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BIOLOGICAL SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING DATURA METEL: EVALUATION OF ANTIMICROBIAL, ANTI-INFLAMMATORY, CYTOTOXIC EFFECTS, AND ZEBRAFISH EMBRYONIC TOXICOLOGY

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Abstract

This study examined the antimicrobial properties and toxicity profiles of Datura metel Zinc Oxide Nanoparticles (ZnO NPs). Characterisation of the nanoparticles was performed using UV and SEM studies, while FTIR analysis was used to identify functional groups. Well-diffusion assays assessed antimicrobial effectiveness against *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis,* and *Pseudomonas sp.* Findings indicated that ZnO NPs exhibited a concentration-dependent effect against these pathogens, with the maximum inhibition zones measured at 100 µg/ml. The nanoparticles' ability to prevent protein denaturation was evaluated through Bovine Serum Albumin (BSA) and Egg Albumin (EA) denaturation assays, which demonstrated significant inhibition, reflecting potent anti-inflammatory properties. Additionally, the membrane stabilisation assay suggested possible cytoprotective effects, as notable inhibition was seen in the lysis of red blood cells. Toxicological evaluations included the Brine Shrimp Lethality Assay and a zebrafish embryonic toxicity study, revealing that while ZnO NPs were non-toxic at lower concentrations, higher doses (≥40 µg/mL) resulted in in vitro cytotoxicity and significant embryotoxicity in zebrafish. This was evidenced by decreased viability and various developmental abnormalities, including pericardial edema and spinal malformations. These findings underscore the dual role of ZnO NPs as potential antimicrobial agents and underscore the need for caution regarding their biological and environmental safety at elevated concentrations. It is crucial to consider the dose-responsive therapeutic and toxic effects associated with any application of ZnO NPs in medical and environmental settings.

Keywords: Zinc oxide Nanoparticles, Datura Metel extract, Antimicrobial efficacy, Biocompatible, Ecofriendly, Zebrafish embryonic toxicology

INTRODUCTION

Nanotechnology, defined as manipulating and controlling matter at the nanoscale between one to one-hundred nanometres, has revolutionized the biomedical field. This innovative technology covers designing, characterizing, producing, and applying structures, devices, and systems by managing shape and size at the nano level(1,2). Frequently termed nanomedicine when utilized for health sciences, it promises significant breakthroughs in diagnostics, therapeutics, and targeted medication distribution systems. The unique properties of nanomaterials, containing their size-dependent phenomena and large surface region relative to volume, allow for novel applications that were previously not achievable with larger particles(3).

Emergence of Zinc Oxide Nanoparticles in Medicine Among various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention owing to their special properties for example high chemical stability, UV light filtering, antibacterial, and anticancer activities(4). These nanoscale particles are used across diverse domains like cosmetic products, food packaging, and textiles, and extensively in medicine because of their biocompatibility and bio-safety. Their ability to generate reactive oxygen species and intrinsic UV-blocking capabilities make them specifically suitable for oncological, dermatological, and antibacterial applications(5).

Green synthesis is the biological method of synthesis of nanoparticles, representing a new source of nanomaterials and an important step towards sustainable alternatives to chemical synthesis approaches(6). This method minimizes environmental and biological hazards of nanoparticle fabrication, relying on biologic pathways.This method minimizes environmental and biological hazards of nanoparticle fabrication, relying on biologic pathways. Green synthesis methods allow for the synthesis of nanoparticles without toxic chemicals and at room temperature and pressure using plant extracts, microbes or enzymes. From the plants used in traditional medicine, the potential of Datura metel in the biosynthesis of nanoparticles was also investigated(7). Datura metel has phytochemicals like alkaloids, flavonoids, and terpenoids that help in the green synthesis of metal nanoparticles and acts as empty stabilizers as natural reducing agents to keep ions in nanoparticle form(8–10).
Zinc oxide (ZnO) nanoparticles (NPs) are well known for their antimicrobial effect and widely used against pathogenic microorganisms(11). Antimicrobial action mechanisms are largely due to reactive oxygen species (ROS) and zinc ion release, which destroy microbial membrane function and barrier(12). Additionally, ZnO NPs have been shown in more than one study to have anti-inflammatory effects by suppressing the expression or production of pro-inflammatory cytokines and other mediators(13). This allows them to serve as effective therapeutic candidates in treating inflammatory diseases and diseases in which inflammation plays a key role(14). With these promising characteristics of ZnO NPs in antimicrobial and anti-inflammatory applications, it must, however, be noted that these materials do also show in some cases cytotoxic behaviours. The same features responsible for their antimicrobial action can also trigger cytotoxicity in mammalian cells, especially at high concentrations and prolonged durations. Thus, one of the principal steps that need to be performed is the evaluation of cytotoxicity (especially using in vivo models, such as zebrafish embryos)(15). Due to a range of advantages, such as high genetic similarity of zebrafish to humans, the transparency of zebrafish embryos in early development, and fast embryonic development, zebrafish embryos present a dynamic model for nanoparticle toxicity investigation. This model allows researchers to capture potential effects on development, viability, and morphological defects after exposure to nanoparticles(16,17).
Although there is considerable knowledge regarding different aspects of ZnO NPs, notably between the biological interactions and safety profiles of the ZnO NPs synthesised with Datura metel, gaps remain at this level. Very few studies have investigated the multifaceted antimicrobial, anti-inflammatory, cytotoxic and embryotoxic effects of such nanoparticles, especially as green methods are used to synthesize them from this plant. Herein, we aim to address these gaps by assessing the biomedical efficacy and safety of ZnO NPs synthesized using Datura metel extract.

MATERIALS AND METHODS

Preparation of Datura Metel extract:
1 gm of Datura Metel was dissolved in 100 ml of distilled water. Then it was heated with heating mantle at a temperature of 60-70 degree Celsius for 15-20 min. The solution was then filtered through Whatmann No:1 filter paper, and the resulting filtered extract was used for synthesizing nanoparticles.
Green Synthesis of Zinc oxide Nanoparticles using Datura Metel Extract.
60 mL of distilled water was used to dissolve 20mM of Zinc nitrate. Then 40mL of filtered Datura Metel extract was added and stirred on magnetic stirrer at 700 rpm for 48 hours. After synthesis, the sample was then centrifuged at 8000 rpm in a centrifuge for 10 minutes. The supernatant was discarded and the pellet was stored for biomedical assays.
Antimicrobial Activity: (Agar Well Diffusion Technique)
The antibacterial effect of the synthesized ZnONPs was determined by agar well diffusion method. Mueller-Hinton agar plates were made sterile by cooling them down to room temperature, and then, suspensions of bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis) were spread evenly on the surface using sterile cotton swabs. Agar was punched with a sterile cork borer to create wells 9 mm in diameter, which were then filled with a solution of ZnONPs at concentrations of 25, 50, and 100 µg/ml. Standard – Commercial antibiotics Amoxyrite were used.Plates were incubated at 37°C for 24h to allow bacterial growth. Mean diameters of the zones of inhibition (in mm), were used to show the antimicrobial potency of ZnONPs.
Anti-inflammatory activity
Egg Albumin denaturation
The reaction mixture contained 0.2 mL of fresh egg albumin, 2.8 mL of phosphate (buffer pH 6.3) and ZnONPs of different concentrations (10,20,30, 40, and 50 µg/mL), and was incubated at room temperature for 10 minutes. The mixture was heat treated at 55 °C for 30 minutes after incubation. The absorbance at 660 nm was measured by a spectrophotometer. Diclofenac sodium was used as the positive control and dimethyl sulfoxide served as the negative control. The inhibitory percentage of protein denaturation was expressed according to the formula:
% inhibition = $\frac{\text{(absorbance of sample} \times 100) - \text{absorbance of control}}{}$

