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Cytotoxic Activity of Zinc Oxide Nanoparticle Synthesis Using Leaves Extract of *Abies webbiana*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft manuscript. Authors MJ and SR managed the analyses of the study. Author SJ managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The present study aimed to analyse the cytotoxicity of zinc oxide nanoparticles (ZnO NPs) that are biosynthesized using extract of *Abies webbiana*
Methodology: Zinc oxide nanoparticles were synthesized by green approach using *A. webbiana* and were characterized by UV- vis spectroscopy. The powdered *A. webbiana* mediated ZnO NPs were subjected to cytotoxic tests for different concentrations by brine shrimp lethality assay.
Results: ZnO NPs synthesized from *A. webbiana* showed reduced toxicity at lower concentrations. After 48 hours, it was found that at a minimal concentration of 5 µl, 90% of the nauplii and in concentration of 10 µl and 20 µl, 80% of the nauplii were alive. At a concentration of 40 µl and 80 µl, 70% of the nauplii were alive. Whereas the control showed 100% of the nauplii to be alive.
Conclusion: Various nanoformulations of *A. webbiana* mediated ZnO NPs can be developed at optimal concentrations for therapeutics that are safe and economical.

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Keywords: Abies webbiana; brine shrimp lethality assay; cytotoxicity; green synthesis; innovative technology; zinc oxide nanoparticles.

1. INTRODUCTION

Nanoparticles are broadly applied in medicine, engineering, agriculture and in almost all other fields [1]. The properties of nanoparticles demonstrate enormous applications compared to those of mass materials because of their incredibly small size [2]. Metal nanoparticles are extensively used in a wide range of applications including chemical and biological sensing, catalysis, energy, drug and gene delivery and electronics [1,3–8]. Nanoparticles have been intensively studied over the last decade due to their characteristics such as the physical, chemical, electronic and electrical properties [9].

Zinc oxide nanoparticles (ZnO NPs), which are known to be nontoxic, chemically stable, biocompatible, and could be used as drug carriers, cell imaging agents, anticancer agents, antimicrobials, biosensors, antidiabetics, and cosmetics, because of their novel physicochemical properties. Crude extracts of *Abies Webbiana* leaf had antibacterial, mast cell stabilizing, anxiolytic, anti-tumor, anti-inflammatory, antitussive and central nervous system (CNS) depressant actions [10–14]. With reference to our previous studies [2,15–22] and other available literature, no report has yet highlighted the synthesis of ZnO NPs using *A. Webbiana.* Cytotoxicity is the nature of being harmful to cells. Brine shrimp lethality assay using the larvae of the crustacean, *Artemia salina* is a more common method which is employed to analyse the cytotoxicity of bioactive compounds [23,24]. Our team has extensive knowledge and research experience that has translated into high quality publications. 25–44]. The present study aimed to analyse the cytotoxicity of the *A. webbiana* mediated zinc oxide nanoparticles.

2. MATERIALS AND METHODS

2.1 Preparation of *A. webbiana* Extract

Dried *A. webbiana* powder was procured from Annai Aravindh Vedic Centre, Chennai. To prepare the *A. webbiana* extract, 50 mL of distilled water was added to 0.5 g of *A. webbiana* powder in a conical flask. The heating mantle was utilized to heat the reaction mixture with the temperature of 50°C for 6-8 minutes. After heating, the solution was filtered using Whatman filter paper to get a clear filtrate.

2.2 Synthesis of ZnO NPs

For synthesizing ZnO NPs, 0.5 g of anhydrous Zinc sulfate was taken and to this 70 mL of distilled water was added. Then the solution was added to the previously prepared 30 mL of plant extract. The solution was brown in color and subjected to an orbital shaker for uniform dispersion of the reaction mixture and the color changes were visually observed at various periods of time. After complete synthesis of ZnO NPs, the solution was then dried by heating. The obtained ZnO NPs were utilized for further examination. The optical properties of the ZnO NPs were determined utilizing UV-vis spectroscopy. The synthesised nanoparticles were centrifuged and pellets were collected. The collected pellets were dried and stored for further analysis. The total weight of nanoparticle pellets was 265 mg.

