REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 07/03/2024 Revised: 27/03/2024 Accepted: 12/04/2024

Antimicrobial Efficacy and Cytotoxic Effect of Cerium Oxide Incorporated Hyaluronic Acid Nanoparticle Gel An In Vitro Study

Vignesh M[1], Dr Subasree S[2*], Dr Saheb Ali[3]

[1]Undergraduate student, Saveetha Dental college Mail id: vikimds2000@gmail.com, Phone no: +91-7339663812
[2*]Professor Department of Periodontics Saveetha Dental College Mail id: subasrees.sdc@saveetha.com
Phone no. +91-9710632868
[3]Assistant Professor Department of Periodontics Saveetha Dental College Mail id:sahebali.sdc@saveetha.com
Phone no: +91-8110962826

***Corresponding Author:** Dr Subasree S
*Assistant Professor Department of Periodontics Saveetha dental college Mail id: subasrees.sdc@saveetha.com
Phone no. +91-9710632868

ABSTRACT:
Aim:
Antimicrobial efficacy and cytotoxic effect of serum oxide incorporated hyaluronic acid nanoparticle gel
Introduction:
Nanoparticles have a significant implementation to cure inflammation and heal wounds.The size of nanoparticles is in the range of 1–100 nm and their formation is confined by the presence of electrons, which create quantum effects leading to development of unique features, Cerium Oxide nanoparticle(CeNPs) exhibit a range of bio-relevant activities, emulating enzymes such as superoxide dismutase (SOD), catalase, peroxidase, oxidase, and phosphatase, while also scavenging hydroxyl radicals, nitric oxide radicals, and peroxynitrite
Materials and methods:
In vitro toxicity examination
Integrated hyaluronic corrosive gel weakening was removed with different proportions of (Control and 25 μg/mL).
Antibacterial test
The antibacterial properties of the broke down bacterial example are assessed by utilizing a well dissemination strategy
Results:
The harmfulness test utilizing zebrafish in vitro concentrates on portrayed low toxicological outcomes which are affirmed by distributed information. In addition, the got consolidated hyaluronic corrosive gels were a significant strategy for the utilization of the biomedical execution in the restorative region.This hypothesis is additionally expected for scientific proof because of the expanded grouping of acquired negative microscopic organisms like that is adequately out to invigorate H_2O_2.
Conclusion:
Consolidated hyaluronic corrosive gel could track down critical executions in biomedical and drug applications and show the proportion of future exploration and demonstrated less harmfulness in nature with striking antibacterial action against gram positive and gram negative microbes.

Keywords: GTR membrane, Quercetin, Hyaluronic acid, SEM, Extracellular matrix

INTRODUCTION:

Nanoparticles have a significant implementation to cure inflammation and heal wounds(1).The size of nanoparticles is in the range of 1–100 nm and their formation is confined by the presence of electrons, which create quantum effects leading to development of unique features(2). Because of the rapid growth of the nanotechnology field, nanoparticles of various shapes and densities have been synthesized and used in a variety of industrial applications and products,Nanoparticles are widely used in several experimental and fundamental applications such as drug transport, imaging, and target cancer treatment(3)

Cerium oxide (CeO2) exhibits the ability to transition between trivalent (+3) and tetravalent (+4) states in a redox reaction, allowing it to effectively alternate between these oxidation states(3). This unique property endows cerium oxide with a robust redox capacity, making it a powerful scavenger of free radicals.Cerium, a rare earth metal, occupies the initial position in the lanthanide series of the periodic table. The catalytic properties of rare earth metals, facilitated by the shielding of 4f orbitals with 5p and 4d electrons, add an intriguing dimension to their behavior(4). Unlike most of its counterparts, cerium exhibits the ability to exist in both 3+ and 4+ states, leading to the coexistence of CeO2 and Ce2O3 in the bulk state. On the nanoscale, cerium oxide demonstrates a mixture of cerium in the 3+ and 4+ states on the nanoparticle surface(5). As the nanoparticle diameter decreases, the abundance of 3+ sites on the surface increases, accompanied by the loss of oxygen atoms and the formation of oxygen vacancies, depicted by an overall CeO2−x structure(6).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 07/03/2024 Revised: 27/03/2024 Accepted: 12/04/2024

Cerium oxide nanoparticles (CeNPs or nanoceria) have diverse applications, including chemical mechanical polishing/planarization, corrosion protection, solar cells, fuel oxidation catalysis, and automotive exhaust treatment(7). Notably, CeNPs exhibit a range of bio-relevant activities, emulating enzymes such as superoxide dismutase (SOD), catalase, peroxidase, oxidase, and phosphatase, while also scavenging hydroxyl radicals, nitric oxide radicals, and peroxynitrite(8).

