive phytochemicals while minimizing thermal degradation. The resulting infusion was cooled to room temperature and filtered sequentially

through Whatman No. 1 filter paper to remove particulate matter, yielding a clear, pale-yellow extract. The extract was stored at 4°C for subsequent synthesis and used within 48 hours to ensure the stability of the phytochemical constituents.

Green Synthesis of Magnesium Oxide Nanoparticles

For the biosynthesis of magnesium oxide nanoparticles, 20 milliliters of the aqueous T. indica leaf extract was added dropwise to 80 milliliters of a 0.1 molar magnesium nitrate hexahydrate solution in a 250 milliliter Erlenmeyer flask. The dropwise addition was performed under continuous magnetic stirring to ensure uniform mixing and to prevent localized concentration gradients that could lead to heterogeneous nucleation. The reaction mixture was maintained at 80°C under continuous stirring to provide the thermal energy required for the reduction reaction and to promote the formation of a stable colloidal suspension.

The pH of the solution was adjusted to 10 by the dropwise addition of 1 molar sodium hydroxide, which facilitated the precipitation of magnesium hydroxide from the reaction mixture. The pH was monitored using a calibrated pH meter to ensure precise adjustment, as the pH significantly influences the precipitation efficiency and the subsequent properties of the final nanoparticles. Stirring was maintained for 2 hours following pH adjustment until a distinct color change from pale yellow to a darker hue was observed, along with the formation of a visible precipitate, indicating the completion of the bioreduction and precipitation process.

The precipitate was collected via centrifugation at 10,000 revolutions per minute for 15 minutes, a speed sufficient to pellet the nanoparticles while allowing unreacted precursor ions and excess phytoconstituents to remain in the supernatant. The obtained pellet was washed three times with double-distilled water to remove unreacted precursor ions and once with ethanol to eliminate any remaining water-soluble impurities and facilitate drying. The purified pellet was dried in a hot air oven at 80°C for 12 hours to remove residual solvents. Finally, the dried precursor was calcined in a muffle furnace at 400°C for 3 hours to convert the magnesium hydroxide to highly crystalline magnesium oxide nanoparticles. The calcination temperature was selected based on preliminary experiments to ensure complete conversion while preventing excessive particle growth or sintering.

Physicochemical Characterization

The optical properties and preliminary confirmation of magnesium oxide nanoparticle synthesis were monitored using a UV-Vis spectrophotometer in the wavelength range of 200 to 800 nanometers. This technique detects the surface plasmon resonance characteristic of metal oxide nanoparticles, providing an initial indication of successful nanoparticle formation. Samples were diluted appropriately with double-distilled water to obtain measurable absorbance values.

Fourier Transform Infrared spectroscopy was employed to identify the biomolecular functional groups responsible for the reduction and stabilization of the nanoparticles. Spectra were recorded in the range of 4000 to 400 reciprocal centimeters using the potassium bromide pellet technique. This method involved mixing the nanoparticle sample with anhydrous potassium bromide, compressing the mixture into a transparent disc under hydraulic pressure, and scanning the disc to obtain the absorption spectrum.

The crystalline phase and purity of the biosynthesized magnesium oxide nanoparticles were analyzed using X-Ray Diffraction with copper K-alpha radiation. The samples were scanned over a 2-theta range of 20 to 80 degrees at a scan rate of 2 degrees per minute. The resulting diffraction pattern was compared with the standard reference pattern for magnesium oxide from the International Centre for Diffraction Data database to confirm the crystal structure and identify any impurity phases.

Morphological features and elemental composition were determined utilizing Field Emission Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy. The samples were mounted on aluminum stubs using double-sided carbon tape and sputter-coated with a thin layer of gold to enhance conductivity. Images were captured at various magnifications to assess particle morphology, size distribution, and agglomeration state. Energy Dispersive X-ray analysis was performed to confirm the elemental composition and to identify any trace elements present in the sample.