2.3 Cytotoxic Effect of ZnO NPs

Brine shrimp lethality assay was performed for determining the cytotoxic effect of ZnO NPs. Brine shrimp eggs were procured from Aquatic Remedies, Chennai. The hatching of eggs was encouraged by adding it to the artificially made seawater by dissolving 36 g of sea salt in 1000 mL of distilled water. This artificial seawater was added to the chamber that had a partition for dark and light areas. Shrimp eggs were added to the dark area of the chamber. Once the eggs hatched, it took 2-3 days for it to mature into larvae. These larvae moved to the light area of the partition. The hatched nauplii was used for the cytotoxicity evaluation, A 6-well ELISA plate was taken and 5 mL of artificial seawater was added to each of the 6 wells and 10 nauplii were added to each well. Then, 5 different concentrations of dried ginger mediated copper nanoparticles (5 µl, 10 µl, 20 µl, 30 µl, 50 µl) were introduced to each of the 5 wells and a control containing only the seawater was taken in one well. The wells were left uncovered under the lamp. The number of surviving nauplii was recorded periodically after 24 h and 48 h of incubation.

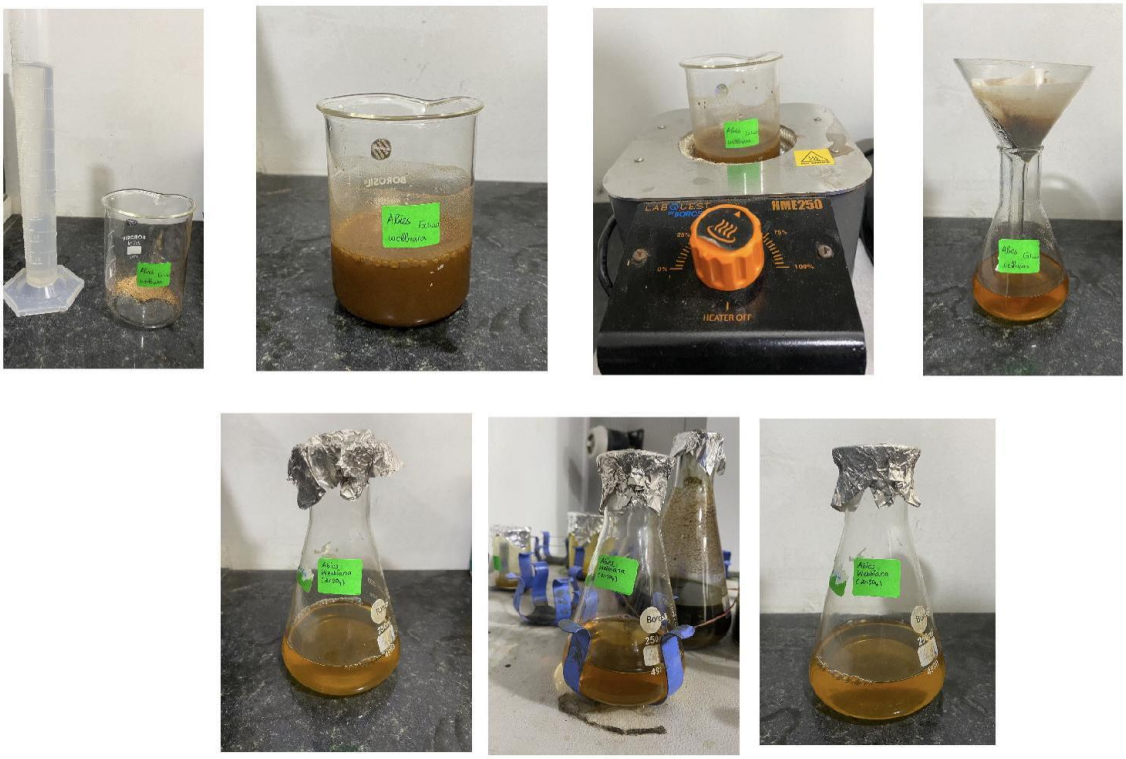

Fig. 1. This picture shows the procedure used to obtain *A. webbiana* mediated ZNO NPs