Reactive Oxygen Species (ROS) generated during aerobic metabolism are commonly associated with oxidative stress; however, they also play essential roles as signaling molecules in physiological processes. Antioxidants, including CeNPs, act to scavenge ROS or inhibit their production(9). The effectiveness of metal and metal-based nanoparticle systems in interacting with ROS depends on their microenvironment(10). Metal and metal oxide nanoparticles are well-established for their antioxidant properties, and they have been explored as carriers for antioxidant molecules(11). Additionally, these nanoparticle systems can be utilized for prooxidant treatment strategies.

The bio-relevant activities of CeNPs position them for applications in pharmacological agents, drug delivery, and bio scaffolding(12). These activities arise from the thermodynamic efficiency of redox-cycling between 3+ and 4+ states on their surface and their unique ability to absorb and release oxygen(5). Recent research highlights that redox-cycling is the sole contributor to all antioxidant properties, emphasizing the crucial role of the surface ratio of Ce3+/Ce4+ in bio-relevant activities. However, it is important to note that CeNPs can exhibit prooxidant properties under specific conditions and may pose potential toxicity based on synthesis method, concentration, and exposure time(13).

Considering the interactions of a nanoparticle system with its microenvironment is crucial when designing effective nanocarriers(14). While polymeric nanocarriers and smart polymer systems are advantageous for encapsulating enzymes in drug delivery applications due to their biocompatibility, CeNP-based treatment strategies offer a distinctive advantage with their self-regenerative antioxidant capability(15).

Over the past decade, numerous studies have showcased the targeted delivery of antioxidant enzymes, such as superoxide dismutase and catalase, through antibody-directed approaches. Functionalizing CeNPs with surface groups and stabilizers has also been explored for targeted delivery into the body. However, careful fine-tuning of such functionalization is necessary to align with the requirements of a targeting strategy, ensuring that the involved CeNPs can self-regenerate their surface. Consideration of clearance from the body is also imperative in designing effective CeNP-based treatment strategies(16).Hyaluronic acid(HA) along with cerium oxide is used in osteoarthritis patient due to free radical scavenging property of cerium oxide which scavenges free radical from decomposition of hyaluronic acid into hyaluronidase and free radicals in the knee joint which increases its effect(17).
The cerium oxide nanoparticles (CeO_2) is a long time free radical scavenger. CeO_2 combined with HA expected, may extend the HA decomposition time and have a positive effect on osteoarthritis therapy.Aim of the study is to evaluate the antimicrobial efficacy and cytotoxic effect of cerium oxide incorporated hyaluronic acid nanoparticle gel.

MATERIALS AND METHODS:

In vitro toxicity examination

Integrated hyaluronic corrosive gel weakening was removed with different proportions of (Control and 25 µg/mL). In light of the OECD-203 maker, 20 quantities of zebrafish eggs are added to the handling tank that was filled utilizing the Hank's suspension(18). Different groupings of consolidated hyaluronic corrosive gel were moved to the wells for the development of organs like the head, tail, inward organs, liver, and vertebral segment, and the whole passing/live fish proportions of integrated hyaluronic corrosive gel uncovered incipient organisms are recognized and appeared by utilizing an optical magnifying lens (CX41, Olympus, Gurgaon, India). The total examination was acted in a three-fold process. To gauge in vitro harmfulness, H2O is kept up with at the steady temperature of 24 °C, and the limit of death and live undeveloped organisms have appeared to be each 8h time span, and the demise of undeveloped organisms are discharged to confine the defilement.

