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Enhancing Therapeutic Efficacy of Cerium Oxide Nanoparticles (CNP) Conjugated with S-Nitroso-N-Acetyl Penicillamine for Multifunctional Biomedical Applications

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ABSTRACT
Nanoparticles are extensively used because their small size, unique orientation, and distinct physical properties can significantly modify the behavior of the materials they interact with. Cerium oxide nanoparticles (CNP) also known as nanoceria are gaining popularity in a multitude of sectors, including Biomedical science as drug delivery, therapeutics, and enzyme mimetics. Nanoceria are preferred over typical antioxidants or enzymes because they can scavenge numerous types of free radicals. Studies such as In vitro and in vivo methods have shown that nanoceria may be employed as a carrier for targeted medication and gene delivery. Nanoceria works as an antioxidant therapeutics in healthy cells by scavenging ROS (at physiological pH) free radicals. Furthermore, S-Nitroso-N-acetyl penicillamine (SNAP), a nitroso thiol derivative, releases nitric oxide (NO) under physiological conditions. This makes SNAP a valuable tool for researching the pharmacological and physiological effects of NO. The conjugation of CNP and SNAP along with bacterial cellulose enhances its antimicrobial, SOD, and antidiabetic activities. CNP was synthesized using the Wet chemistry method and optimized using a UV/Vis Spectrophotometer. Further, the conjugate solution was characterized by UV-Vis (i.e., within the range of 230-280 nm) and SEM shows a perfect conjugate solution by its morphology and elements. The anti-microbial activity was administered, and a moderate zone was observed. The zone of inhibition activity was shown 4 mm against gram-negative bacteria and 7 mm against gram-positive bacteria. The conjugate solution examined cellular viability of approximately 50.6 % in HACAT cells which shows less toxicity. These observations concluded that the procedure for nanoparticle synthesis is advantageous and synthesized nanomaterial was found to be highly Antioxidative.

1. Introduction

The study of nanoparticles is a rapidly expanding topic that is attracting interest and curiosity because of its distinctive qualities and wide range of applications in many academic fields. Because of their small size and unique physical properties, nanoparticles have become important players that may significantly affect the behaviour and functionality of nearby substances. Out of all the nanoparticles in the universe, cerium oxide nanoparticles (CNP), also known as nanoceria, have become the talk of science,

enthralling scientists and practitioners with their extraordinary adaptability and use in a variety of fields. (**1**)

The great interest in CNPs stems from their emerging potential as multifunctional agents with a wide range of uses in the fields of therapeutics, drug transport, enzyme mimetics, and biomedical science. Unlike traditional antioxidants or enzymes, CNP has a special set of characteristics that make them effective in scavenging various kinds of free radicals, giving them a significant advantage in the fight against issues connected to oxidative stress. Because CNP can replicate the enzymatic functions of Catalase like (H_2O_2inhibiton) and SOD like, it may stabilize free radicals and reduce oxidative damage in biological systems with unmatched effectiveness. (**2**)

Furthermore, CNP's extraordinary capacity to scavenge nitric oxide radicals emphasizes their therapeutic potential even more, making them invaluable resources in the search for cutting-edge pharmacological treatments and therapeutic approaches. Numerous aspects of CNP's pharmacological capabilities have been revealed by in vitro and in vivo research, demonstrating their ability to act as carriers for targeted drug administration and gene transfer. Because of its antioxidant qualities, CNP has an amazing ability to protect healthy cells from harm caused by oxidative stress, creating an environment that promotes cellular homeostasis and vitality. (**3**)

CNP has been wisely coupled with S-Nitroso-N-acetyl penicillamine (SNAP), a nitroso thiol derivative known for its capacity to produce nitric oxide (NO) under physiological circumstances, in a ground-breaking synergy of scientific innovation. (**4**) Because of this strategic partnership, the resultant composite has improved antibacterial, superoxide dismutase (SOD), and antidiabetic properties. It also generates a strong amalgam of medicinal potential. The addition of bacterial cellulose enhances this composite's multifunctionality even further and gives it access to a wide range of treatment modalities that have the potential to completely transform clinical practice and scientific research. (**5**)

In conclusion, the discovery of CNP signals the beginning of a new phase in scientific research and the development of novel therapeutics. It presents exciting opportunities to tackle a wide range of biological problems and push the boundaries of medical knowledge. CNP is well-positioned to drive revolutionary advances in drug delivery, therapies, and enzyme mimetics with their unmatched adaptability and diverse range of functions, paving the way for a more promising and health-conscious future for humanity.