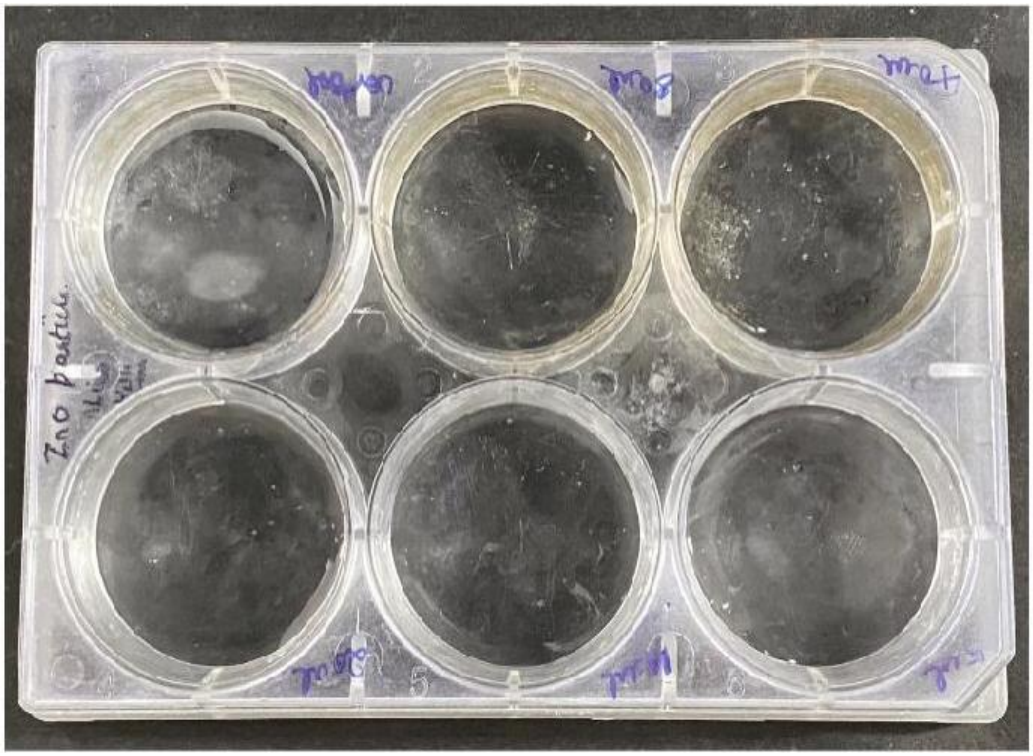

Fig. 2. Brine shrimp lethality assay comprising ELISA plate wells with different concentrations of *A. webbiana* mediated ZnO NPs and a control observed for the presence of live nauplii after 24 h and 48 h incubation

