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Green Synthesis of Magnesium Oxide Nanoparticles using *Cresentia cujete* Leaf Extract and its Toxicity Assay on *Danio rerio*

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ABSTRACT

Nanomaterials have been widely applied in different fields of science, such as biotechnology, microbiology, environmental remediation, medicine, various engineering, and material sciences, because of their peculiar properties. For biomedical and ecotoxicological concerns, it is necessary to evaluate their biocompatibility at the molecular level. The green production of magnesium oxide nanoparticles is investigated in this study using *Crescentia cujete* leaf extract (MgONP). The synthesized MgONP has a dimension of 626 nm and a hydrodynamic diameter of 246 ± 12, according to FE-SEM and dynamic light scattering (DLS). MgONP was stable in fish medium, with a zeta potential of 19 ± 10 mV. The current work assesses the biocompatibility of produced nanoparticles using the zebrafish model, which is a well-established animal model for biocompatibility due to its quick development, transparency, and strong genetic similarity to humans. The influence of the produced nanoparticles on development was studied to determine biocompatibility.

Key words: *Danio rerio*, *Cresentia cujete*, Toxicity, Nanoparticles, Leaf extract

In recent decades, nanomaterials have been widely applied in different fields of science, such as biotechnology, microbiology, environmental remediation, medicine, various engineering, and material sciences, because of their peculiar properties (e.g., large area to volume ratio) [10], [13]. The Food and Drug Administration, United States, has authorized numerous nanomedicines. Many of them have advanced to the concluding stages of development [17]. Green synthesis of MgO is considered as a potential and eco-friendly way towards creation of Inorganic metal oxide nanoparticle. Inorganic materials such as metal and metal oxides have attracted a lot of attention over the past decades due to their ability to withstand harsh process conditions [7], [11]. Metal oxide is of particular interest as it is not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals [5]. There are numerous chemical methods available to synthesize MgO nanoparticles but as the usage of chemicals are highly toxic and hazardous it may lead to the environmental problems [21]. Solution combustion, co-precipitation, sol-gel, hydrothermal, Solvo thermal, micro aided sol-gel, and the green technique are currently accessible methods for synthesizing metal oxide nanoparticles [3]. When compared to other methods, green synthesis of metal oxide nanoparticles is reported to be more favourable in terms of cost, less use of harmful chemicals, and the product and by products being environmentally benign in nature [9]. Magnesium oxide is a fascinating basic oxide with numerous uses in catalysis, adsorption, and refractory ceramic production [1], [18], [20], [24]. The large number of edge/corner sites and structural flaws on the surface of nanocrystalline magnesium oxide particles has been found to have a high specific surface and reactivity due to their shape and size. Apart from all of this, Magnesium Oxide Nanoparticles are crucial since they possess distinct features in comparison to bulk materials. MgO nanoparticles are particularly distinctive due to their exceptional qualities such as strong chemical stability, high photocatalytic activity, high electrical permittivity, and non-toxic nature. Catalysis, toxic waste cleanup, paints, superconducting goods, optical, electrical, electronic, antiseptic, antibacterial properties, semiconductors, and catalytic devices are among its many uses [4]. Given these characteristics, the current work assesses the biocompatibility of produced nanoparticles using the zebrafish model, which is a well-established animal model for biocompatibility due to its quick development, transparency, and strong genetic similarity to humans. The influence of the produced nanoparticles on development was determined to determine biocompatibility.

In the present study, *Crescentia cujete* L. leaf extract was used for synthesizing magnesium oxide nanoparticles. *Crescentia cujete*, often known as the calabash tree, is a

