MICROWAVE AIDED SYNTHESIS OF ZINC OXIDE NANOCERAMICS: A POTENT BIOACTIVE AGENTS WITH THERAPEUTIC CHARACTERS

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ABSTRACT

Nanoparticles have been inextinguishably utilized in Nanotechnology cohere with Bio-Technology to upgrade the immobilization and movement of catalysts in pharmaceutical nanotechnology for conveyance of helpful specialists in incessant illness diagnostics and in sensors. In our communication we report microwave irradiation technique implemented for the preparation of novel Zinc oxide nanoparticles with beneficial bio-efficacy wherein the method employed has the advantages of producing small particle size metal oxide with high purity owing to short reaction time. We successfully synthesized and characterized two Zinc oxide based C3-doped ZnO and Li2-doped ZnO nanoparticles and were subjected to in vitro free radical scavenging assay wherein all the particles could scavenge DPPH and Nitric oxide radicals with less IC50 value over the positive control. Micronical particles of nanoparticles were proved by its effectiveness against both Gram positive and Gram negative bacteria tested besides its action against Candida albicans. Both nanoparticles synthesized showed a prominent inhibition of erythrocyte lysis by PhospholipaseA2 in a concentration dependent manner indicating better anti-inflammatory agent with possibility of preventing cancer. In this study we demonstrate by shell less CAM assay that synthesized nanoparticles could inhibit in vivo angiogenesis which would otherwise under abnormal conditions causes the acceleration of several inflammatory diseases in spite of its role in normal growth and wound healing process. Inhibition of such process is the promising methodology to hinder the progression of diseases. Present characteristic features make use of ZnO based doped nanoparticles to be a potential approach as a therapeutic molecule with favourable biological performance.

Keywords: Zinc oxide, Antioxidant, Anti-inflammatory, Anti-angiogenic, Antimicrobial, CAM-Chick Chorioallantoic membrane assay

INTRODUCTION

Nanoparticles have been copiously utilized in clinical Bio-Technology to upgrade the immobilization and action of catalysts (Wang, 2006) in pharmaceutical nanotechnology for conveyance of restorative operators (Zhang et al., 2008), in invertebrate infection diagnostics and in sensors and imaging strategies (Hong et al., 2008). Nano oxide materials have found wide extending applications especially as catalysts and as beginning materials for making progressed auxiliary ceramics (Kokila et al., 2008). Amid sintering and forming of oxidic materials are utilized for viable applications. Utilize of nano measured particles as beginning materials can be of awesome advantage since of the accessibility of huge surface regions compared to its bulk partner (Anil Reddy, 2009). A few strategies have been created for the planning of nanomaterial’s (Pramila et al., 2023); procedures like splash pyrolysis, warm decay, atomic bar epitaxy, and chemical vapor statement have been broadly utilized within the amalgamation of nanomaterial’s, one such prepare is the work of microwave illumination strategy. Chemical vapor decomposition and forming of oxidic materials are utilized in treatment of dermatitis, diaper rashes, diaper wipes, rankles, and open skin bruises (Voicu et al., 2013). The explore for new strategies to bargain with the illnesses causing microscopic organisms heighten as a result of resistance improvement by the pathogens against ordinary anti-microbial. Analysts have to be distinguish and create the another era of drugs or operators to control bacterial diseases. Antibacterial considers on natural materials are frequently not steady especially at tall temperatures and/or weights compared to inorganic antibacterial specialists (Sawal et al., 2003). Reports are accessible on the impressive antibacterial action of inorganic metal oxides like TiO2, MoO3 (Shivaganga et al., 2022), ZrO2, (Deepakumari et al., 2022) ZnO (Mallikarjunaswamy et al., 2022) SiO2, MgO, NiO (Mallikarjunaswamy et al., 2022) CaO, Bi2O3 (Mallikarjunaswamy et al., 2023) CeO2, Bi2O3 (Pramila et al., 2020) and ZnO showing bacteriostatic, antimicrobial, or biocidal activity. Particularly, TiO2, ZnO (Lakshmi et al., 2022), MnWO4 (Shivaganga et al., 2023), MgO, and CaO are of specific concern since they are not as it were steady beneath cruel prepare conditions, but moreover are considered as secure materials to people (Narayanan et al., 2012). Besides, ZnO shows up to successfully stand up to microorganisms for the display think about is that it could be a metal oxide, which is much steadier and features a longer life than organic-based disinfectants and antimicrobial specialists (Hewith et al., 2001). Zinc is additionally a basic constituent for cell development and in repressing bacterial proteins like dehydrogenase and certain defensive proteins such as thiolperoxidase and glutathione reductase (Priyanka et al., 2009). In this study we have carried out antibacterial measure against four pathogenic microbes and an organism wherein Cesium and lithium doped ZnO are strong against all the tried strains. Indeed in spite of the fact that meager reports are accessible with respect to antioxidant property of nanoceramics we tried for in vitro antioxidation capacity of doped ZnO nanoparticles to rummage receptive free radicals and anti-inflammatory action was gotten to by Roundabout hemolytic test.
utilizing PLAs as a proinflammatory chemical and antiangiogenic test were moreover carried out utilizing shell less CAM (Chorioallantoic film) test to assess the viability of doped ZnO nanoparticle which makes it to be a conceivable approach to utilize as restorative atom with positive natural exercises. Our objective is to synthesize Zinc oxide based nanoparticles doped with Cs2 and Li2 characterization of the same and its natural applications.