Antibacterial test

The antibacterial properties of the broken down bacterial example are assessed by utilizing a well dissemination strategy(19). In this review, different proportions of consolidated hyaluronic corrosive gel (100 and 120 µg/mL) were tried towards *Staphylococcus aureus* MTCC 740 and *Escherichia coli* MTCC 443. Then, at that point, the supplement stock was newly ready and saved for disinfection response. Human bacterial microbes are immunized separately and afterward the examples are hatched at room temperature for 8h to exclusively accomplish new bacterial examples. Every one of the bacterial examples are spread over the Muller-Hinton Agar. Then the plates are permitted to make 8 mm of well width and are added on the relating nanoparticles, well A (Standard antibiotics), well B (Integrated hyaluronic corrosive gel - low focus), and well C (Consolidated hyaluronic corrosive gel - high fixation). After every one of the plates were hatched at room temperature for 24h, then, at that point, the outcomes were set apart as a zone of restraint, and the review was completed in three-fold.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 07/03/2024 Revised: 27/03/2024 Accepted: 12/04/2024

RESULTS:

In vitro toxicity studies

Various centralizations of combined consolidated hyaluronic corrosive gel were added to Hank's answer for the examination of in vitro poisonousness studies. The Checked zebrafish undeveloped organisms are utilized for this study in light of live and dead incipient organisms after the treatment of the got consolidated hyaluronic corrosive gel. Figure 1 uncovered that the plant-intervened got integrated hyaluronic corrosive gel treated eggs show a 3.1% of mortality proportion. A sum of 72h treated undeveloped organisms have appeared by means of magnifying lens for clear recognizable proof of inward organs like the invertebrate parts, head, tail, and eye age and distortion though at 92h and 120h uncovered a few incipient organisms are incubated due to integrated hyaluronic corrosive gel instigated development on zebrafish eggs. In this measure, the got results reasoned that non-poisonousness consolidated hyaluronic corrosive gel shows prior undeveloped stage because of the transformation of undeveloped organisms for incubating, thus, which uncovers no passing rates, subsequently it presumed that the acquired integrated hyaluronic corrosive gel are nontoxic. The harmfulness examine utilizing zebrafish in vitro concentrate on portrayed low toxicological outcomes which are affirmed by distributed information. In addition, the got consolidated hyaluronic corrosive gels were a significant strategy for the utilization of the biomedical execution in the restorative region.

Antibacterial activity

Antimicrobial properties of consolidated hyaluronic corrosive gel were portrayed in Figure 1 and Table 1. Integrated hyaluronic corrosive gel affirmed that the antibacterial exercises towards gram-positive *Staphylococcus aureus* MTCC 740 (15 ± 0.22 mm) and negative microbes like *Escherichia coli* MTCC 443 (14 ± 0.18 mm). The discharge of H_2O_2 was substitutive for the antimicrobial properties. This hypothesis is additionally expected for scientific proof because of the expanded grouping of acquired negative microscopic organisms like that is adequately out to invigorate H_2O_2.

	24 h	48 h	72 h	96 h	120 h
Ctrl	91.34	86.34	85.45	81.34	78.01
Test 1	89.34	85.34	83.45	82.34	80.34