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flowering plant that can be found in Africa, Central America, South America, the West Indies, and the far south of Florida. [8]. It is a member of the Bignoniaceae family and has a wide range of therapeutic applications [15]. The pulp of this fruit is used as a laxative and for the treatment of several respiratory diseases, such as asthma and catarrh, according to traditional medicine. The bark is used to treat mucoid diarrhoea and to disinfect wounds. The leaves are used to cure tumours and hematomas, as well as a diuretic and a poultice for headaches and hypertension [2]. Bronchitis, asthma, colds, coughs, stomachaches, diarrhoea, and urethritis are all treated with a decoction of its fruit [14]. Naphthoquinones, aucubin, iridoid glycosides, asperulo-side, and plumerialumieride are all found in the plant's leaves [12], tannins, citric acid, alpha and beta marina, beta-sitosterol, triacontanol, apigenin, stigmasterol, p-hydroxybenzoyloxy-gluco-esteric acid, 3-hydroxyoctanol glycosides, palmitic acid, and flavonoids-quercetin. *Crescentia cujete*'s biomolecules and phytochemicals have been demonstrated to have therapeutic effects in various clinical trials. [14]. It's possible that nanoparticles made with *Crescentia cujete* have *Crescentia cujete*'s qualities and, as a result, can be employed for a variety of medical reasons while maintaining good biocompatibility. Furthermore, the procedure is simple, safe, and environmentally beneficial. For the first time, a unique approach of producing magnesium oxide nanoparticles using *Crescentia cujete* proteins and evaluating their toxicity effect on Danio rerio is revealed in this paper.

MATERIALS AND METHODS

Green synthesis of magnesium oxide nanoparticles

Crescentia cujete leaf extract was used as a reducing agent, and magnesium sulphate (MgSO4) was used as a metal salt precursor in the green synthesis of magnesium oxide nanoparticles. Fresh leaves of *Crescentia cujete* were taken from the Botanical Garden, Vinoba Bhave University, Hazaribagh, India, and cleaned thoroughly using tap water, followed by distilled water, to eliminate any dirt material. Cut into little pieces, these were air-dried at ambient temperature. The extract was made by boiling 10 g of leaves in 100 mL distilled water for 20 minutes. To obtain a clear extract, the extract was filtered twice with Whatman No. 1 filter paper. The reaction solution was made by combining a 1:1 ratio of an aqueous solution of 1 mM magnesium sulphate (MgSO4) with the leaf extract and leaving it at room temperature for 24 hours. After 24 hours, the solution was centrifuged three times at 5000 rpm for 15 minutes to obtain the MgONP powder, and the pellet containing the MgONP was dried in a hot air oven.

Physiochemical characterization

Standard procedures were used to characterise MgONP's physiochemical properties. The optical characteristics of the nanoparticles were determined using UV-Vis spectroscopy. The scanning was done using a UV-visible spectrophotometer (Cary 5000, Agilent, Santa Clara, CA, USA) and a spectral scan between 200 and 800 nm. The MgONP's hydrodynamic size and zeta potential were also assessed using Zetasizer and dynamic light scattering (DLS) (Malvern, UK). The size of the MgONP was also evaluated using FE-SEM electron microscopy (Carl Zeiss, Jena,Germany). The materials were dried in the sun and photographed at a voltage of 20 KV.

Maintenance of zebrafish

Adult zebrafish were kept in an aquarium system bought at a local market in Hazaribagh, Jharkhand, India. Fish water with 75 g NaHCO3, 18 g sea salt, and 8.4 g CaSO4 per 1000mL was managed in the system to maintain physiological parameters [16]. Fish meal containing bloodworms was fed to the fish three times a day. All animal procedures followed the applicable criteria of VBU University's Institutional Animal Ethics Committee (IAEC). The appropriate criteria of the VBU University's Institutional Animal Ethics Committee approved all animal procedures (IAEC). All investigations were carried out in accordance with IAEC, VBU University laws and applicable animal management standards.

Toxicological assay

In adult zebrafish, toxicological estimate of MgONP was detected. The size and charge of the nanoparticle were determined. 3-month-old fish of proportionate sizes were subjected to different concentrations of MgONP (10, 50, 200 g/mL) in 200 mL of filter-sterilized fish water for the toxicological study [19]. The trial lasted for 96 hours. Photographs were collected of the morphological abnormalities, and the mortality rate was calculated. All of the studies were done in triplicate, and the results were provided as mean standard deviation.