Absorbance of control

Bovine Serum Albumin Denaturation

The BSA and ZnONPs (10-50 µg/mL) were mixed, and after 10 min at room temperature, incubated at 55°C for 30 min. Diclofenac sodium was used as the standard and dimethyl sulfoxide was used as a control. Absorbance was measured at660 nm using a spectrophotometer. Protein denaturation was measured in the same way as mentioned in the egg albumin assay as the following equation:

% inhibition = (absorbance of sample ×100) − absorbance of control
Absorbance of control

Membrane Stabilization Assay

This study isolated red blood cells (RBCs) from human samples, which were collected in sterile tubes containing an anticoagulant and centrifuged at 3000 rpm for 10 min. The RBCs were washed thrice with PBS and suspended in lipid-free Tris-HCl buffer (50 mM, pH 7.4) at a 10% suspension. Then 1 mL of each RBC suspension was incubated with ZnONPs at different concentrations of 10, 20, 30, 40 and 50 µg/mL for 30 minutes at 37 °C and subsequently centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatant was determined at 560 nm. The following equation was used to calculate Percentage inhibition of hemolysis:

The calculation for % inhibition is given by the following formula

% inhibition = [(OD control – OD sample) / OD control] x 100

Absorbance of the RBC suspension without (test compound[–]) is called OD control, and absorbance of the RBC suspension post-testing (test compound[+]) is called OD sample.

Brine shrimp lethality assay: a cytotoxic effect

Brine Shrimp Lethality Assay was conducted to screen the cytotoxicity of Datura metel extract mediated ZnONPs. The test organisms were Artemia salina nauplii that were hatched from cysts in synthetic seawater. Different concentrations (5, 10, 20, 40 and 80 µg/mL) of the green synthesized ZnONPs were prepared by dilution of the stock solution in seawater. The control group consisted of only seawater without any herbal oral rinse, serving as the baseline to compare with all test and control groups.

Ten nauplii were added in separate vials containing 5 mL of each test solution for each concentration. From there these vials were kept under continuous light and monitored for 24 and 48 hours respectively. At each time point, the survival of the nauplii was recorded, and the mortality rate was calculated by comparing the number of dead nauplii from each test vial with the control group.

Embryonic toxicology evaluation

To evaluate the acute cytotoxicity of ZnONPs, we conducted a zebrafish embryonic toxicology study. Wild-type zebrafish (*Danio rerio*) were sourced from local vendors in India and maintained under controlled conditions, with a stable temperature of 28±2°C, a 14:10-hour light/dark cycle, and a pH range between 6.8 and 8.5. The fish were fed a nutritious diet of dry blood worms or optimal feed twice daily to promote healthy growth and reproduction. Fertilized eggs were collected by pairing one female with three males in a breeding tank, with the viable eggs carefully rinsed in E3 medium to remove any residues before use in the experiment.

The zebrafish embryos were then transferred to culture plates with varying well sizes (6, 12, and 24 wells), placing 20 embryos in each well with 2 mL of the respective treatment solution. Embryos were exposed to different concentrations of theDatura metel mediated ZnONPs, alongside a commercial dental varnish for comparison. Treatment concentrations ranged from 5 to 80 µg/L, with untreated control groups included to monitor baseline development. Each treatment and control group were replicated three times to ensure reliable results. The treated plates were covered with foil to prevent light interference and maintained at a constant temperature of 28°C. Every 12 hours, dead embryos were removed to maintain culture conditions.

Throughout the 24–78-hour post-fertilization period, the zebrafish embryos were observed using a stereo microscope to track developmental progress and to record any mortality, hatching rates, and abnormal morphology. Observations focused on identifying any potential dose-dependent toxicity effects of the ZnONPs particularly in higher concentrations. The percentage of embryo/hatchling mortality was documented every 24 hours, and hatching rates were recorded to identify any developmental delays or deviations. Any abnormalities, including spinal deformities, yolk sac edema, or craniofacial malformations, were also noted. Images of malformed embryos were taken using a COSLAB - Model: HL-10A light microscope, and the percentage of abnormal embryos was documented daily to provide a comprehensive overview of potential toxicological effects.

Characterization of ZnO NPs

The structure of synthesized ZnO NPs from datura metal was determined by SEM analysis (JSM 6510 LA,JEOL Ltd.) using gold (99.9%) coating with sputtering for 90 sec at room temperature, and the size of nano particles were analysed by using imageJ software version 1.53t (ImageJ.org). The optical absorption spectrum of ZnO NPs was recording using a UV- visible spectrophotometer ELICO corporation: cat mo SL-159) at a range of 300-700nm. Diffraction patterns was determined by XRD at 1°/min with two angles from 20 to 80°(Bruker D8 Advance). Cu-K radiation (1.54060 A°) were operated at 40 kV and 40 mA. The XRD result was analyzed using Origin Lab version software 2023b (originlab.com) for the identification of type, morphology and the crystal size

of measure ZnO NPs. The diffraction peak maximum was observed at the plane and the crystalline size was determined by using Scherrer's physical formula as followed: D=0.94λ/βcosθ here D is crystalline size, is the X-ray wavelength, and is the full width at half the maximum of the peak (Sharma et al., 2018). The functional groups and the various compound classes of datura metal extracts and synthesized ZnO NPs was identified using FTIR (Digital Excalibur 3000 series, Japan) at room temperature with frequencies of 400-4,000 cm-1 (Singh et al., 2019).

RESULT AND DISCUSSION

Characterization of ZnO NPs

UV-Visible Spectroscopy

The absorption spectrum of ZnO NPs was represented with ranged from 300 to 700nm. The ZnO NPs showed absorption bands at 350 and 372nm respectively. Similar absorption peaks were noted after 24, 48, 72, 96 and 120hrs, by conforming their stability. The purity of ZnO NPs was determined by the absence of any peak in the range of the absorption spectrum. ZnO NPs synthesised from datura metal leaf extract exhibited theri absorption peak at the lower wavelength was supposed to have smaller size than that synthesized from datura, when the size of bulk molecules get reduced to nano range, their absorption peak get shifted towards UV range from visible range. Here datura leaf extract were obtained in UV wavelength range at 350 and 372nm respectively which confirmed their size in nanto range (Figure 1), comparatively the ZNO NPs synthesized from leaf extract of hibiscus rosa-sinensis had their absorption peak at 352 and 375nm, similarly during the synthesis of ZnO NPs using Pyrus pyrifolia leaf extract shows the absorption peak was found to be 378nm.