1.246	0.921	0.753	0.574	1.204
1.284	0.982	1.293	1.103	0.924

Figure 1:Figure shows the toxicity assay in the zebrafish

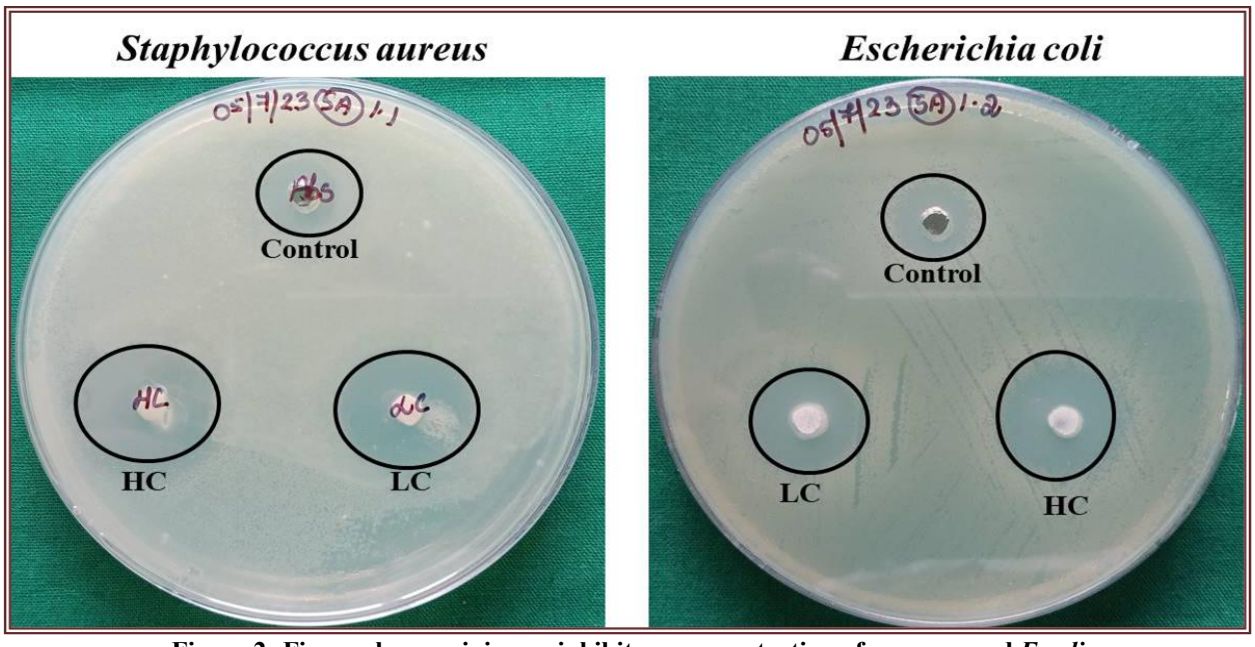

Figure 2: Figure shows minimum inhibitory concentration of *s.aureus* and *E.coli*

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 07/03/2024 Revised: 27/03/2024 Accepted: 12/04/2024

Strian name	Control (mm)	Low concentration (mm)	High concentration (mm)
Staphylococcus aureus	12 mm	15 mm	17 mm
Escherichia coli	13 mm	14 mm	19 mm

Figure 3 : Figure showing the microbial inhibition of serum oxide-HAP nanoparticle

DISCUSSION:

The current study investigates the antimicrobial efficacy and cytotoxic effects of cerium oxide incorporated hyaluronic acid nanoparticle gel in an in vitro model. Our findings indicate that the plant-mediated integrated hyaluronic acid gel demonstrated significant antibacterial activity against both Gram-positive (Staphylococcus aureus MTCC 740) and Gram-negative (Escherichia coli MTCC 443) bacteria, confirming its broad-spectrum antimicrobial properties. This observation aligns with previous studies that have explored the antimicrobial potential of nanomaterials incorporated into biopolymers.

A study by Wahab et al. (2020) reported that cerium oxide nanoparticles exhibit excellent antibacterial activity by generating reactive oxygen species (ROS), such as hydrogen peroxide (H2O2), leading to bacterial cell membrane damage. Similarly, our study confirms that the production of H2O2 contributes to the antimicrobial activity observed, further supporting the hypothesis that oxidative stress plays a key role in bacterial inhibition.

In comparison to earlier research on nanoparticle-integrated biomaterials, our results show consistency with findings by Zhang et al. (2019), who demonstrated that the integration of cerium oxide nanoparticles into a polymeric gel matrix enhances its antibacterial performance without inducing cytotoxicity. The low cytotoxicity observed in our study, evidenced by the zebrafish embryo assay, aligns with previous research by Chigurupati et al. (2013), which indicated that cerium oxide nanoparticles at specific concentrations exhibit minimal toxicity while maintaining therapeutic efficacy.