In Vitro Anti-Inflammatory Evaluation: BSA Denaturation Assay

The anti-inflammatory potential of the green-synthesized magnesium oxide nanoparticles was evaluated utilizing the bovine serum albumin denaturation assay with minor modifications to established protocols. This assay is based on the principle that inflammation is associated with the denaturation of tissue proteins, and agents that inhibit this process may possess anti-inflammatory properties.

The reaction mixture consisted of 0.5 milliliters of 1% aqueous bovine serum albumin solution and 0.5 milliliters of varying concentrations of the magnesium oxide nanoparticles, specifically 20, 40, 60, 80, and 100 micrograms per milliliter. The bovine serum albumin solution was prepared fresh before each assay to ensure protein integrity. The pH of the reaction mixture was adjusted to 6.3 using a small amount of 1 normal hydrochloric acid, as this pH is optimal for detecting protein denaturation and its inhibition.

The samples were incubated at 37°C for 20 minutes to allow for interaction between the nanoparticles and the protein, and subsequently heated to 71°C in a water bath for 15 minutes to induce protein denaturation. This temperature was

selected based on preliminary experiments to achieve consistent denaturation while allowing for detection of inhibitory effects. After cooling to room temperature, the turbidity of the solutions was measured spectrophotometrically at 660 nanometers, where denatured proteins exhibit increased absorbance due to aggregation.
Double-distilled water served as the negative control, and standard ibuprofen at concentrations ranging from 20 to 100 micrograms per milliliter was used as the positive reference drug. The experiment was performed in triplicate for each concentration to ensure statistical reliability. The percentage inhibition of protein denaturation was calculated using the following equation:

Inhibition (%) = (A_control - A_test) / A_control × 100

where A_control represents the absorbance of the control reaction without nanoparticles, and A_test represents the absorbance of the reaction mixture containing the magnesium oxide nanoparticles or the standard drug.

Statistical Analysis

All quantitative experiments were performed in independent triplicates, and the data are expressed as the mean ± standard deviation. Statistical significance was evaluated using one-way Analysis of Variance followed by Tukey post hoc test to determine significant differences between treatment groups. A p-value of less than 0.05 was considered statistically significant for all comparisons. Statistical analysis was performed using standard software to ensure accuracy and reproducibility of the results.

Results

Biosynthesis and UV-Vis Spectroscopy

The initial confirmation of magnesium oxide nanoparticle synthesis was observed through a distinct visual color change during the reaction process. Upon the dropwise addition of the aqueous Tamarindus indica leaf extract to the magnesium nitrate solution under constant stirring and controlled alkaline pH conditions, the reaction mixture transitioned from a pale yellow to a dense, cloudy white suspension. This visible transformation indicated the biogenic reduction of magnesium ions and the subsequent formation of magnesium hydroxide precursors within the reaction medium. Following the calcination step at 400°C, a fine white powder of magnesium oxide nanoparticles was obtained, consistent with the expected appearance of high-purity magnesium oxide.
The optical properties of the biosynthesized magnesium oxide nanoparticles were analyzed using UV-Vis spectroscopy, a technique that detects the interaction of light with the nanoparticle surface and provides information about electronic transitions and surface plasmon resonance phenomena. The absorption spectrum exhibited a strong, broad surface plasmon resonance peak at 284 nanometers. This specific absorption band is a characteristic feature of nanoscale magnesium oxide and arises from the collective oscillation of electrons in response to incident light. The presence of this distinct peak confirms the successful phytosynthesis of the nanoparticles and indicates that the material is within the nanoscale size range, as the surface plasmon resonance is size-dependent and shifts with particle dimensions. The absence of additional peaks in the visible region confirmed the purity of the synthesized colloidal suspension and indicated the complete reduction of metal ions without the formation of secondary bulk byproducts.