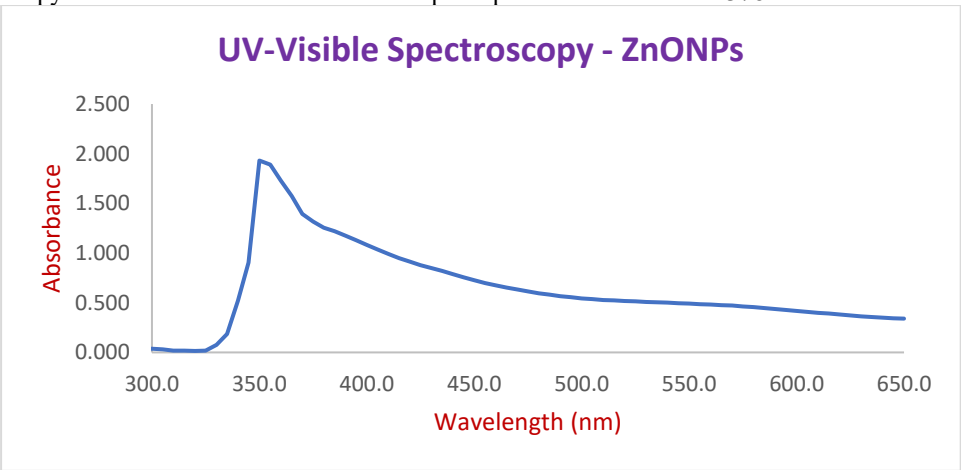
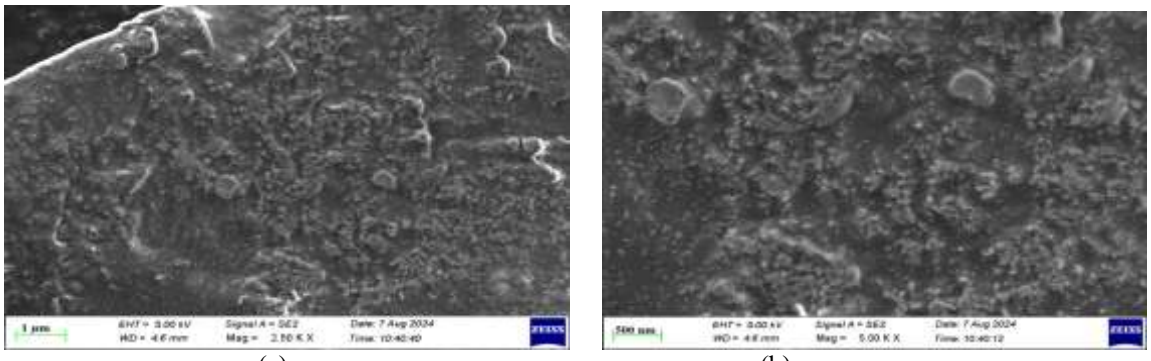


Figure 1. Absorbance peak of ZnO NPs synthesized from *datura metel* leaf extract in UV-Spectroscopy

Scanning Electron Microscopy (SEM) analysis

Particle size, alone with surface morphology of the ZnO NPs, was estimated that 1um and 500nm utilizing a scanning electron microscopy (SEM). Representative images are shown in figure 2, and 3. A typical scanning electron micrograph reveals that particle possess spherical shape with some degree of aggregation compared with our findings specifically, ZnO Nps synthesized from shilajit extract exhibited a morphology resembling nano-flakes with size ranging from 75 to 400nm (Parthasarathi Perumal et al., 2024).



(a) (b)

Figure 2. SEM analysis of ZnO NPs at different magnifications (a) 1um and (b) 500 nm

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FTIR analysis of ZnO NPs

The FTIR spectra of ZnO NPs from datura metals leaf extract are shown in figure 3. FTIR of ZnO NPs from datura metel extract exhibited a broad peak about at 3416.75cm^{-1} which can be attributed to stretching vibration of OH group (alcohols, phenols) and a peal at 2852.81cm^{-1} confirmed the stretching vibration of C–H stretch (alkanes), the presence of peak at 1623.16cm^{-1}, 1384.48cm^{-1} confirmed the presence of C=O of carbonyl group and C=C of aromatic system, respectively. C-O stretching was absorbed in 1099.73cm^{-1} which is further in zinc oxide nanoparticles synthesized from datura metel exhibited a characteristic peak at 3416.75cm$^{-1,}$ 2852.81cm^{-1}, 2923.60cm^{-1},2852.81cm^{-1}, 2066.11cm^{-1} and which was resembles of shilajit extract. The absorption peak associated with Zn-O stretching band is clearly absorbed at 618.45cm^{-1} which confirm the creation of ZnO-NPs. FTIR analysis revealed the presence of alkanes, phenol, alcohol, aromatic, alkenes, alkylhalides and aliphatic amines vibrations (Song et al., 2011). Furthermore, -C=O-, C-O-C and C-O stretching vibrations were shown to generate maximam in carboxylic acid, polysaccharide and amino acid respectively (Stanciu et al., 2019).

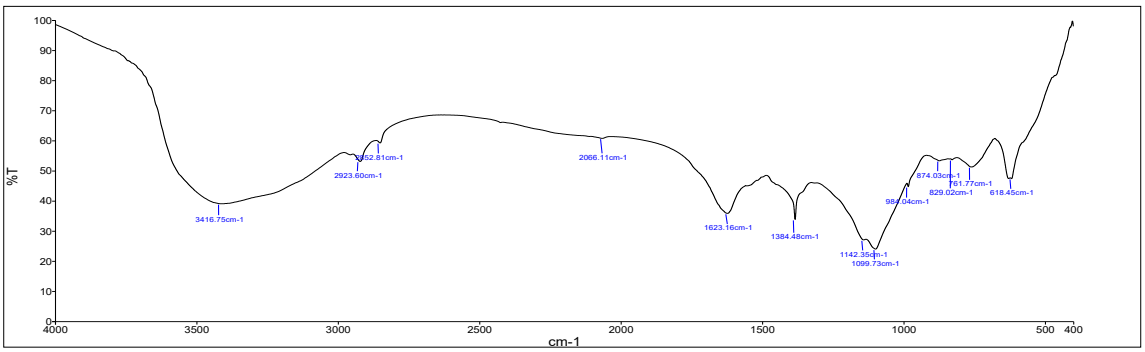

Figure 3 FTIR spectrum of ZnO NPs from datura metel.

Antimicrobial activity:

Figure 1: Antibacterial activity against different oral pathogens using agar well diffusin technique. A) Pseudomonas aeruginosa B) E.coli C) S.aureus D) E.faecalis