2. Materials and Methods

Materials: Cerium Nitrate Hexahydrate, 30% H_2O_2, Ascorbic acid, NBT, DMSO, Methionine, Phosphate buffer saline (PBS), Gram-positive strain, Gram-negative strain, Riboflavin, EDTA, LB Broth, α-amylase, starch, dinitro salicylic acid, and Acarbose, Make- Sigma Aldrich), Distil water, SNAP- S-nitroso-N-acetyl penicillamine, jelly of Nata de coco

Fabrication method: CNP is prepared by the wet-chemical protocols. Ce $(NO_3)_3 \cdot 6\ H_2O$ (Cerium precursor) was mixed to D/W steadily and vortexed continuously for 3-4 minutes on a vortex machine. Cerium nitrate hexahydrate was dissolved through continuous vertexing. The quantity of mixture was accustomed , and Oxidizer- H_2O_2 was added and again vortexed for a minute or two. Again, a few drops of distilled water were added to balance the volume. The product fabricated was of yellow colored and then turned colorless in 6-7 days. It leads to the formation of liq CNP (5mM). No precipitate should be formed. pH should be maintained at 4. (**6**)

UV-Vis Spectrophotometry

UV-visible spectra of cerium oxide nanoparticles dissolves aq mixture was read against blank of the reaction which is taken as Distilled water. Different concentrations of CNP- cerium oxide nanoparticles were taken (3mM, 1mM, 0.5mM, and 0.25 mM) absorbance is measured and the graph is plotted. (**7**)

Conjugation of materials for preparation of Nanocomposite

The procedure for preparing a composite solution with CNP, activated SNAP combined with crosslinkers, and bacterial BC begins by adjusting the concentration of CNP to be 3-4 folds higher than the SNAP solution. (**8**) The mixture is then stirred for 40 minutes using a magnetic stirrer. Next, a 20 ml volume of the conjugated Nanoceria-Activated SNAP solution was prepared. For further mixing, the volume of CNP-SNAP and BC are equal in ratio (1:1). SO, 20 ml of bacterial cellulose solution mixed with 20 ml of CNP-SNAP conjugate to obtain a total volume of 40 ml containing all three components. (**9**)

Antimicrobial activity of composite (CNP-SNAP-BC)

In the sterile Petri plate, about 25 ml of nutrient agar was added. Using sterile spatulas, 200 µL of the overnight culture of the pathogenic gram-positive (*S.aureus*) and gram-negative (*Escherichia coli*) bacteria of interest was spread onto separate plates. Make 5 wells into the plates, in which 2 wells for positive and negative controls and 3 for different concentrations of samples. The plates were incubated at 37 ± 1 °C for a full day before the results were determined. The diameter of inhibition zone was measured and reported in mm. (**10**)

Cell Viability using MTT assay:

The MTT-based cytotoxicity assay for cerium oxide nanoparticles (CNP) was conducted using the HACAT cell line. In this assay, 5,000 cells/well put into a 96-well plate and incubated for two days in a 5% CO2 incubator to allow for cell attachment and growth. Five measurements were taken to ensure reproducibility. Each well was then treated with the specified concentration of CNP. Following a 48-hour incubation period, 5 mg/ml of MTT reagent was added to wells. After incubation, the MTT solution was washed out and 120 µl of DMSO was used for dissolving the formazan crystals. The dissolution was aided by gently pipetting five times. The OD was measured at 570 nm to assess cell viability. (**11**) Cell viability (%) was calculated using the following formula.

$$\text{Viability \%} = [\text{OD (Treated)} / \text{OD (control)}] * 100$$

3. Results

UV-visible characterization of CNP and composite solution (CNP-SNAP-BC)

UV-visible characterization was performed. It aimed to decipher optical properties and band gap energy. We were studying synthesized nanoceria that provide crucial insights into the structural and functional attributes. The absorption spectrum unveiled distinctive features. These features signaled the compositional and size-dependent optical behavior of the nanoparticle. Notably, observed absorption in the UV region was seen. This absorption stemmed from charge transitions. Transitions were between oxygen (O) and cerium (Ce) orbitals. It prompted the formation of O^{2-} and Ce^{4+} species. It highlights the intrinsic electronic structure of nanoceria. A notable outcome from UV-visible characterization was a witness of a well-defined peak. This absorbance peak was within the wavelength range of 230 nm to 260 nm. Pronounced peak punctuates a narrow and uniform particle size distribution. This can be indicative of precise control of the synthesis process. Such uniformity is important. It ensures consistent performance and efficacy across diverse applications. They include catalysis and biomedicine.