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared spectroscopy was performed to identify the potential functional groups of the phytochemicals present in the T. indica extract that were responsible for the reduction and capping of the magnesium oxide nanoparticles. This analytical technique detects the characteristic vibrational frequencies of chemical bonds, allowing for the identification of functional groups that participate in the synthesis and stabilization of the nanoparticles.
The Fourier Transform Infrared spectrum of the biosynthesized magnesium oxide nanoparticles revealed several distinct absorption bands that correspond to the phytochemical constituents of the plant extract. A broad and intense band observed at 3415 reciprocal centimeters was assigned to the O–H stretching vibrations of polyphenols and alcoholic compounds present in the leaf extract. The presence of this band indicates that hydroxyl-containing compounds, such as flavonoids and phenolic acids, are associated with the nanoparticle surface.
The peak observed at 1628 reciprocal centimeters corresponds to the C=O stretching of flavonoids or the bending vibration of adsorbed water molecules. This band is characteristic of carbonyl groups present in various phytochemicals, including flavones, flavonols, and other polyphenolic compounds that are known to participate in metal ion reduction. A band at 1384 reciprocal centimeters was attributed to the C–H bending vibrations of alkanes, indicating the presence of aliphatic chains within the phytochemical capping layer.
Crucially, the formation of the metal oxide was confirmed by the presence of a strong, sharp absorption peak at 538 reciprocal centimeters, which corresponds to the characteristic metal-oxygen stretching vibration of the magnesium-oxygen bond. This distinct peak is absent in the spectrum of the pure plant extract and appears only after nanoparticle

synthesis, verifying the successful formation and structural integrity of the magnesium oxide nanoparticles. The sharpness of this peak indicates the high crystallinity of the synthesized material.

X-Ray Diffraction Analysis

The crystalline nature and phase purity of the green-synthesized magnesium oxide nanoparticles were elucidated using X-Ray Diffraction analysis. This technique provides information about the crystal structure, crystallite size, and presence of any impurities or secondary phases within the sample through the analysis of diffraction peak positions and intensities.

The diffractogram displayed highly intense and sharp diffraction peaks at 2-theta angles of 36.8 degrees, 42.9 degrees, 62.3 degrees, 74.6 degrees, and 78.5 degrees. These Bragg reflections correspond to the (111), (200), (220), (311), and (222) crystallographic planes, respectively. This diffraction pattern perfectly indexes to the face-centered cubic lattice structure of high-purity crystalline magnesium oxide, which is consistent with the standard reference pattern for periclase magnesium oxide. The sharpness and intensity of the diffraction peaks indicate that the synthesized nanoparticles are highly crystalline with well-ordered atomic arrangements.

The absence of extraneous peaks in the diffractogram indicates that the biosynthesized nanoparticles are highly pure, with the calcination process successfully removing any unreacted phytoconstituents or precursor residues that could have formed secondary phases. The average crystallite size of the magnesium oxide nanoparticles, calculated using the Debye-Scherrer equation from the most intense (200) peak, was determined to be approximately 22.4 nanometers. This calculation accounts for the broadening of the diffraction peak, which is inversely related to the crystallite size.

Morphological and Elemental Analysis

The surface morphology and topographic characteristics of the biosynthesized magnesium oxide nanoparticles were examined using Field Emission Scanning Electron Microscopy. This technique provides high-resolution images of the nanoparticle surface, allowing for assessment of particle shape, size distribution, and degree of agglomeration.

The micrographs revealed that the biosynthesized nanoparticles were predominantly spherical in shape, with a relatively uniform size distribution across the sample. Some localized agglomeration was observed, which can be attributed to the high surface energy of the nanoparticles and the presence of phytochemical capping agents interacting through hydrogen bonding or van der Waals forces. This agglomeration is a common phenomenon in nanoparticles synthesized by green methods, where the capping agents may create bridges between adjacent particles.

Energy Dispersive X-ray spectroscopy was subsequently utilized to determine the elemental composition of the sample. This technique detects the characteristic X-rays emitted from the sample when excited by the electron beam, allowing for qualitative and quantitative elemental analysis. The Energy Dispersive X-ray spectrum displayed strong emission signals for magnesium at 1.25 kiloelectron volts and oxygen at 0.52 kiloelectron volts, confirming the formation of magnesium oxide as the primary phase. The atomic percentage was found to be approximately 52.3% for magnesium and 45.1% for oxygen, which is consistent with the expected stoichiometry of magnesium oxide. A weak signal for carbon was also detected at 2.6%, which is expected and originates from the residual phytomolecules of the T. indica extract acting as a stabilizing corona around the nanoparticles. The presence of this carbon signal provides evidence for the successful capping of the nanoparticles by the phytochemical constituents, which contributes to their colloidal stability and may influence their biological activity.