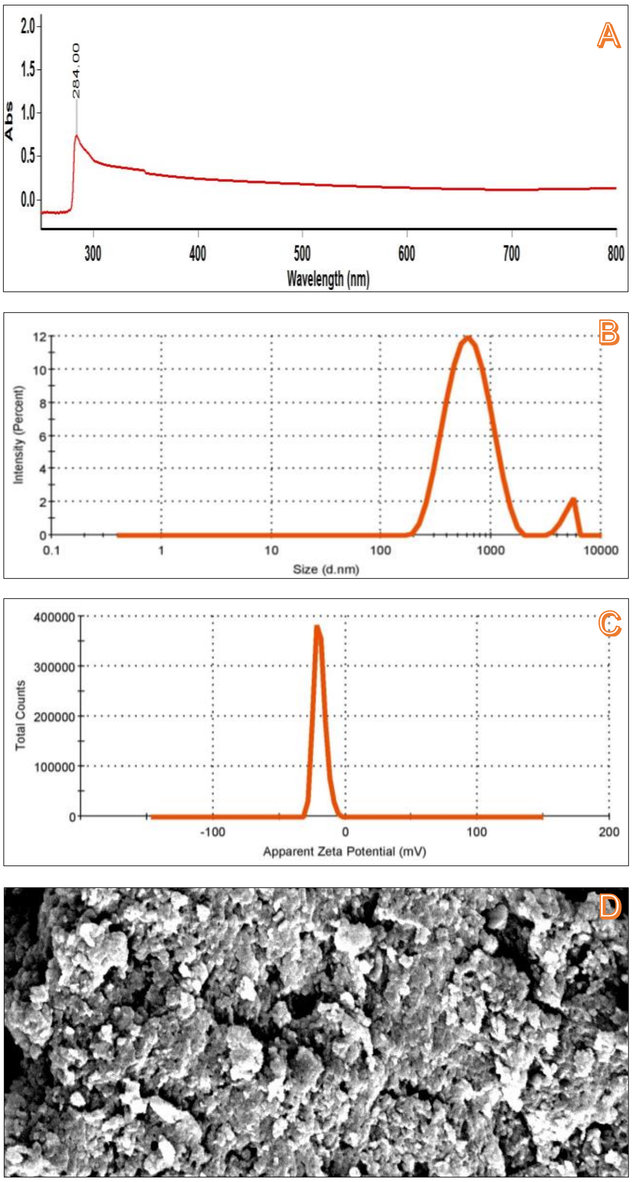

Fig 1 Physiochemical characterization of green synthesized CaONP. (A) UV–Vis spectrum of CaONP;(B) zeta-potential of CaONP determined by dynamic light scattering; (C) hydrodynamic diameter of CaONP as determined by dynamic light scattering; (D) optical image of CaONP as determined by FE-SEM

RESULTS AND DISCUSSION

Green synthesis and characterization of MgONP

The successful green production of magnesium oxide nanoparticles (MgONP) was carried out utilising *Crescentia cujete* leaf extract. For characterization, the produced nanoparticles were dried and dispersed in an HF medium. As illustrated in (Fig 1A), the nanoparticles were evaluated for their physical and optical properties. A sharp peak at 284nm was seen in the UV-Vis spectrum study. The appearance of an absorbance peak has been described as a distinguishing property of nanoparticles that may be used to categorize their optical properties [22]. The stability of the nanoparticles in the HF medium was further tested by calculating their zeta potential using dynamic light scattering. The nanoparticles hydrodynamic diameter was discovered to be 246 ± 12 nm as shown in (Fig 1B), indicating the stability of CaONP. The zeta potential of MgONP was determined to be −19 ± 10 mV, as shown in (Fig 1C). FE-SEM investigation of synthesized CaONP revealed a size of 626 nm (Fig 1D). The nanoparticles' physicochemical evaluation validated their nano characteristics and the efficient fabrication of nanoparticles.