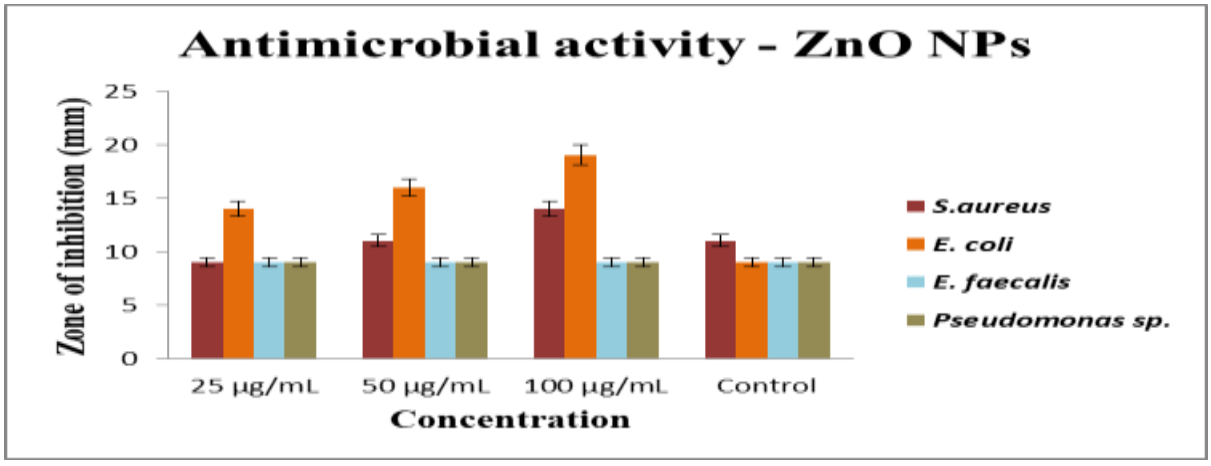

Figure 2: Antimicrobial activity of Zinc Oxide Nanoparticles (ZnO NPs) against different microbial strains. The graph illustrates the zones of inhibition (in mm) for Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Pseudomonas sp. across varying concentrations (25, 50, and 100 µg/mL) compared to a control group,

Well-diffusion assays were performed to verify the antimicrobial effect of zinc oxide nanoparticles (ZnO NPs) with a range of microbial strains, including Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Pseudomonas sp. Results- The test results illustrated in Figure 2A to 2D represents the zones of inhibition formed by different concentrations of ZnONPs on Mueller- Hinton agar plates.

Panel A shows the inhibition of the Pseudomonas sp. all showing clear, distinct zones that expanded in diameter with higher nanoparticle concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL). inhibition in E. coli (Panel B). In a similar way, the effects on E. faecalis are shown in panel C, whereby zones enlarged with increasing concentrations of ZnO NPs. Panel D is for S. aureus, showing strong inhibition with increased nanoparticle doses. The inhibition zones for appropriate dilution of ZnO NPs against each of the strains tested and control, was quantitatively assessed and illustrated in the bar chart where the categorical performance of NPs against different microbial species at varying concentrations is summarized. The percentage of inhibition by pathogen is evident from the graph (Figure E), which illustrates that the highest zone of inhibition was observed at 100 µg/mL for all tested pathogens, with S. aureus demonstrating the highest response followed by Pseudomonas sp., E. coli and E. faecalis in that order.

The antimicrobial activity of ZnONPs obtained from Datura metel mainly occurs through their release of zinc ions and the generation of reactive oxygen species (ROS) and through the generation of physical damages in cells. A key mechanism responsible for this is the release of zinc ions, which undermine the integrity of bacterial cell membranes and interfere with vital metabolic processes, ultimately culminating in bacterial cell death. Such release mechanism of ions is consistent with that obtained from the Park et al. (2024), which highlighted the disturbance of cellular homeostasis(18).

Furthermore, the ROS generation was verified by the studies of Nan et al. (2024), which mediates oxidative stress by damaging key cellular components like lipids, proteins, and nucleic acids in the bacterial cells(19). The oxidative stress mechanism is consistent with Park et al. (2024) for its broader application to various microbial strains(18).

The bactericidal mode of action of ZnONPs is further highlighted by their physical interactions with bacterial cells, such as penetration and compromise of structural components of the cell walls. These results are in line with those reported by Udayagiri et al. (2024), demonstrating that ZnONPs directly exerted physical destruction of the integrity of the bacterial cell(20).

ZnONPs performance is significantly impacted by their size and surface characteristics. Nanoparticles with smaller size have a higher surface area-to-volume ratio, providing more extensive interactive sites on the bacterial membranes leading to enhanced antibacterial efficacy. El-Masry et al. (2022), found that the antibacterial efficiency increased with a decrease in size of ZnONPs. Likewise, the specific surface properties of ZnONPs, which can be customized to improve ROS production, also contribute to their pathogenesis(21).

The antibacterial activity of ZnONPs has been improved by; combinations with other antimicrobial agents and manipulation of the addition(4). For example, the decoration of ZnONPs with antimicrobial peptides was found to enhance ROS generation and increase the bactericidal effect. These interactions improve the basic characteristics of ZnONPs and open other avenues related to regulating infections such as infections involving multidrug-resistant pathogens(22).

ZnONPs have also been very well investigated in their combination with clinically available antibiotics to enable their therapeutics. Hao et al. (2024) further demonstrated this synergistic approach that not only enhances the antimicrobial activity but also broadens the clinical use of the ZnONPs, especially in wound management(23).The results obtained on biocompatibility and environmental benignity of ZnONPs indicated the need for sustainable utilization of these nanomaterials for medical and industrial applications.

These findings indicate a strong antimicrobial property of ZnO NPs depending on concentration and species. The microbial growth-disrupting ability of the nanoparticle shows that it could be a powerful agent in the field of antimicrobials.

Anti-inflammatory activity:

Bovine Serum Albumin (BSA) Assay for Protein Denaturation Inhibition

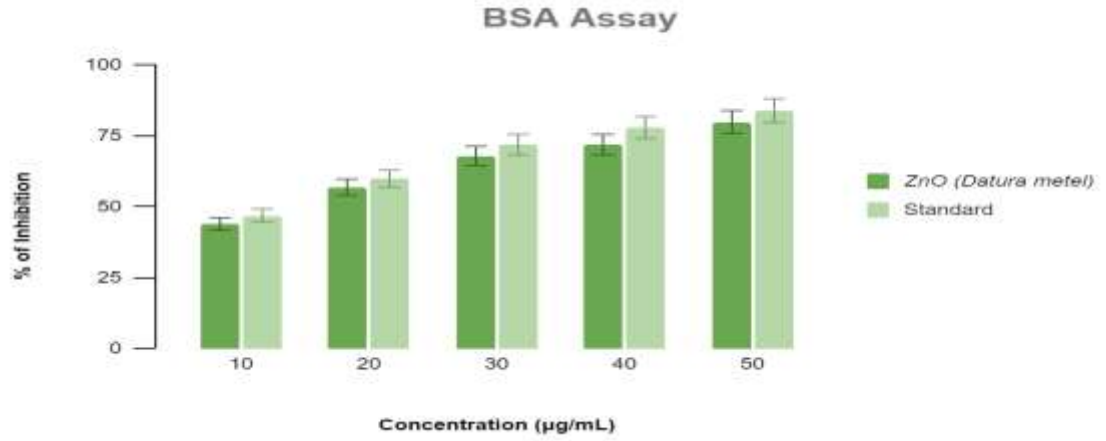

Figure 3: Bovine Serum Albumin (BSA) Assay comparing the anti-inflammatory effects of Zinc Oxide Nanoparticles (ZnO) synthesized from *Datura metel* against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.

Posteriorly, the anti-inflammatory potential of Datura metel mediated ZnO NPs was determined in Bovine Serum Albumin (BSA) denaturation assay. The assay determined the percent inhibition of protein denaturation at different concentrations of ZnO NPs with respect to a standard anti-inflammatory agent.

As shown in Figure 3, the inhibition of BSA denaturation was dose-dependent, as represented by the results. ZnO NPs at a concentration level of 10 µg/mL provided inhibition of protein denaturation in the order of nearly 27%. The inhibition continually increased in parallel with the concentration of the nanoparticle, measuring approximately 75% at a concentration of 50 µg/mL. This trend observed for the standard anti-inflammatory agent provided an additional confirmation to the efficiency of the methanol extracts of Datura metel synthesized ZnO NPs inhibition of denaturation.