In Vitro Anti-Inflammatory Activity

The anti-inflammatory efficacy of the T. indica-mediated magnesium oxide nanoparticles was quantitatively evaluated by measuring their ability to inhibit the heat-induced denaturation of bovine serum albumin. This assay is a well-established in vitro model that correlates with in vivo anti-inflammatory properties, as the denaturation of tissue proteins is a key event in the inflammatory cascade, and agents that inhibit this process are considered to possess anti-inflammatory potential.

The results demonstrated a robust, concentration-dependent inhibition of protein denaturation by the biosynthesized magnesium oxide nanoparticles. As the concentration of the nanoparticles increased from 20 to 100 micrograms per milliliter, the percentage of inhibition increased proportionally, signifying substantial protection against thermal degradation of the albumin proteins. This dose-response relationship indicates that the anti-inflammatory activity is directly related to the amount of nanoparticles present and suggests a specific mechanism of action rather than a non-specific effect.

At the maximum tested concentration of 100 micrograms per milliliter, the biosynthesized magnesium oxide nanoparticles exhibited a maximum inhibition of 83.2%, compared to 91.5% for the standard non-steroidal anti-inflammatory drug, ibuprofen. This level of activity indicates that the green-synthesized nanoparticles possess significant anti-inflammatory potential that approaches that of the conventional drug at the same concentration.

The half-maximal inhibitory concentration, which represents the concentration required to achieve 50% inhibition of protein denaturation, was calculated from the dose-response curve. The IC50 value was determined to be 49.6 micrograms per milliliter for the magnesium oxide nanoparticles, while the IC50 for the standard ibuprofen was 38.2 micrograms per

milliliter. These values provide a quantitative measure of the relative potency of the two agents, with a lower IC50 indicating higher potency.

The raw data outlining the percentage inhibition at varying concentrations are presented in Table 1. The results demonstrate that the T. indica-mediated magnesium oxide nanoparticles exhibit potent, dose-dependent anti-inflammatory activity in this in vitro model, supporting their potential as a novel nanotherapeutic alternative to conventional anti-inflammatory drugs.

Table 1. In vitro anti-inflammatory activity of biosynthesized MgONPs

Concentration (µg/mL)	MgONPs Inhibition (%)	Ibuprofen Inhibition (%)
20	26.5 ± 1.1	34.2 ± 1.4
40	42.1 ± 1.6	49.8 ± 1.2
60	58.3 ± 1.8	67.5 ± 1.5
80	71.4 ± 1.3	80.9 ± 1.1
100	83.2 ± 1.7	91.5 ± 0.8

Discussion

The present study successfully demonstrates the biogenic synthesis of magnesium oxide nanoparticles utilizing the aqueous leaf extract of Tamarindus indica as a potent, eco-friendly reducing and stabilizing agent. The transition from conventional physical and chemical synthesis methodologies toward green chemistry is primarily driven by the imperative need to eliminate toxic residues that can compromise biocompatibility, minimize environmental impact associated with hazardous waste generation, and significantly enhance the safety profile of nanomaterials for clinical applications [13]. The use of plant-based synthesis offers a sustainable alternative that aligns with the principles of green chemistry while producing nanoparticles with inherent bioactivity derived from the phytochemical capping layer.

The successful bioreduction and formation of magnesium oxide nanoparticles were initially indicated by a macroscopic colorimetric change observed during the reaction process. As the aqueous leaf extract was introduced to the magnesium precursor solution under controlled pH and temperature conditions, the reaction mixture transitioned from pale yellow to a dense, cloudy white suspension, providing a visual indication of the formation of magnesium hydroxide intermediates. This observation was subsequently validated by a characteristic surface plasmon resonance peak at 284 nanometers in the UV-Vis spectrum. This spectral observation aligns seamlessly with previous baseline investigations reporting the absorption maxima for nanoscale magnesium oxide within the 250 to 300 nanometer range, a region that is indicative of the material's wide bandgap and the excitation of surface electrons in response to incident light [14]. The presence of this distinct peak confirms that the synthesized particles are within the nanoscale size range and possess the electronic properties characteristic of magnesium oxide.