MATERIAL AND METHODS

Chemicals

All chemicals were purchased from Sisco Research Laboratories, Mumbai, India, and they were of analytical grade. Pathogenic Microorganisms were procured from Department of Molecular Biology, Yuvaraja’s College, University of Mysore, Mysuru and were maintained in the laboratory in Mueller hinton agar slants which were subjected repeated sub culturing for every 8 days.

Chemistry

Synthesis of nanoparticles was carried out according to the method described previously by Arunkumar et al., (2007) with slight modification wherein the hydrated metal oxide precursors were prepared by dissolving equimolar proportions of the respective metal salts (Zinc Oxalate and Lithium oxalate were used for Li2ZnO2 synthesis; Zinc Oxalate and Cesium oxide for Cs2ZnO2 Synthesis) and oxalic acid in minimum volume of water and was stirred for about 15 min on a magnetic stirrer. The different metal salts show different coloured precipitate at different pH range of 2–6. The precipitates of respective metal oxalates were washed with cold distilled water till it was free from the respective sulphate and excess oxalic acid. Finally, the precipitate was washed repeatedly with dry acetone and then dried under vacuum and was transferred into a crucible with dry acetone and then dried under vacuum and was transferred into a crucible and ignited in an electrical oven individually for partial decomposition. Then it was placed in a microwave oven synthesizer having frequency 5.0 GHz for about 0–30 min at various power levels 0–90. The solids burn by producing different coloured light depending upon the metal present in the carboxylate precursors and leaving behind respective metal oxides. The nano sized fillers so obtained were characterized by SEM, EDS, DLS and UV-Visible Spectral studies.

Biological assays for nanoparticles

Antioxidant assays

DPPH radical scavenging assay

The DPPH radical scavenging activity was performed using the methodology described by Scherer & Godoy (2009). In summary, distinct aliquots of Cs2ZnO2 and Li2ZnO2 nanoparticles independently, with concentrations ranging from 2–10μg/ml, were combined with 1 millilitre of DPPH solution (0.1 millilitres in 95% ethanol). As a negative control, DPPH alone was used in another reaction, and the combination was left to stand at room temperature for 20 minutes. The resulting solution’s absorbance was measured at 517 nm using an HITACHI, U-2900 UV-VIS spectrophotometer. As a positive control, butylated hydroxyl toluene (BHT) solution’s absorbance was measured at 517 nm using an HITACHI, U-2900 UV-VIS spectrophotometer. Each experiment was run in triplicate.