Egg Albumin Denaturation (EA) Assay for Anti-inflammatory Evaluation

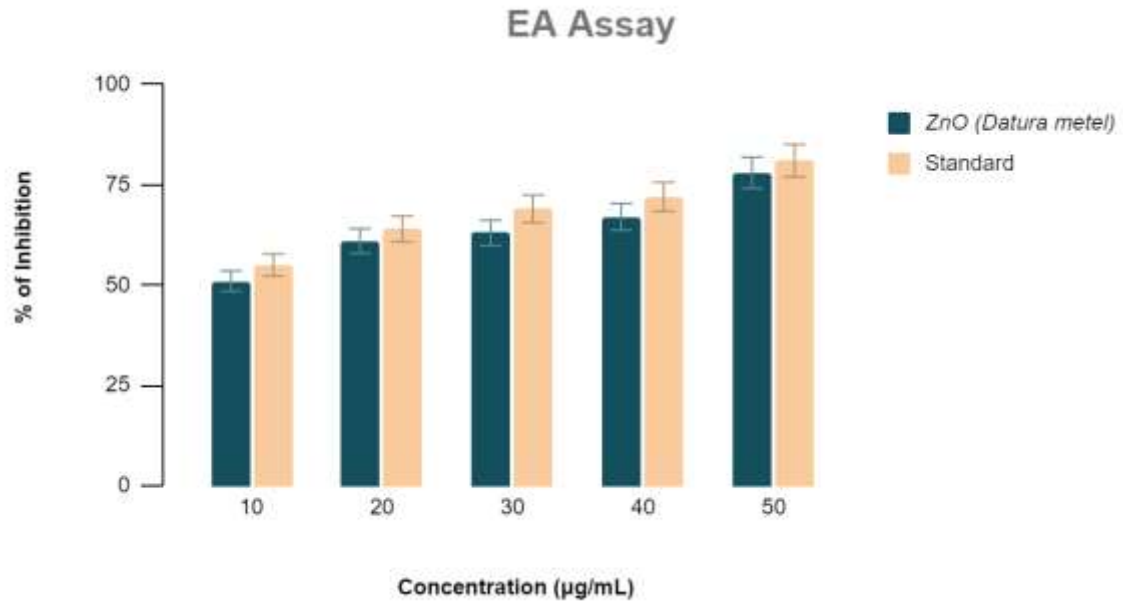

Figure 4: Egg Albumin (EA) Assay comparing the anti-inflammatory effects of Zinc Oxide Nanoparticles (ZnO) synthesized from *Datura metel* against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.

The Egg Albumin Denaturation (EA) assay further confirmed the anti-inflammatory activity of Datura metel synthesized Zinc Oxide Nanoparticles (ZnO NPs). This assay gives clues on the ability of ZnO NPs to prevent thermal denaturation of proteins, a central event of inflammation.
Inhibition of egg albumin denaturation was concentration-dependent, (Figure 4), for both ZnO NPs and standard anti-inflammatory agent. The nanoparticles began an inhibition rate of about 55% at 10 µg/mL, and then increased to approximately 75% at 50 µg/mL. The standard agent followed this pattern in parallel, indicating equivalent effectiveness.

Membrane Stabilization Assay

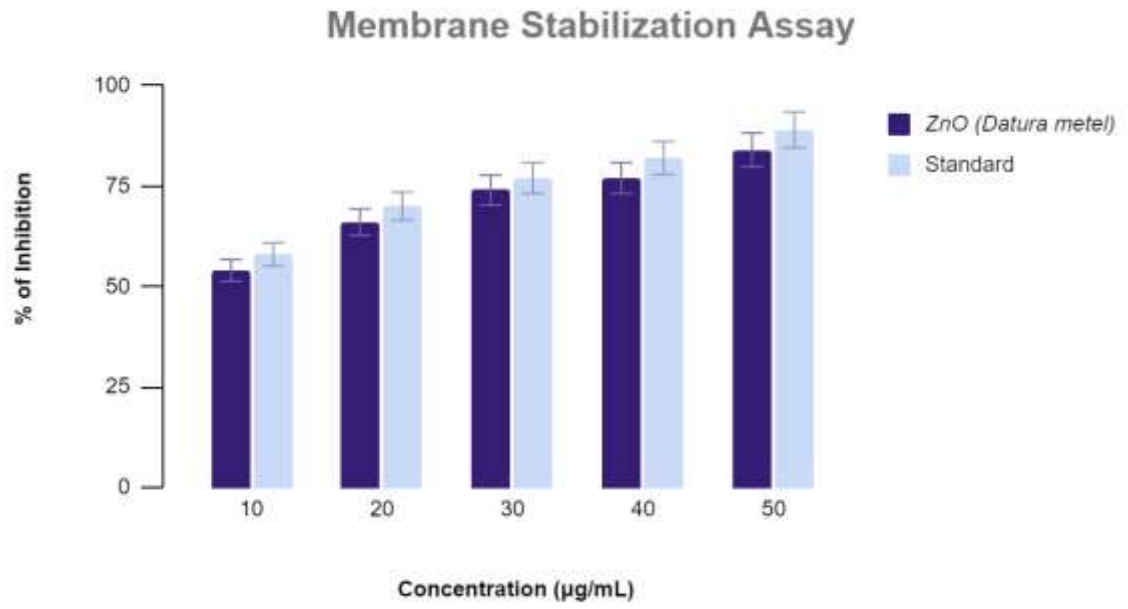

Figure 5: Membrane Stabilization Assay (MSA) comparing the anti-inflammatory effects of Zinc Oxide Nanoparticles (ZnO) synthesized from *Datura metel* against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.

The capacity of synthesized Datura metel ZnO NPs to stabilize the membrane under stress conditions was evaluated using a Membrane Stabilization Assay. Compounds are assessed for their anti-inflammatory properties based on their ability to inhibit the lysis of red blood cell membranes, which develops after exposure to various cell-damaging agents; thus this assay serves as a convenient model for screening cytoprotective activities.
Inhibition of ZnO NPs against standard diclofenac sodium is shown in Figure 5. It is evident from the results that the inhibition percentage for ZnO NPs shows a significant stabilization effect with a decrease of around 53% at 10 µg/mL while the inhibition percentage continuously increased up to 73% at 50 µg/mL. The similar trend was observed with the standard agent which ensured a sound comparison and clearly highlighted the efficacy of ZnO NPs.

Comparative Analysis :

The anti-inflammatory activities of Datura metel-synthesized Zinc Oxide Nanoparticles (ZnO NPs) were assessed using three different methods: Bovine Serum Albumin (BSA) denaturation, Egg Albumin (EA) denaturation, and Membrane Stabilization (MSA). The findings from each assay contribute to understanding the therapeutic potential of ZnO NPs in combating inflammation and providing cellular protection.
An increase in the percentage of inhibition was observed as the concentration of ZnO NPs increased for this assay, reaching a maximum of 75% inhibition at 50 µg/mL. The findings demonstrate that ZnO NPs effectively inhibit thermal induced denaturation of proteins, a major indication of their anti-inflammatory properties.
There was also a dose-dependent inhibition with the EA assay that was consistent with the data of the BSA assay. The egg albumin denaturation inhibition was not variable and near 75% inhibition was reached at 50 µg/mL. These results highlight the stability of ZnO NPs in preserving protein conformations from heat alterations.
In the MSA, the potential of ZnO NPs to stabilize cell membrane against induced lysis was observed, and inhibition was also concentration dependent. The nanoparticles inhibited the membrane disruption in a dose-dependent manner, with an inhibition of around 73% at 50 µg/mL and clearly showed its possible role in cellular protective mechanisms during inflammatory stress.