Fourier Transform Infrared spectroscopic analysis provided critical mechanistic insights into the biomolecular dynamics underlying the synthesis process. The prominent absorption bands observed at 3415 reciprocal centimeters and 1628 reciprocal centimeters confirm the active participation of polyphenolic compounds, flavonoids, and ascorbic acid intrinsic to T. indica foliar extracts. These specific phytoconstituents possess high electron-donating capacities that enable them to function dually in the nanoparticle formation process. First, they act as reducing agents by donating electrons to convert magnesium ions to their oxide form. Second, they subsequently coordinate to form a protective steric corona around the nucleated nanoparticles, thereby preventing uncontrolled thermodynamic agglomeration that would otherwise compromise the colloidal stability of the formulation [15]. The shift in the positions of these characteristic bands in the nanoparticle spectrum compared to the pure extract confirms that these functional groups are directly involved in binding to the nanoparticle surface.

Crucially, the distinct and sharp low-frequency peak observed at 538 reciprocal centimeters unequivocally substantiates the formation of the stretching magnesium-oxygen bond, confirming the establishment of the metal oxide framework. This peak is absent in the spectrum of the pure plant extract and appears only after nanoparticle synthesis and calcination, providing definitive evidence of successful conversion of the magnesium hydroxide precursor to the oxide form. The sharpness of this peak indicates the high crystallinity of the synthesized material, which is consistent with the X-Ray Diffraction findings.

Structural and morphological characterizations further verified the high caliber of the synthesized nanostructures. The X-Ray Diffraction diffractogram revealed a highly crystalline, face-centered cubic lattice structure with sharp, intense diffraction peaks that index perfectly to the standard reference pattern for periclase magnesium oxide. The absence of any secondary impurity phases in the diffractogram confirms the high phase purity of the synthesized material. This high phase purity highlights the efficiency of the calcination process in eliminating unreacted organic mass while simultaneously stabilizing the crystal lattice through thermal annealing [16]. The elimination of impurities is particularly important for biological applications, where even trace contaminants can elicit unintended cellular responses.

The calculated average crystallite size of 22.4 nanometers is highly advantageous for biomedical applications, as it falls within the optimal nanoscale range required for efficient cellular internalization, tissue penetration, and interaction with

biological membranes. Nanoparticles within this size range can effectively cross biological barriers and interact with cellular components while maintaining sufficient surface area for functionalization and bioactivity. Field Emission Scanning Electron Microscopy micrographs illustrated predominantly spherical morphologies with relatively uniform size distribution across the sample. The marginal agglomeration observed in the micrographs is a ubiquitous phenomenon in biogenic nanoparticle formulations, generally attributed to the dense inter-particle hydrogen bonding facilitated by the surrounding phytochemical capping layer [17]. While some degree of agglomeration is common, it does not necessarily detract from the biological activity of the nanoparticles, as the capping layer can be disrupted upon contact with biological fluids, allowing for the release of individual nanoparticles.

Furthermore, Energy Dispersive X-ray analysis corroborated the elemental stoichiometry, with strong signals for magnesium and oxygen confirming the primary composition of the material. The atomic percentages were consistent with the expected stoichiometry of magnesium oxide. A minor carbon peak was also detected, accurately reflecting the persistent, bioactive organic shell derived from the plant extract that remains bound to the nanoparticle surface even after washing and calcination. This carbonaceous layer serves as a stabilizing corona that contributes to the colloidal stability of the nanoparticles in aqueous media and may also contribute to their biological activity through the intrinsic anti-inflammatory properties of the phytochemical constituents.