Nitric oxide scavenging assay

Sodium nitroprusside was converted to nitric oxide, which was then quantified using the Griess reagent. In an aqueous solution at physiological pH, sodium nitroprusside spontaneously produces nitric oxide (Marcocci Etal 1994). This nitric oxide then combines with oxygen to produce nitrite ions, which are quantifiable using Griess reagent. Because nitric oxide scavengers compete with oxygen for available nitrite, nitric oxide synthesis is decreased. Different amounts of Cs2ZnO2 and Li2ZnO2 nanoparticles individually (2–10 μM/ml) were mixed with 1 ml of sodium nitroprusside (5 mM) in phosphate buffer saline (0.1M, Ph-7.4) and incubated at room temperature for 2 hours. Without the sample, a parallel reaction mixture was maintained as a control. Subsequently, 0.5 millilitres of Griess reagent containing 0.1% N-1-naphthyl ethylenediaminedihydrochloride, 2% H3PO4, and 15% sulphanilamide was added. A standard solution’s absorbance was used to measure the absorbance of the chromophore created when nitrate was diazotized with sulphanilamide and then coupled with naphthylethylene diamine. This absorbance was measured at 546 nm. The mean values are computed after the experiment was run in triplicate. The radical scavenging activity was calculated similarly as described earlier for DPPH radical scavenging assay.

Anti-inflammatory assay

Indirect hemolytic assay for PLAs inhibition

By utilising bovine serum albumin as a reference (0-75μg/ml), the protein concentration in the venom was determined using Lowry's technique. The method used was a semi-quantitative indirect hemolytic assay (Boman and Kaletta, 1957). In short, a solution of egg yolk, packed human erythrocytes, and phosphate buffer saline (1:1.8 V/V) was made. Varespladib was employed as the usual medication, and a reaction mixture devoid of nanoparticles served as the control. One millilitre of this mixture was incubated with 20μg of enzyme primed with Cs2ZnO2 and Li2ZnO2 independently at varied concentrations (2, 4, 6, 8, 10 nM). After the reaction was halted after 10 minutes at 37 °C by adding 9 ml of ice-cold phosphate buffer saline, the mixture was centrifuged for 10 minutes at 6000 rpm. The amount of hemoglobin released by the action of PLAs in the supernatant was measured at 540 nm. Lysis of erythrocytes by adding 9 ml of distilled water to the control reaction mixture was taken as 100%. The experiment was carried out in triplicates and the mean values are calculated.

Anti Angiogenic activity

Chick Chorioallantoic membrane (CAM) assay

With a few changes, the CAM assay was performed using the previously published Chen et al. (2014) methodology. The fertilised chicken eggs were placed in a humidified incubator and incubated at 37 °C. After removing the egg shell on the eleventh day of development, the premature embryo was placed in a culture vial and saturated with 25 nm/ml of vascular endothelial growth factor (VEGF). Discs containing 10μg of each of the Cs2ZnO2 and Li2ZnO2 nanoparticles were placed on the developing blood vessel, and the culture vials were sealed with sterile polymer wrap before being incubated for 48 hours at 37 °C in a humidified environment. We looked for variations in the microvessel density around the nanoparticle-saturated disc in the culture vials.