A correlation was noted across all assays where ZnO NPs showed that higher concentrations exhibited greater anti-inflammatory activity, indicating a correlation between concentration and efficacy. The common trend in BSA and EA assays indicates that ZnO NPs have a wide range of protection effect on protein structures, which is important for an anti-inflammatory application. Moreover, the MSA also indicates that cell structures are maintained better under stress, which is critical to prevent inflammation-related cell death.

The physicochemical properties of ZnONPs are unique and broad spectrum constitute its potential application in treating inflammatory diseases. The mechanisms through which they modulate inflammatory pathways are closely linked to their ability to impact biological processes at the most fundamental and cellular levels.

Studies have shown that ZnONPs are useful to inhibit the enzymes involved directly in the inflammatory process, such as collagenase and elastase. When uncontrolled, these enzymes can cause the degradation of tissues and inflammation. Previous studies (Dwivedi & Singh, 2024) have quantified these effects, where collagenase and elastase were inhibited by 68.72% and 65.16%, respectively. This inhibitory behavior reflects the promise of ZnONPs as pharmacological agents in conditions involving overexpression of enzymes such arthritis and skin-related disorders(24).

The antiinflammatory mechanisms of ZnONPs are largely dependent on its antioxidative properties. ZnONPs protect at cellular level and assist in reducing the inflammatory mediators including ROS and other free radicals. The superoxide radicals and nitric oxide suppression rates reported by Dwivedi & Singh (2024) are notably high as well, suggestive of substantive anti-inflammatory properties(24,25).

ZnONPs are known to activate several mechanistic pathways like Nrf2/HO-1, which in turn fortifies the GR adopted by the cells against oxidative stress. Guo et al. (2023) illustrate this interaction and underscore the importance of the capability of ZnONPs to promote cytoprotective responses, which is important for the resolution of prolonged inflammation and chronic inflammatory states(26).

This is further contributing to their therapeutic profile is the ability of ZnONPs to modulate apoptosis-related proteins. ZnONPs can help to both promote cell survival in inflammatory environments and prevent the pathological sequences induced by chronic inflammation as described by Cheng et al. (2024)(27).

In addition to their anti-inflammatory activity, therapeutic applications of ZnONPs are also being researched for cancer therapy and antibacterial applications. This versatility further increases their possible utility in a wide range of biomedical applications.

Although ZnONPs show great promise as therapeutic agents, the long-term safety and biocompatibility of ZnONPs is still under concern. Due to the complexity of biotic interactions with biological systems, extensive in vivo studies are required to accurately characterize their safety profiles.

In general, the comparative studies of these assays confirm the widespread anti-inflammatory and cytoprotective properties of ZnO NPs synthesized from Datura metel. These properties render ZnO NPs an ideal candidate for research into new anti-inflammatory treatments requiring broad-spectrum protection against multiple biochemical and cellular stresses.

Cytotoxic Effects of Zinc Oxide Nanoparticles (ZnONPs) on Brine Shrimp

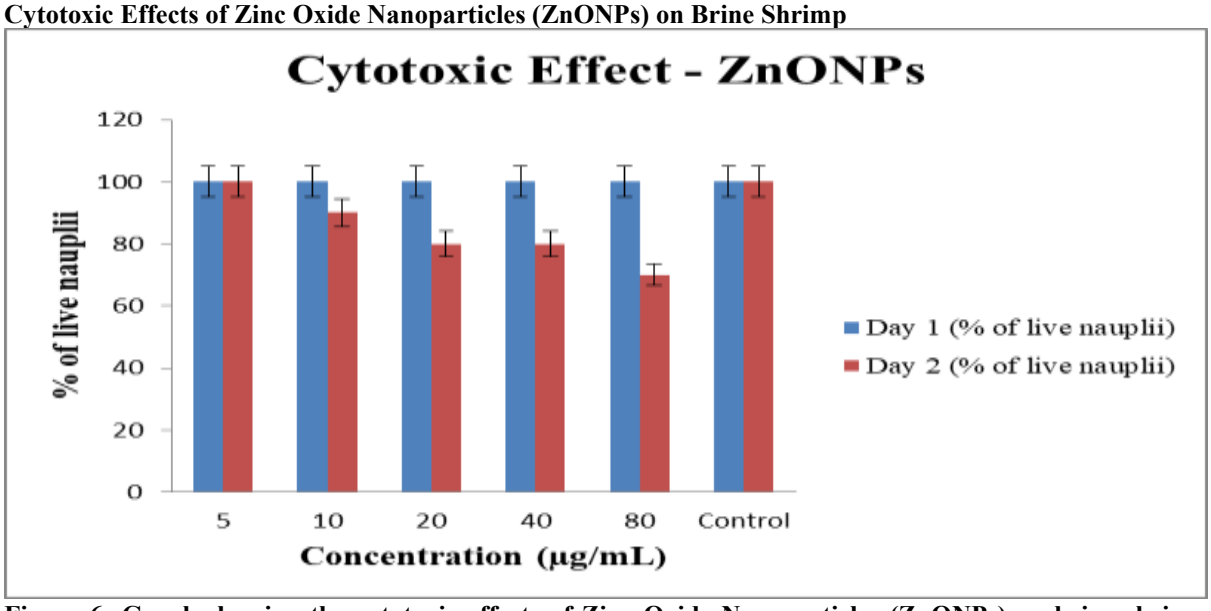

Figure 6: Graph showing the cytotoxic effects of Zinc Oxide Nanoparticles (ZnONPs) on brine shrimp nauplii over two days. The bars represent the percentage of live nauplii at various concentrations (5, 10, 20, 40, 80 µg/mL) alongside a control group, illustrating both day-to-day survival and dose-dependent toxicity.

Brine Shrimp Lethality Assay is considered to be a quick and efficient preliminary toxicity assessment tool that was used to investigate the cytotoxic potential of Zinc Oxide Nanoparticles (ZnONPs). A two-day bioassay was performed to determine the survival of brine shrimp nauplii exposed to all concentrations of ZnONPs.

The corrective capacity exposure about percentage of live nauplii after exposure to ZnONPs range from 5 µg/mL to 80 µg/mL along with in control without NP is shown in figure (6). Nauplii had a minimal reduction in survival rates on Day 1 with increasing concentrations of ZnONPs, indicating that they were fairly well-tolerated (over 80% survival) up to 40 µg/mL, with a minor reduction in survival at 80 µg/mL. Survival rates of all concentrations began to decrease from Day 2 onwards, which was particularly evident at higher concentrations, indicating that the inhibitory effects of ZnONPs started to appear from Day 2.

The survival rate of the control group remained close to 100% on both days, confirming that the cytotoxicity of ZnONPs observed in the treated groups was replicable. This indicates a toxic effect that is dependent on the dose as the higher concentrations experienced are more lethal. Importantly, the toxic effects of ZnONPs were significant even at lower concentrations and with increased time.