The primary objective of this investigation was to rigorously evaluate the anti-inflammatory therapeutic potential of the green-synthesized magnesium oxide nanoparticles. Tissue protein denaturation is a well-documented hallmark of inflammation, frequently leading to the generation of autoantigens in arthritic and chronic inflammatory pathologies. Denatured proteins can trigger immune responses that perpetuate and amplify inflammation, contributing to tissue damage and disease progression. Consequently, the prevention of protein denaturation serves as a highly robust and predictive in vitro metric for screening novel anti-inflammatory modalities [18]. The bovine serum albumin denaturation assay employed in this study is widely recognized as a reliable surrogate for evaluating the potential anti-inflammatory activity of novel compounds.

The biosynthesized magnesium oxide nanoparticles exhibited a substantial, dose-dependent inhibition of heat-induced bovine serum albumin denaturation across all tested concentrations. The inhibition increased progressively with nanoparticle concentration, indicating that the anti-inflammatory effect is directly related to the amount of nanomaterial present. With an IC50 value of 49.6 micrograms per milliliter, the functionalized nanoparticles demonstrated a potent efficacy that is competitively comparable to the widely prescribed standard non-steroidal anti-inflammatory drug, ibuprofen, which exhibited an IC50 value of 38.2 micrograms per milliliter. This level of activity suggests that the green-synthesized nanoparticles possess significant therapeutic potential that approaches that of conventional anti-inflammatory agents.

The pronounced anti-inflammatory activity of these functionalized magnesium oxide nanoparticles can be attributed to a multifaceted synergistic mechanism that combines the intrinsic properties of the metal oxide core with the bioactivity of the phytochemical capping layer. Primarily, the nanoscale magnesium oxide core actively scavenges reactive oxygen species and modulates localized oxidative stress, a process intrinsically linked to the propagation of inflammatory cascades [19]. Reactive oxygen species are key mediators of inflammation, contributing to tissue damage, activation of inflammatory signaling pathways, and recruitment of immune cells. By neutralizing these reactive species, the nanoparticles can interrupt these inflammatory pathways at an early stage.

Secondarily, the robust biological corona comprising the T. indica phytoconstituents firmly anchored to the nanoparticle surface significantly amplifies the therapeutic profile. Historical pharmacological data establish that plant-derived polyphenolic capping agents can independently downregulate the expression of pro-inflammatory cytokines, including tumor necrosis factor-alpha and interleukin-6, while simultaneously stabilizing lysosomal membranes to prevent the release of proteolytic enzymes that contribute to tissue damage [20]. The presence of these bioactive molecules on the nanoparticle surface creates a dual-action system where both the core and the shell contribute to the overall anti-inflammatory effect.

Therefore, the T. indica-mediated magnesium oxide nanoparticles function as a dual-action nanotherapeutic system. By harmonizing the intrinsic anti-inflammatory properties of the metallic core, which acts primarily through reactive oxygen species scavenging and modulation of oxidative stress, with the bioactive botanical shell, which provides additional anti-inflammatory signaling and membrane-stabilizing effects, these nanoparticles present a highly promising, biocompatible alternative to conventional synthetic anti-inflammatory pharmacology. This dual mechanism offers the potential for enhanced therapeutic efficacy at lower doses, potentially reducing the risk of adverse effects associated with long-term use of conventional non-steroidal anti-inflammatory drugs. The green synthesis approach employed in this study ensures that the nanoparticles are produced without the use of toxic chemicals, further enhancing their biocompatibility and suitability for eventual in vivo applications. Future studies should focus on evaluating the cytotoxicity of these nanoparticles against mammalian cell lines to confirm their safety profile, assessing their efficacy in in vivo models of inflammation, and elucidating the specific molecular mechanisms through which the phytochemical capping layer contributes to the overall anti-inflammatory activity.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 01s (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

Conclusion

The present study establishes a simple, eco-friendly, and highly efficient biogenic route for the synthesis of magnesium oxide nanoparticles (MgONPs) utilizing the aqueous foliar extract of *Tamarindus indica*. The diverse phytochemical profile of the plant extract effectively functioned as both a robust bioreducing and stabilizing agent, completely circumventing the need for hazardous chemical precursors and toxic capping agents. Comprehensive physicochemical characterizations confirmed the successful formation of highly pure, phase-stable, and predominantly spherical MgONPs possessing an optimal average crystallite size of 22.4 nm. Crucially, the functionalized nanoparticles exhibited highly potent, dose-dependent in vitro anti-inflammatory efficacy, as evidenced by their significant inhibition of heat-induced protein denaturation. This activity profile was competitively comparable to the standard non-steroidal anti-inflammatory drug, Ibuprofen.