Antimicrobial activity (Disc diffusion method)

Antimicrobial activity was determined by disc diffusion method. Two Gram negative bacteria Escherichia coli, Pseudomonas fluorescense and two Gram positive bacteria Micrococcus luteus, Bacillus subtilis and a fungal culture Candida albicans were inoculated and maintained under aseptic condition in Mueller hinton broth and Sabouraud dextrose broth respectively for the assay. Culture media primed with agar was poured into sterile petriplates followed by Microbial swab was streaked on the medium for a lawn of growth for respective microbial culture. The sample discs containing different concentrations of doped ZnO nano particles (5, 10, 20, 40 μg/ml) were placed on the surface of the agar medium at the correct distance apart using flamed sterilized forceps and a standard antibiotic of concentration 40μg/ml placed at centre and were incubate for 24hours at 37 °C. Zone of inhibition in diameter (mm) was measured. And minimum inhibitory concentration (MIC) was determined, where the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism.

RESULTS

Characterization

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The domain size of the synthesized nano particles were assessed by SEM analysis (Figure 1A and 1B) and DLS measurements (Figure 1C and 1D), which reveals all the synthesized metallic particles are within upper threshold limit (100nm), with an average domain size of 60nm and 50nm for Cesium and Lithium Zincate respectively. Furthermore, the success of green synthesis of Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles affected by microwave irradiation technique was ascertained by Energy Dispersive X-ray spectral studies (Figure 1E and 1F). As can be seen from the figure, the cesium/lithium zincate nano particles show corresponding EDS peaks at different X-ray energies, owing to the difference in their binding potentials.

Figure 1 Physical Characterization of Cs₂ZnO₂ and Li₂ZnO₂ nano particle. 1A & 1B Scanning electron microscopy images; 1C &1D Dynamic light scattering; 1E & 1F Energy Dispersive X-ray spectral studies.

The synthesized nano particles were also subjected to UV-visible spectral studies to ascertain their optical absorption behaviors. The UV-visible spectrum of Cs₂ZnO₂ nano particles exhibit a broad shouldered 325 nm, however the integration of Cs into the zinc lattice is found to red shift the UV absorption maximum towards higher wavelength regions.

Figure 2 UV-Visible Spectrophotometric analysis of Cs₂ZnO₂ and Li₂ZnO₂ nano particles

The UV-visible spectrum of Cs₂ZnO₂ nano particles exhibit a broad shouldered 325-425 nm, however the integration of Cs into the zinc lattice is found to red shift the UV absorption maximum towards higher wavelength regions.

Anti-Oxidant Assays

Figure 3 DPPH radical scavenging assay of Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles

The experiment was carried out in triplicates and the results indicates Mean ± SE concentration depended manner with the low IC₅₀ value of 0.013 comparatively (Figure 3).

Figure 4 Nitric oxide radical scavenging assay of Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles

The experiment was carried out in triplicates and the results indicates Mean ± SE concentration depended manner with the low IC₅₀ value of 0.013 comparatively (Figure 3).

Nitrre oxide radical scavenging was another method for In vitro antioxidant assay wherein Li₂ZnO₂ has almost same percentage of radical scavenging except at concentration of 2 and 6µg to that of positive control (Figure 4). Cs₂ZnO₂ showed high percentage of scavenging at the concentration of 6µg with lowest IC₅₀ value compared to other positive control (Table 1).

Table 1 IC₅₀ Value of ZnO₂ doped nanoparticles for Nitric oxide radical scavenging assay

<table>
<thead>
<tr>
<th>DRUG</th>
<th>BHT</th>
<th>Cs₂ZnO₂</th>
<th>Li₂ZnO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ Value (µmoles/ml)</td>
<td>0.023</td>
<td>0.016</td>
<td>0.051</td>
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</tbody>
</table>

Anti Inflammatory Assay

Since the compounds showed positive results for antioxidant activities they were also examined for Anti Inflammatory activity (Indirect Haemolytic Assay) by using PLA2 enzyme which usually causes hemolysis as a consequence of inflammatory reactions.

Figure 5 Indirect Hemolytic Assay of Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles.

The experiment was carried out in triplicates and the results indicates Mean ± SE concentration depended manner with lowest IC₅₀ value (Table 2).