Significance was on the discernible peak at 240 nm. It is indicative of nanoparticles. These contain a mixture of Ce^{3+} and Ce^{4+} species. Cerium other than Ce^{3+} is predominantly present in the 3+ oxidation state. This observation has deep implications for the understanding of redox chemistry. It also affects the catalytic activity of nanoceria. Ce^{3+} species' presence provides oxygen vacancy formation. The identification of Ce^{3+}/Ce^{4+} species is prominent, and they are within a nanoparticle ensemble. It highlights the dynamic nature of cerium oxidation states. As a result, it affects the physiochemical properties of nanoceria.

A deep understanding of properties is provided by these insights. It helps tailor the synthesis process. And we can optimize nanoparticle performance. It is for specific applications. Consequently, the UV-visible characterization is a crucial step. It isn't just to shed light on the structural properties and optical properties of nanoceria. It offers valuable insights. Insights into size-dependent optical behavior and electronic structure of nanoceria. (**12**)

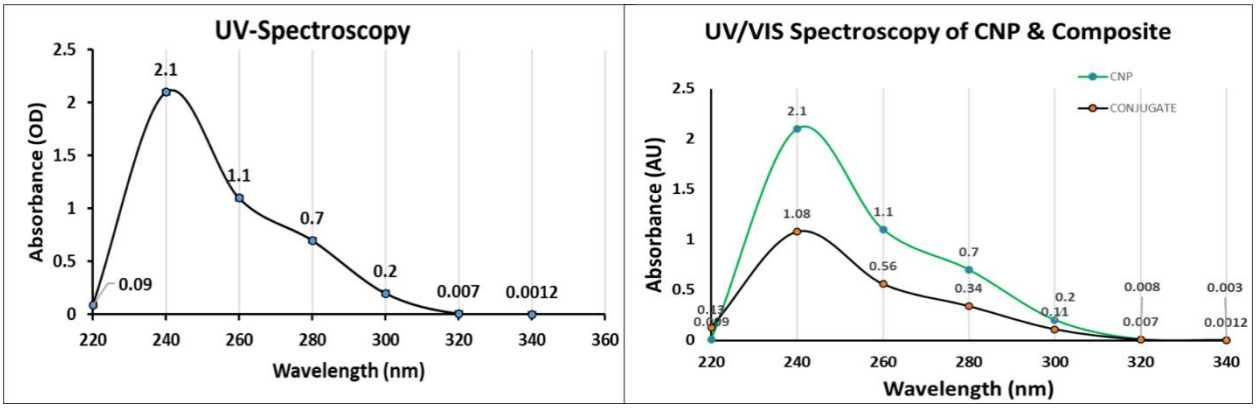

Figure 1 UV spectroscopic examination of the synthesized cerium oxide nanoparticles (CNPs) at various concentrations displayed discernible absorption peaks within the UV spectrum, suggesting transitions between energy levels predominantly associated with Ce3+ and C4+ oxidation states.

Antimicrobial Analysis of Conjugate Solution (CNP-SNAP-BC)

This study defined antimicrobial importance of a compound solution with cerium oxide nanoparticles (CeO_2 NPs), S-nitroso-N-acetyl penicillamine- SNAP, and bacterial cellulose against gram-negative (*Escherichia coli*) and gram-positive *(S.aureus)* bacterial strains. Disk Diffusion assays were determined to inhibit visible bacterial growth. Results showed that a 1 mM concentration exhibited a maximal zone of inhibition around 6 mm of bacterial proliferation, indicating the robust antimicrobial activity of nanocomposite. Conjugate exerts its antimicrobial effects through several pathways leading in inhibition of growth. (**13**) The conjugate solution at 1mm concentration exhibited inhibition zone of 4 mm against gram-negative bacteria and 7 mm against gram-positive bacteria. Apart from this concentration, other concentrations either show very little zone of inhibition or no inhibition. This discrepancy is attributed to differences in cell wall composition, with Gram-positive bacteria being more susceptible due to their thinner cell wall and lower peptidoglycan content. (**14**)