The brine shrimp (Artemia) lethality assay has been widely used to further reveal and provide details on the cytotoxic effects of ZnO NPs, displaying toxicological effects that were dose-dependent as well as time dependent. This assay therefore represents a strong model to be able to use for assessing the ecological and human health risk of NP substances.

Concentration and exposition time strongly influence the lethality of ZnO NPs to Artemia nauplii. According to Sarkheil et al. (2018), the 96hr median effective concentration (EC50) for zinc oxide nanoparticles was designated as 4.86 mg/L, which can be interpreted as the concentration at which half the population of nauplii is predicted to be affected, highlighting the relatively high toxicity of these particles with chronic exposures(28).

ZnO NPs size is responsible for cytotoxicity: smaller NPs (10–30 nm) are more toxic than larger (200 nm) ones. The mechanism of toxicity in a size-dependent manner can be explained by the large surface area-to-volume ratio of smaller nanoparticles, thus increasing their interaction with biological membranes and subsequently bioactivity and cellular toxicity.Therefore, the major pathway for exerting toxic effects of ZnO NPs in brine shrimp is most likely to be through oxidative stress. Ates et al. (2013) reported increased lipid peroxidation, an oxidative damage marker, indicating that generation of reactive oxygen species (ROS) is a contributory aspect of the toxicity mechanism. Such oxidative stress can interfere with integrity of cells, resulting in death to the exposed organisms(29).

Notably, the plant extract-mediated biosynthesis of ZnO NPs has been reported to reduce their toxicity. The lower toxicity may be attributed to the capping agents originating from the plant extracts that alter the surface characteristics of ZnO NPs, leading to deactivation and lower toxicity(30).

Zebrafish Embryonic Toxicology Evaluation of Zinc Oxide Nanoparticles Synthesized Using *Datura metel*

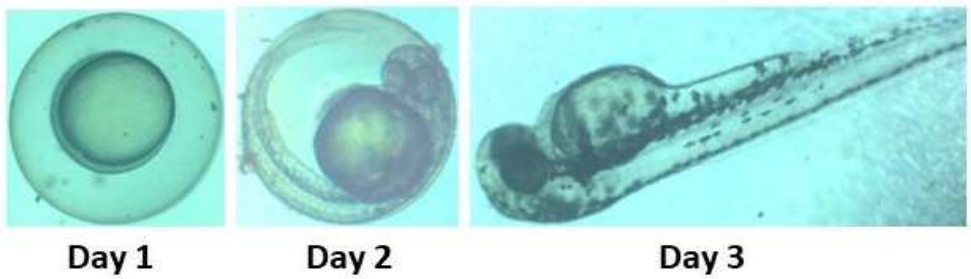

Figure 7: **Progressive developmental stages of zebrafish embryos exposed to environmental conditions. Day 1 shows a fertilized embryo, Day 2 features early developmental changes, and Day 3 displays advanced growth and the onset of major organ development.**

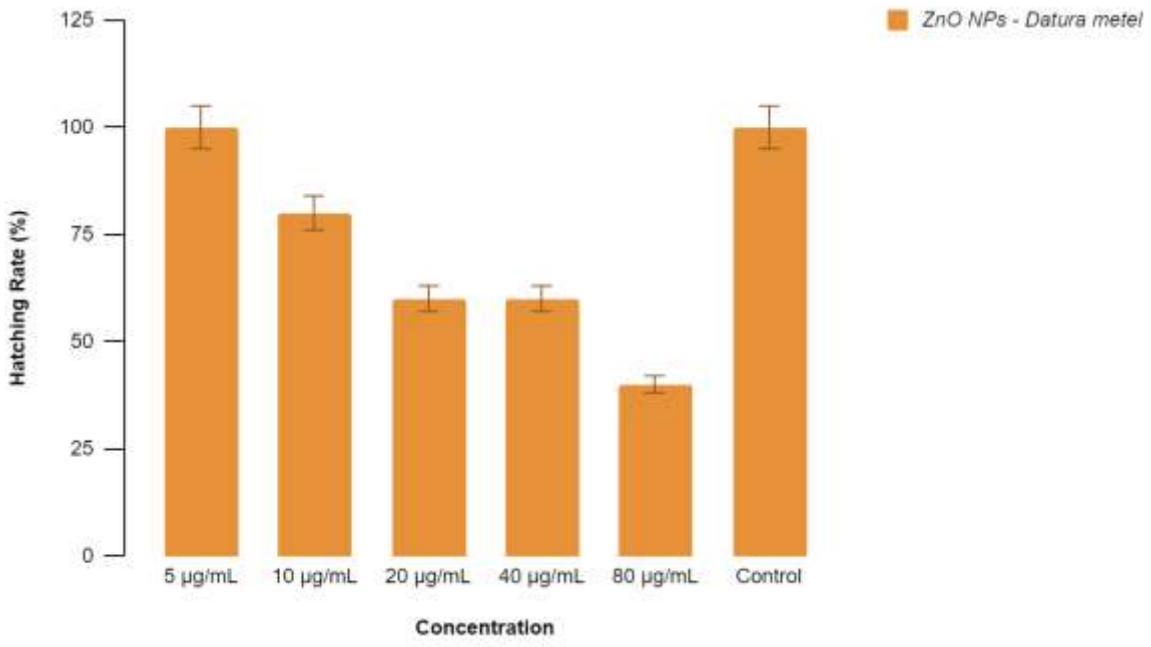

Figure 8: Hatching rates of zebrafish embryos exposed to varying concentrations of Zinc Oxide Nanoparticles (ZnO NPs) synthesized from *Datura metel*. The graph displays the percentage of successful hatching at concentrations of 5, 10, 20, and 40 µg/mL compared to a control group, illustrating the impact of nanoparticle concentration on embryonic development.