Table 2 IC₅₀ value of Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles for Indirect hemolytic assay

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Varespladib</th>
<th>Cs₂ZnO₂</th>
<th>Li₂ZnO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ Value(nM)</td>
<td>0.02</td>
<td>0.017</td>
<td>0.055</td>
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</table>

Anti-Angiogenic Activity Assay

Nanoparticle based chemotherapeutic specialists are outlined such that they can laterally or effectively target cancer cells. Shell less CAM test was performed to know the conceivable activity of ZnO₂ on angiogenesis wherein both Cs₂ZnO₂ and Li₂ZnO₂ nanoparticles at the concentration of 10µg was utilized to immerse the circle in that it might repress the assist development of blood vessel within the locale where the nanoparticles were stacked (shown by arrows) compared to
DISCUSSION

Nanoparticles can be functionalized to carry and convey restorative operators such as drugs, qualities, or proteins. Lithium-doped zinc oxide nanoparticles have been examined as sedate conveyance vehicles due to their biocompatibility, controlled discharge properties, and potential for focused on conveyance to particular cells or tissues. Nanoparticles can act as differentiate specialists in different imaging modalities, counting fluorescence imaging, attractive reverberation imaging (MRI), and computed tomography (CT). Helpful parts for zinc in numerous infections have been built up in later a long time. ZnO contains a exceptionally great potential to move into the clinic (Shopsin et al., 1999). The improvement of builds to diminish oxidative harm caused by free radicals in natural substances with more prominent productivity can be accomplished by mixing of fabric science with Bio-Nanotechnology (Kannan & Hyun 2013). Lithium-doped zinc oxide nanoparticles have been investigated as imaging specialists due to their fluorescence properties, biocompatibility, and potential for focused on imaging of particular tissues or cellular structures (Marco et al., 2020). In this examination such sort of builds were made and were tried for in vitro antioxidant tests which shows the capacity of nano ZnO2 to give electrons and in this way extinguishing steady free radical like DPPH and was able compete with oxygen driving to decreased generation of nitric oxide (NO) which was assessed utilizing Griess reagent. Upon rummaging DPPH Li2ZnO2 appeared maximum percentage (80% at the concentration of 10 µg/ml) of rummaging with comparatively higher concentration required to induce 50% restraint (Figure 3). Li2ZnO2 has practically equivalent to capacity in decreasing the generation of nitric oxide by 80% at 10µg/ml concentration (Figure 4) with shifting IC50 esteem (Table 1). In spite of the fact that NO is utilized by the antigen handling cells like macrophages upon incitement by administrative flag particles called interleukins and intergalactic to guard against pathogens its overabundance action amid typical condition; it acts as a receptive radical, specifically harms typical tissues. Assist, nitric oxide can too respond with superoxide anion radical to create an indeed more grounded oxidant peroxynitrite (Lau et al., 1999). In this way ZnO nanoparticles might proficiently rummage these responsive atoms which may diminishes oxidative harm to typical cells and have to be tried in vivo. NO created peroxynitrite by reacting with superoxide anion. Peroxynitrite, as a capable oxidant, can either specifically connected with or oxidize different kinases and translation components, subsequently irritating the cellular signaling organize and advancing inflammation-drained cellular change (Joydeb & Young 2012). Aggravation when hoisted leads to a few disarranges of which cancer is the extreme result. Aggravation was primarily caused by the action of Lipoxygenase (LOX) and Cycloxygenase (COX) which depends on the accessibility of arachidonic corrosive as a subst. This substrate was determined by endogenous inhibitors, which can be advantageous within the treatment of provocative clutters. These materials have the potential to tweak safe reactions and diminish aggravation, making them appropriate candidates for the improvement of medicate conveyance frameworks and anti-inflammatory treatments (Sanvuk et al., 2022). Comparable comes about are gotten by both Cs2ZnO2 and Li2ZnO2 integrated by microwave light strategy wherein Cs2ZnO2 displayed promising result by restraining PLA2 action up to 70% (Figure 5) indeed at mo concentration of 10nm comparatively (Table 2) there by diminishing incendiary reaction.