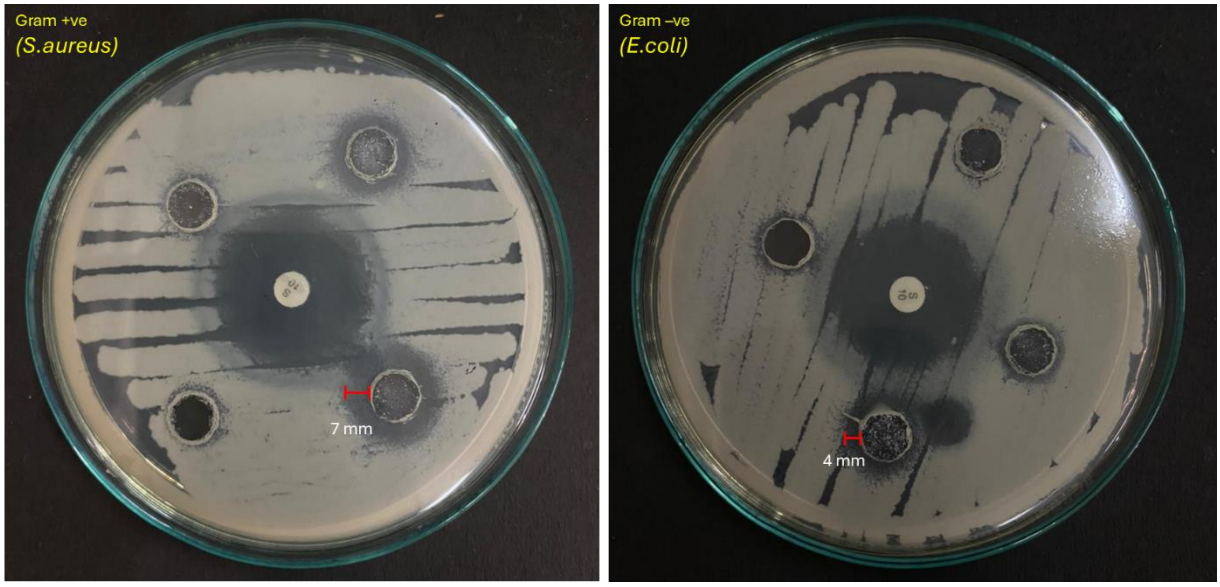

Figure 4 In the figure, the effectiveness of the conjugate solution of CNP-SNAP-BC as an antimicrobial agent was examined against gram+ and gram- bacteria. The zone of inhibition was noted as 4 mm in gram-negative and 7 mm in gram-positive bacteria.

Biocompatibility assay of conjugate solution

The study aimed to evaluate the cellular biocompatibility of conjugate incorporating CNP-SNAP-BC through MTT assessment, using the HACAT cell line for compatibility analysis. The HACAT cell line, derived from human skin keratinocytes, was specifically chosen for this study due to its similarity to skin tissues. Treatment with the conjugate solution resulted in a significant cellular viability of 50.6% in HACAT cells. This finding suggests moderate cytotoxicity, highlighting the hybrid nanofiber's promising safety profile for potential biomedical uses. (**15**)