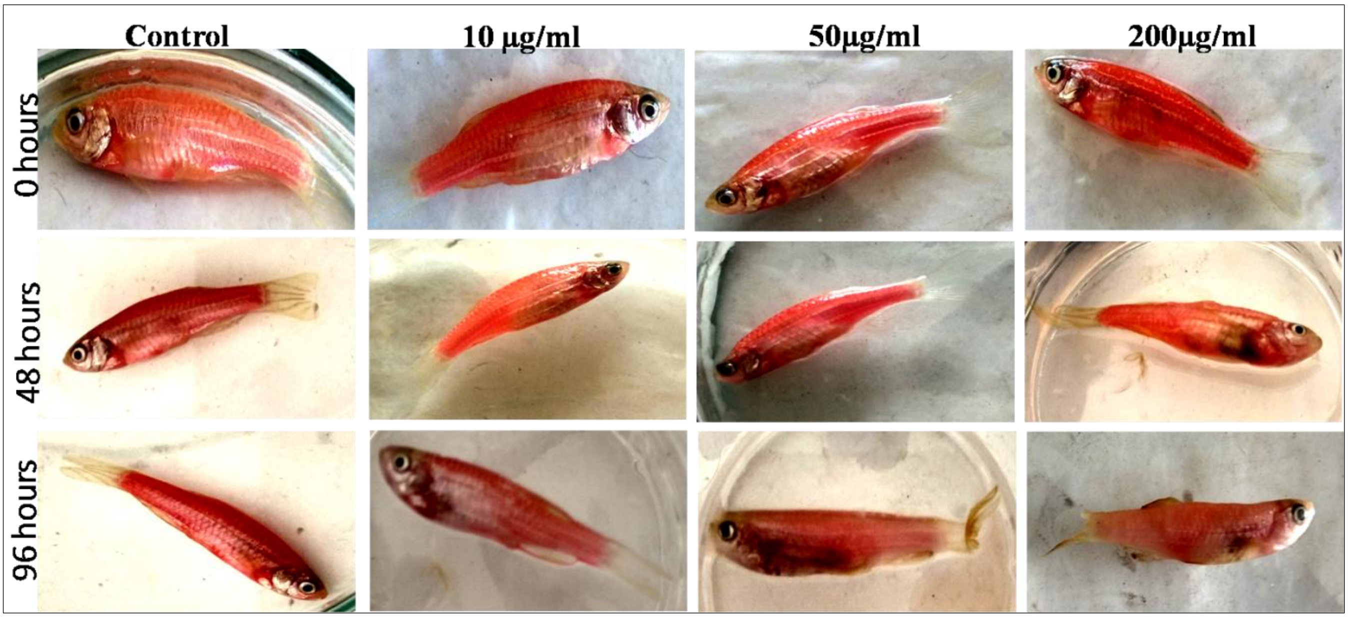

Fig 2 Morphological change observation of adult zebrafish exposed to CaONP with adult Zebrafish. The fish were exposed to different concentrations of CaONP at a time point of 24h, 48h, and 96 h. The formation of the lesion with an accumulation of nanoparticles was observed