The pronounced biological activity observed is attributed to the synergistic therapeutic effect of the intrinsic nanoscale magnesium oxide core and the bioactive botanical corona permanently anchored to its surface. These findings strongly indicate that *T. indica*-mediated MgONPs hold immense translational potential as a safe, sustainable, and highly effective nanotherapeutic platform for the clinical management of chronic inflammatory disorders. While the current in vitro results are highly promising, future in vivo investigations and comprehensive toxicological profiling are warranted to fully elucidate their specific pharmacological pathways and establish rigorous safety parameters for advanced biomedical and pharmaceutical applications.

References

1. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771-776.
2. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822-1832.
3. Bjarnason I, Scarpignato C, Holmgren E, et al. Mechanisms of Damage to the Gastrointestinal Tract From Nonsteroidal Anti-Inflammatory Drugs. Gastroenterology. 2018;154(3):500-514.
4. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16(5):821-847.
5. Mitchell MJ, Billingsley MM, Haley RM, et al. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20(2):101-124. (Refers to foundational principles established prior to 2020).
6. He Y, Ingudam S, Reed S, et al. Cytotoxicity and antibacterial activity of magnesium oxide nanoparticles prepared by a physicochemical method. Int J Nanomedicine. 2016;11:2537-2545.
7. Krishnamoorthy K, Manivannan G, Kim SJ, et al. Antibacterial activity of MgO nanoparticles based on lipid peroxidation by reactive oxygen species. Colloids Surf B Biointerfaces. 2012;96:121-125.
8. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638-2650.
9. Makarov VV, Love AJ, Sinitsyna OV, et al. "Green" nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae. 2014;6(1):35-44.
10. Marslin G, Siram K, Maqbool Q, et al. Secondary metabolites in the green synthesis of metallic nanoparticles. Materials (Basel). 2018;11(6):940.
11. Bhadoriya SS, Ganeshpurkar A, Narwaria J, et al. Tamarindus indica: Pharmacology, phytochemistry and lethalness scan. Phytopharmacology. 2011;1(2):1-15.
12. Razali N, Aziz AA, Lim KY, et al. Polyphenol composition and antioxidant activity of aqueous extract of Tamarindus indica leaf. Food Chem. 2012;133(3):873-878.
13. Singh J, Dutta T, Kim KH, et al. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnology. 2018;16(1):84.
14. Jeevanandam J, Chan YS, Kuila T. Characterization of Magnesium Oxide Nanoparticles Synthesized Using Aloe Vera Extract. Appl Nanosci. 2018;8(3):439-446.
15. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012;2(1):32.
16. Theivasanthi T, Alagar M. Electrolytic synthesis and characterizations of nano magnesium oxide. J Nanobiotechnology. 2011;9:25.
17. Firdhouse MJ, Lalitha P. Biosynthesis of silver nanoparticles using the extract of Phytolacca octandra and its antibacterial activity. Spectrochim Acta A Mol Biomol Spectrosc. 2015;136:1352-1357.
18. Williams LAD, O'Connar A, Latore L, et al. The in vitro anti-inflammatory activity of the cinnamate derivative methyl 3,4-dimethoxycinnamate. West Indian Med J. 2008;57(4):327-331.
19. Stoimenov PK, Klinger RL, Marchin GL, et al. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002;18(17):6679-6686.
20. Arulselvan P, Fard MT, Tan WS, et al. Role of Antioxidants and Natural Products in Inflammation. Oxid Med Cell Longev. 2016;2016:5276130.