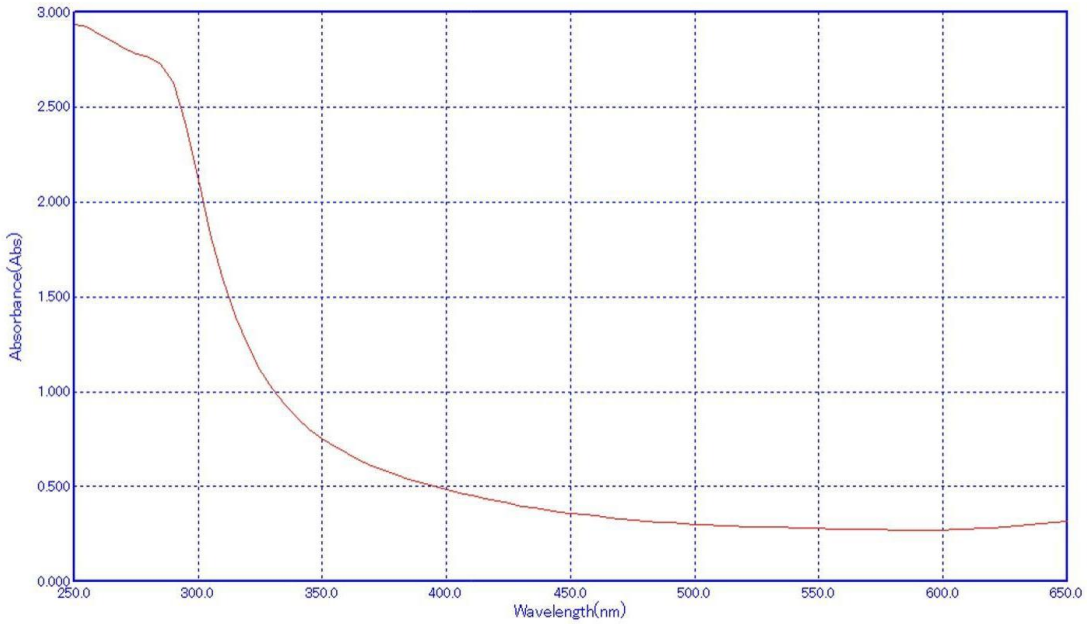

Fig. 3. UV- vis spectroscopic analysis of ZnO NPs synthesized from *A. webbiana* recorded as function of time

3. RESULTS AND DISCUSSION

Nanobiotechnology is the merge between biotechnology and nanotechnology for developing biosynthetic and ecofriendly technology for the synthesis of nanomaterials [45]. Among the metal nanoparticles ZnO.NPs are interesting due to its impressive properties from which the wide band gap, large binding energy and high piezoelectric property [46]. ZnO.NPs which can exhibit a wide variety of nanostructures are believed to be biosafe, non toxic and biocompatible, been used in various technologies and industries such as optoelectronics, piezoelectric and magnetic sensors, biological labelling, ceramic and rubber processing, environmental protection, biology and medicinal [8,47,48]. Chemically, the surface of ZnO is rich in -OH groups, which permit ZnO to slowly dissolve in both acidic (e.g., the tumor cells and tumor microenvironment) and strong basic conditions. Based on this property, ZnO.NPs have gained immense interest in biomedical applications [49].

3.1 Visual Observation

In the visual identification, there was color change at various intervals of the incubation time. The first color change was observed when the Zinc sulphate was reduced to ZnO NPs giving a brown color. After 2 hours of the incubation period there was change from brown to light yellow (Fig. 1). Similar visual observations of colour change were found in the study done by Bisht et al and Sonia et al [50,51].

3.2 The UV- Spectrophotometer Analysis

Spectrophotometer analysis depends on the arising color of the reaction mixture due to transition of ions from ground state to excited state. The reduction process of the solution was optically measured at different wavelengths and was recorded. The UV- vis spectroscopy analysis of the present study showed that the surface plasmon resonance band peak was positioned at the wavelength of 290 nm (Fig. 3).

3.3 Cytotoxicity Analysis

Brine shrimp lethality assay is an important test in the study of toxicity, that gives us the information about the cytotoxic effect exhibited by a bioactive compound to cells. The viability of the nauplii was analysed for different concentrations of ZnO NPs that are synthesized from *A. webbiana* (Fig. 2). After 24 hours, 100% of the nauplii were alive in concentration of 5 µl,10 µl, 20 µl, 40 µl and 80 µl. The control also showed 100% of the nauplii to be alive. After 48 hours, it was found that at a minimal concentration of 5 µl, 90% of the nauplii and in concentration of 10 µl and 20 µl, 80% of the