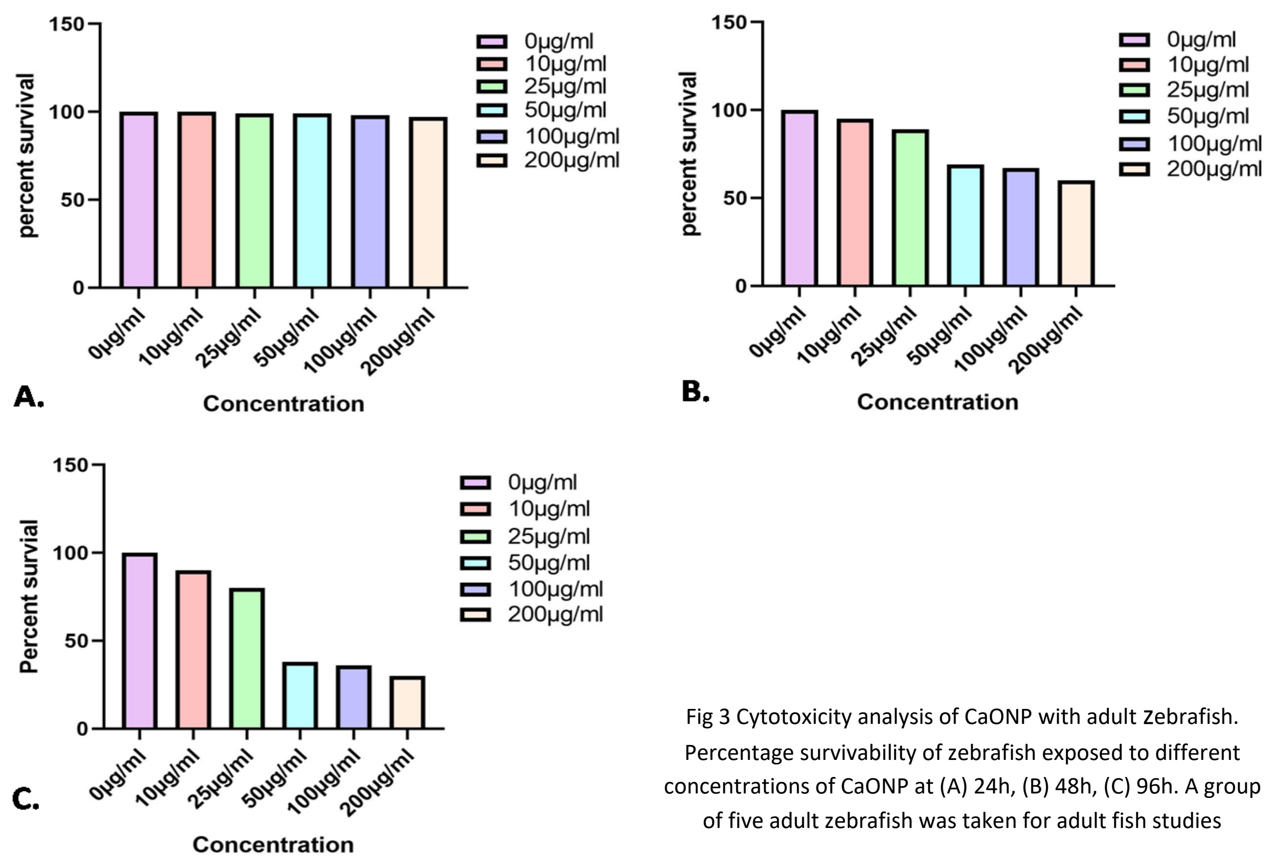

Fig 3 Cytotoxicity analysis of CaONP with adult zebrafish. Percentage survivability of zebrafish exposed to different concentrations of CaONP at (A) 24h, (B) 48h, (C) 96h. A group of five adult zebrafish was taken for adult fish studies

In vivo biocompatibility of green synthesized MgONP

A nanoparticle's biocompatibility and eco-compatibility are essential factors in determining their potential utility in biomedical and ecological applications [6]. As a result, it's critical to figure out what effect nanoparticles have in vivo as a result of their accumulation at the cell surface and internalization within the cells. An in vivo investigation with zebrafish was conducted to test the biocompatibility of synthesized MgONP. The effect of the nanoparticles on the metabolism and survival of adult zebrafish was investigated. Visual inspection of fish exposed to varied amounts of MgONP was used to confirm the hypothesis. The buildup of nanoparticles on the surface was obvious in the form of black dots, as seen in (Fig 2). The percentage survivorship of zebrafish was shown to be concentration and time-dependent exposure to MgO nanoparticles. Surprisingly, mortality increased at a faster rate in the 50 g/mL and higher range. (Fig 3A-C) show the proportion of zebrafish that survived after being exposed to different concentrations of MgONP for 24 hours, 48 hours, and 96 hours. As can be shown, the MgONP had the greatest fatal effect after 72 hours of exposure. The deadly impact can be linked to the concentration of nanoparticles on the surface of the zebrafish's outer shell, which can then be accounted for by their absorption inside cells [19].

The creation of lesions was noticed in fish exposed to larger amounts, and the fishes' overall health had deteriorated. Surprisingly, the negative impacts grew stronger as the exposure period increased. In previous investigations, similar effects were identified in adult zebrafish exposed to various metallic oxide nanoparticles, such as MgONP [23]. The harmful effect of the nanoparticles was assessed in a dose-dependent manner based on visual inspection and experimental investigation. The findings revealed that MgONP concentration plays an important function. As a consequence of the prior literature and our experimental findings, the mechanism of MgONP biocompatibility with zebrafish may be deduced as an effect of MgONP accumulation and internalization within the zebrafish body. Future research could focus on modifying the characteristics of nanoparticles using biomolecules that have been studied for toxicity mechanisms in order to improve biocompatibility. The study also looked into a unique approach for producing MgONP in the green utilizing medicinal plant extract.

CONCLUSION

MgONP was successfully synthesized and characterized in a green environment. With a size of 82 nm and SPR of 284 nm, the synthesized MgONP was shown to be stable. The synthesized MgONP was proven to be biocompatible with Zebrafish at a certain concentration range. Furthermore, the usage of the medicinal plant resulted in a green approach to the synthesis of MgONP.

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