Table 3 Minimum Inhibitory Concentration values of Cs2ZnO2 and Li2ZnO2 nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>Escherichia coli</th>
<th>Pseudomonas fluorescence</th>
<th>Bacillus subtilis</th>
<th>Micrococcus luteus</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>6.1 µg/ml</td>
<td>7.02 µg/ml</td>
<td>5.35 µg/ml</td>
<td>4.16 µg/ml</td>
<td>--</td>
</tr>
<tr>
<td>Cs2ZnO2</td>
<td>7.32 µg/ml</td>
<td>8.21 µg/ml</td>
<td>7.55 µg/ml</td>
<td>7.82 µg/ml</td>
<td>6.12 µg/ml</td>
</tr>
<tr>
<td>Li2ZnO2</td>
<td>6.25 µg/ml</td>
<td>7.12 µg/ml</td>
<td>----</td>
<td>5.82 µg/ml</td>
<td>5.12 µg/ml</td>
</tr>
</tbody>
</table>

Figure 6 Shell less CAM assay for Anti-Angiogenic property of Cs2ZnO2 (A) and Li2ZnO2 (B).

Anti microbial activity

The synthesized Cs2ZnO2 and Li2ZnO2 nano particles were screened for its antimicrobial activities against two Gram positive and two Gram negative bacteria including fungal pathogen Candida albicans. Invitro studies by disc diffusion method clearly shows the antimicrobial efficacies of the nanoparticles.

Figure 7 Invitro Antimicrobial activities of Cs2ZnO2 and Li2ZnO2. 7A1, 7A2, 7A3 were triplicates for Cs2ZnO2 nanoparticles and 7B1, 7B2, 7B3 were triplicates for Li2ZnO2 nanoparticles.
It is well known that irritation is exasperated by ROS generation, in which ROS act as auxiliary delivery people in signaling and acceptance of proinflammation go betweens. Expanded levels of free radical NO by proinflammatory inducible nitric oxide synthase (iNOS) contribute to incessant irritation. Nanoceria managed as a nanotherapeutic treatment smoothes the expression of iNOS and diminishes NO generation in J774A.1 murine macrophages as well as extinguishes ROS generation. Fiery reactions play conclusive parts at diverse stages of tumor advancement, counting start, advancement, harmful change, intrusion, and metastasis (Sergei et al., 2010). For all of these forms it needs the supply of supplements through the arrangement of unused blood vessels by a handle of angiogenesis which is being a complex prepare, including different quality items communicated by diverse cell sorts leads to tumor improvement beneath extraordinary conditions (Sanggliyandi et al., 2009). Tumor advancement initiated by irritation may happen early or late in tumor improvement and can lead to actuation of premonlicant injuries that were topird for numerous a long times. The aggravation advances tumor advancement by means of various instruments and, in expansion to upgraded survival, can moreover include the so-called angiogenic switch, which permits a small topird tumor to get the blood supply vital for the development phase (Lewis & Pollard, 2006). Preventing this stage may be distant better or improved distant better target for tumor control by nanocomposites.

With suitable surface alterations Metallic and non-metallic Nanomaterials counting liposomes, polymeric nanoparticles, carbon nanotubes, nanowires, viral nanoparticles and cross breeds, quantum specks and dendrimers are now for tumor control by nanocomposites. The present study clearly indicated that CsZnO2 nanoparticles would expand the surface range of Cesium/lithium zincate nano particles (Figure 1) showing tumor smothering property by means of the blockage of unu hindered microvessel can be watched within the culture vials contained untimely (Lewis et al., 2012). Emerging avenues linking inflammation and cancer. Free Radical Biology and Medicine, 52, 2013-2037. http://dx.doi.org/10.1016/j.freeradbiomed.2012.02.047