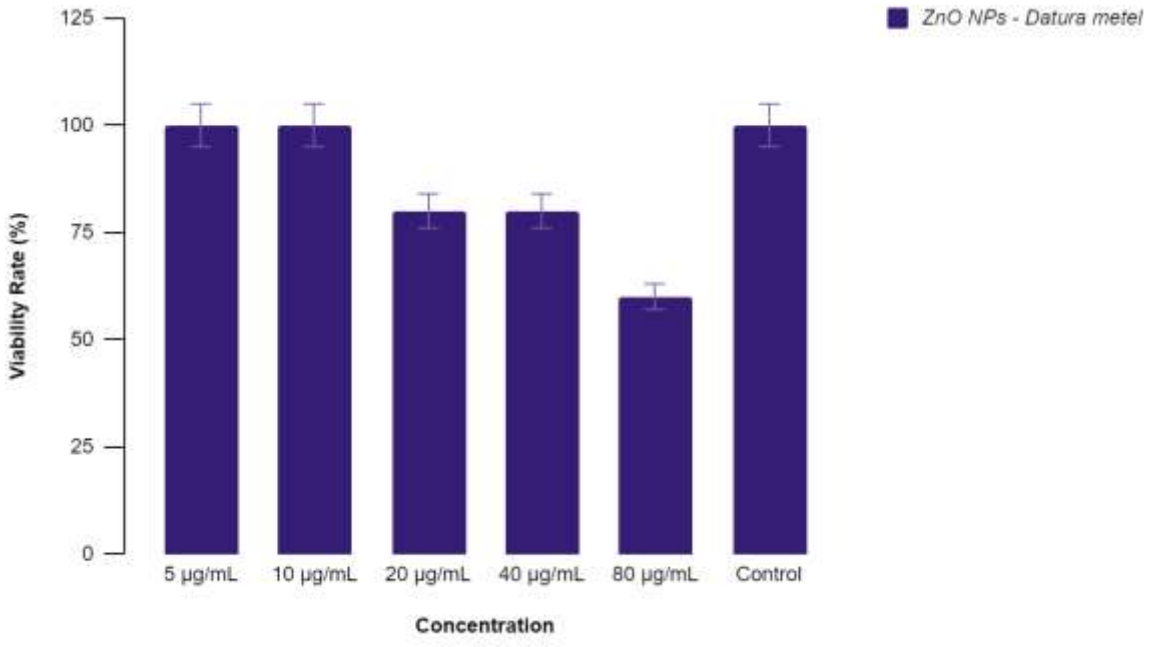

Figure 9: Viability rates of zebrafish embryos exposed to various concentrations of Zinc Oxide Nanoparticles (ZnO NPs) synthesized from *Datura metel*. The graph shows the percentage of viable embryos at concentrations ranging from 5 to 80 µg/mL compared to a control group, demonstrating the effects of nanoparticle concentration on embryonic survival.

The current study was carried out to examine the developmental and toxicological effects of Datura metel-derived Zinc Oxide Nanoparticles (ZnONPs) using a zebrafish embryonic toxicology approach. The objective of this study was to investigate embryonic viability, hatching rates and morphological development for three days in zebrafish embryos exposed to multiple concentrations of ZnONPs.

ZnONPs were administered to zebrafish embryos at concentrations of 5 µg/mL to 80 µg/mL. Viability rates were evaluated daily and controls were included for comparison. Figure 9 exhibited a concentration-dependent

reduction in the viability of embryos exposed to ZnONPs. Only at relatively lower concentrations (5 µg/mL and 10 µg/mL) were the viability rates comparable to those of the control group, reflecting minimal toxic effects. Conversely, following treatment with 40 µg/mL and 80 µg/mL of the tested nanoparticles, the viability rates significantly decreased pointing to the higher embryotoxicity of the tested nanoparticles in the higher concentrations.

Figure 8 showed the effects of ZnONPs on embryonic development assessed by monitoring hatching rates (sub-lethal effect). The results showed that 5 µg/mL had no effect on hatching, as it was more than 95% similar to the control. Notably, hatching rates dropped with increasing concentrations. The hatching rate was significantly reduced at 40 µg/mL and completely reduced at 80 µg/mL, indicating high levels of developmental delays or defects due to exposure to the nanoparticles.

Embryos were observed daily for morphological changes and developmental abnormalities (Figure 7). Embryos appeared morphologically normal on Day 1 following all treatments. On Day 2 of exposure, individuals exposed to higher concentrations (40 µg/mL and 80 µg/mL) began to present morphological deformities, most notably pericardial edema and spinal abnormalities. The knock-on effects were more apparent by Day 3 with clear malformation evident in the groups with the highest concentrations of ZnONPs, confirming the teratogenic potential of ZnONPs at high levels of exposure.

The dependence of toxicity with increase in doses of ZnONPs was further reflected through the comparative analysis of viability and hatching rates. This is especially crucial for the possible therapeutic and environmental implications of these nanoparticles, where low concentrations manifest little to moderate effects but high concentrations depict considerable toxicity levels(31).

The potential ecological effects of ZnO NPs, particularly in aquatic organisms like zebrafish, are needed due to the increasing presence of NPs in a range of industrial and biomedical applications. Developmental toxicity and behavioral changes following exposure to ZnO NPs have been a consistent focus of these studies(32).

During key stages of early development in zebrafish, exposure to ZnO NPs causes significant physical deformities. Notably, tail malformations, fluorescence-positive pericardial edema, and yolk sac edema are phenotypical characteristics of disrupted embryogenesis along with potential long-term physiological deficits(33). These type of defects, visible during both the embryonic and larval stages, are indicative of developmental processes that have been disrupted, perhaps through mediated mechanisms of oxidative stress relation and apoptosis. These abnormalities are shown to persist in later stages, illustrating the long-lasting health consequences of early exposure to nanoparticles on the organism(34).

The effect of ZnO NPs on hatching rates indicates its developmental toxicity. For example, higher sensitivities have generally been tied to higher concentrations of nanoparticles, providing a clear dose-dependent toxicity; these reductions in hatching success may obviously inhibit the viability of many newly-hatched larvae(31). This inhibition of hatching indicates not only acute toxicity but also a possible sub-lethal effect which could affect population sustainability in the wild(35).

ZnO NP exposure has been shown to influence zebrafish behaviors including locomotion or sensory function. Nanoparticle exposure alters larval swimming behavior, suggesting that neuromuscular or sensory system performance may be altered(36). These changes in locomotor activity are also important in the context of fish because this behaviour affects feeding, predator avoidance and social interactions, ultimately affecting survival and indirect fitness(37).

Especially concerning, are the neurodevelopmental effects of ZnO NPs.as indicated by alterations in neurotransmitter levels and neuronal differentiation. Neurogenesis is the process by which neural stem cells produce haploid neurons and its disruption in early development may result in long-lasting behavioral impairment and deficits in cognition and social behaviors (both of which are essential for optimal ecological fitness in zebrafish)(38).

Although the current evidence base has highlighted some risks of ZnO NP exposure, more work is required to determine the mechanisms underlying these effects. In addition, investigation to encourage the potential toxicity reduction potentiality of biosynthesized ZnO NPs could provide alternate safer way to use ZnO NPs for their affirmed advantageous applications(39,40).

These extensive data show that ZnONPs concentration governs zebrafish embryo development and survival, and thus presents novel conclusions regarding the risk–benefit profile of nanomaterials in terms of safety and environmental outcomes. Therefore, this study highlights the importance of performing detailed toxicity evaluations and implementing strict control measures regarding the use of ZnONPs of biomedical and environmental applications.

CONCLUSION

The Datura metel-derived Zinc Oxide Nanoparticles (ZnO NPs) demonstrated significant antimicrobial properties against a spectrum of pathogenic bacteria, including Staphylococcus aureus, Escherichia coli, Enterococcus

faecalis, and Pseudomonas sp. The concentration-dependent inhibition observed underscores the potential of ZnO NPs as effective antimicrobial agents. Additionally, the nanoparticles exhibited considerable anti-inflammatory effects, as evidenced by their ability to inhibit protein denaturation in BSA and EA assays and stabilize cell membranes under stress conditions. However, the toxicological assessments through Brine Shrimp Lethality and zebrafish embryonic toxicity studies highlighted concerns regarding the safety of higher concentrations of ZnO NPs, which showed cytotoxic and embryotoxic effects. These findings suggest that while ZnO NPs hold promising therapeutic applications due to their antimicrobial and anti-inflammatory properties, their use must be judiciously managed to mitigate potential toxicological risks. Future studies should focus on optimizing the concentration and delivery mechanisms of ZnO NPs to maximize their beneficial effects while minimizing adverse outcomes, ensuring their safe and effective application in biomedical and environmental fields.

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