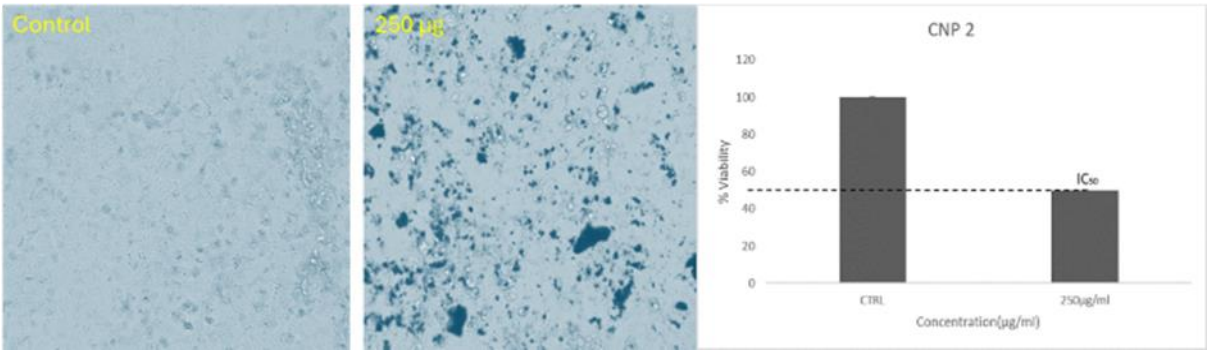

Figure 5 The MTT assay determined the cellular biocompatibility of conjugate (CNP-SNAP-BC) using HaCat cells, yielding a cellular viability of approximately 50.6%.

Discussion

Due to their small size, special orientation, and physical characteristics, nanoparticles greatly improve the performance of materials they come into contact with. The findings of this study highlight the potential applications of cerium oxide nanoparticles, also known as nanoceria, in the fields of biomedical science, medicine, and enzyme mimetics. The greater capacity of nanoceria to scavenge a broad variety of free radicals explains why they are preferred over conventional antioxidants or enzymes. The

therapeutic effectiveness of nanoceria has been highlighted by the confirmation of their potential as carriers for targeted medication and gene delivery through both in vitro and in vivo experiments.

Because they can scavenge reactive oxygen species (ROS) at physiological pH, nanoceria have antioxidative capabilities that make them useful antioxidant therapies in healthy cells. This work presented the coupling of CNP with S-Nitroso-N-acetylpenicillamine (SNAP), a nitroso thiol derivative that, in physiological settings, is known to release nitric oxide (NO). SNAP is a useful technique for researching the physiological and pharmacological effects of NO. The conjugation process improved the antibacterial, superoxide dismutase (SOD) mimic, and antidiabetic properties of the resulting composite material by using bacterial cellulose (BC). Wet chemistry was used to synthesize cerium oxide nanoparticles, and UV/Vis spectrophotometry was used to tune their characteristics within the 230–280 nm absorption range.

The conjugate solution's antibacterial efficacy was assessed against strains of Gram+ -*S. aureus* and Gram- *Escherichia coli* bacteria. The conjugate solution showed, the inhibition zone as 4 mm in gram-negative and 7 mm in gram-positive bacteria, which showed a considerable suppression of bacterial growth. The electrostatic interactions between nanoceria and bacterial cells, which result in the formation of ROS and physical damage to the bacterial membrane, are responsible for this note worthy antibacterial characteristics. Gram+ and Gram- bacterias differ in their structural vulnerability to reactive oxygen species (ROS). This is because Gram-positive bacteria have a thinner cell wall and less peptidoglycan, which makes them more susceptible to damage from ROS.

The conjugate solution demonstrated about 50.6% vitality in HACAT cells concerning cellular viability, showing low cytotoxicity and pointing to possible therapeutic uses. The CNP-SNAP-BC combination is a viable contender for several biological applications, especially in wound healing and infection control, due to its strong antibacterial activity and low toxicity.

Conclusion

Using cerium oxide nanoparticles (CNP), especially as nanoceria, is a prospective application in several sectors, including biomedical research. Nanoceria demonstrate their adaptability and usefulness by playing a multifarious role in medication administration, therapies, and enzyme mimetics, all thanks to their unique features. The promise of nanoceria as an enhanced substitute for conventional antioxidants is highlighted by their capacity to scavenge several kinds of free radicals and their resemblance to vital antioxidant enzymes such as Catalase like and superoxide dismutase like property. Furthermore, increased antibacterial, superoxide dismutase, and antidiabetic properties have been established by the effective conjugation of CNP with S-Nitroso-N-acetyl penicillamine (SNAP) and bacterial cellulose. This highlights the synergistic benefits that may be achieved by new nanoengineering technologies.

Cerium oxide nanoparticles were synthesized, optimized, and thoroughly characterized using UV-VIS spectrophotometry in conjunction with wet chemical techniques. The noteworthy antibacterial activity that has been noted emphasizes the potential of conjugate materials based on CNP in the fight against microbial illnesses. Taken together, these results validate the benefits of the synthesis process for nanoparticles and the strong antioxidant characteristics of the produced nanomaterial, opening doors for more research and use in the biomedical and associated fields.