Table 1. Brine shrimp lethality assay for different concentrations of *A. webbiana* mediated ZNO NPs observed after 24 h and 48 h incubation

	5 µL	10 µL	20 µL	40 µL	80 µL	control
Day 1	10	10	10	10	10	10
Day 2	9	8	8	7	7	10

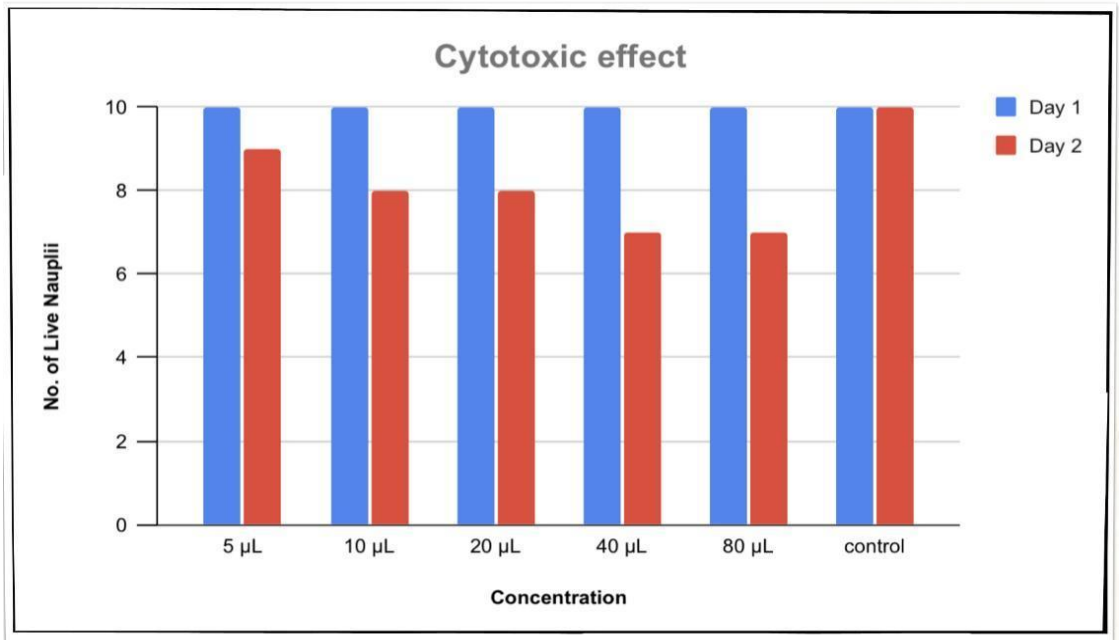

Fig. 4. This graph depicts the cytotoxic effect of *A. webbiana* mediated ZNO NPs

nauplii were alive. At a concentration of 40 µl and 80 µl, 70% of the nauplii were alive. Whereas the control showed 100% of the nauplii to be alive (Table 1) (Fig. 4). Thus, the increase in concentration increased the cytotoxicity. These findings were found to be in agreement with the findings of research on cytotoxicity by Rishey et al, Renugadevi et al and Begum. A et al. [52–54]. More specific cytotoxicity studies need to be undertaken in order to study the exact mechanism of action. Cytotoxicity was seen to be higher in 40 µl and 80 µl concentrations in the present study.

4. CONCLUSION

Within the limitations of the study we can conclude that *A. webbiana* enabled the synthesis of a stable Zn-NPs. The results indicate that the zinc nanoparticles synthesized from *A. webbiana* showed reduced toxicity. Thus, \various nano-formulations at lower concentrations of these nanoparticles can be developed that are safe, eco friendly and economical. Future research in *A. webbiana* mediated ZNO NPs evaluating its biological properties like antimicrobial, antiinflammatory and antioxidant activities can bring about the development of nano-formulations like mouthwash, toothpaste and oral gels as therapeutics in various oral diseases.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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