References

1. Sim, S., & Wong, N. K. (2021). Nanotechnology and its use in imaging and drug delivery. Biomedical reports, 14(5), 1-9.
2. Xu, C., & Qu, X. (2014). Cerium oxide nanoparticle: a remarkably versatile rare earth nanomaterial for biological applications. NPG Asia materials, 6(3), e90-e90.
3. Masadeh, M. M., Karasneh, G. A., Al-Akhras, M. A., Albiss, B. A., Aljarah, K. M., Al-Azzam, S. I., & Alzoubi, K. H. (2015). Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram positive and gram negative biofilm bacteria. Cytotechnology, 67, 427-435.
4. Lee, S. J., Kim, K. M., Namkoong, S., Kim, C. K., Kang, Y. C., Lee, H., ... & Kim, Y. M. (2005). Nitric oxide inhibition of homocysteine-induced human endothelial cell apoptosis by down-regulation of p53-dependent Noxa expression through the formation of S-nitrosohomocysteine. Journal of Biological Chemistry, 280(7), 5781-5788.
5. Gorgieva, S., & Trček, J. (2019). Bacterial cellulose: Production, modification and perspectives in biomedical applications. Nanomaterials, 9(10), 1352.
6. Farias, I. A. P., Dos Santos, C. C. L., & Sampaio, F. C. (2018). Antimicrobial activity of cerium oxide nanoparticles on opportunistic microorganisms: a systematic review. BioMed research international, 2018.
7. Chen, B., Jiao, X., & Chen, D. (2010). Size-controlled and size-designed synthesis of nano/submicrometer Ag particles. Crystal Growth & Design, 10(8), 3378-3386.
8. Estes, L. M., Singha, P., Singh, S., Sakthivel, T. S., Garren, M., Devine, R., ... & Handa, H. (2021). Characterization of a nitric oxide (NO) donor molecule and cerium oxide nanoparticle (CNP) interactions and their synergistic antimicrobial potential for biomedical applications. Journal of colloid and interface science, 586, 163-177.
9. Pandey, S., Kumari, S., Manohar Aeshala, L., & Singh, S. (2024). Investigating temperature variability on antioxidative behavior of synthesized cerium oxide nanoparticle for potential biomedical application. Journal of Biomaterials Applications, 38(7), 866-874.
10. Senthilkumar, R. P., Bhuvaneshwari, V., Ranjithkumar, R., Sathiyavimal, S., Malayaman, V., & Chandarshekar, B. (2017). Synthesis, characterization and antibacterial activity of hybrid chitosan-cerium oxide nanoparticles: as a bionanomaterials. International journal of biological macromolecules, 104, 1746-1752.
11. Anand, S., Rajinikanth, P. S., Arya, D. K., Pandey, P., Gupta, R. K., Sankhwar, R., & Chidambaram, K. (2022). Multifunctional biomimetic nanofibrous scaffold loaded with asiaticoside for rapid diabetic wound healing. Pharmaceutics, 14(2), 273.
12. Singh, S., Kumar, U., Gittess, D., Sakthivel, T. S., Babu, B., & Seal, S. (2021). Cerium oxide nanomaterial with dual antioxidative scavenging potential: Synthesis and characterization. Journal of Biomaterials Applications, 36(5), 834-842.
13. Kumari, S., Pandey, S., Aeshala, L. M., Kumar, A., & Singh, S. (2024). Augmenting Antioxidative Properties of Cerium Oxide Nanomaterial with Andrographis paniculata Mediated Synthesis and Investigating its Biomedical Potentials. Nanofabrication, 9.
14. Zhang, M., Zhang, C., Zhai, X., Luo, F., Du, Y., & Yan, C. (2019). Antibacterial mechanism and activity of cerium oxide nanoparticles. Sci. China Mater, 62(11), 1727-1739.

15. Kalyanaraman, V., Naveen, S. V., Mohana, N., Balaje, R. M., Navaneethakrishnan, K. R., Brabu, B., ... & Kumaravel, T. S. (2019). Biocompatibility studies on cerium oxide nanoparticles–combined study for local effects, systemic toxicity, and genotoxicity via implantation route. Toxicology Research, 8(1), 25-37.