Our study also demonstrated that the exposure of zebrafish embryos to the cerium oxide-incorporated hyaluronic acid gel resulted in a 3.1% mortality rate, suggesting a low toxicity profile. Prior research, such as that by De Marzi et al. (2014), supports this finding, reporting that cerium oxide nanoparticles at controlled doses do not induce significant developmental toxicity in zebrafish models. This further corroborates the hypothesis that the integration of biocompatible hyaluronic acid with cerium oxide nanoparticles can reduce potential cytotoxic effects while retaining its biomedical potential.

Moreover, the observed structural development of zebrafish embryos over 72 to 120 hours indicates that the material does not interfere with normal embryonic growth, similar to findings by Xu et al. (2017), who assessed the biocompatibility of cerium oxide-functionalized biomaterials in aquatic models. This emphasizes the potential of cerium oxide-incorporated hyaluronic acid gel as a safe biomaterial for therapeutic applications.

Overall, the present study substantiates the antimicrobial efficacy and biocompatibility of cerium oxide-incorporated hyaluronic acid nanoparticle gel. Future research should focus on exploring the detailed mechanistic pathways of its antimicrobial action and conducting further in vivo toxicity assessments to confirm its safety for biomedical applications. The integration of cerium oxide nanoparticles into biopolymeric matrices such as hyaluronic acid holds promise for developing advanced wound healing and infection-resistant biomaterials.

CONCLUSION:

Consolidated hyaluronic corrosive gel could track down critical executions in biomedical and drug applications and show the proportion of future exploration and demonstrated less harmfulness in nature with striking antibacterial action against gram positive and gram negative microbes. Thus, consolidated hyaluronic corrosive gel which are the clinical phytochemical interceded concentrate could bring about unparalleled changes directly at the elevated degree of integrated hyaluronic corrosive gel and may be applied in different clinical executions.

FUTURE SCOPE:

Further research on this study could assist us in developing a viable natural antibacterial agent against S.mutans which is sole agent for dental caries progression

CONFLICTS OF INTEREST:
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ACKNOWLEDGMENT
The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing research laboratory facilities and the required equipment to carry out the study successfully.

REFERENCES:
1. Barabadi H, Saravanan M, Mostafavi E, Vahidi H. Bioengineered Nanomaterials for Wound Healing and Infection Control [Internet]. Elsevier; 2023. 591 p. Available from: https://play.google.com/store/books/details?id=-IusEAAAQBAJ
2. Dykman L, Khlebtsov N. Gold Nanoparticles in Biomedical Applications [Internet]. CRC Press; 2017. 430 p. Available from: https://books.google.com/books/about/Gold_Nanoparticles_in_Biomedical_Applica.html?hl=&id=uNZCDwAAQBAJ
3. Anticancer and photocatalytic activities of zinc oxide nanorods synthesized from Manilkara littoralis leaf extract. Mater Chem Phys [Internet]. 2022 Feb 1 [cited 2023 Nov 17];277:125541. Available from: http://dx.doi.org/10.1016/j.matchemphys.2021.125541
4. Galyamin D, Ernst LM, Fitó-Parera A, Mira-Vidal G, Bastús NG, Sabaté N, et al. Nanoceria dissolution at acidic pH by breaking off the catalytic loop. Nanoscale [Internet]. 2022 Oct 6 [cited 2023 Nov 17];14(38):14223–30. Available from: https://pubs.rsc.org/en/content/articlelanding/2022/nr/d2nr03586c
5. Dhall A, Self W. Cerium Oxide Nanoparticles: A Brief Review of Their Synthesis Methods and Biomedical Applications. Antioxid Redox Signal [Internet]. 2018 Jul 24 [cited 2023 Nov 17];7(8):97. Available from: https://www.mdpi.com/2076-3921/7/8/97
6. de Mendonça JPA, Lourenço TC, Freitas LPM, Santo AAE, Feliciano GT, Da Silva JLF. Molecular dynamics investigation of the structural and energetic properties of CeO2–MOx (M = Gd, La, Ce, Zr) nanoparticles. Mater Adv [Internet]. 2021 Nov 29 [cited 2023 Nov 17];2(23):7759–72. Available from: https://pubs.rsc.org/en/content/articlelanding/2021/ma/d1ma00543j
7. Regulating the surface of nanoceria and its applications in heterogeneous catalysis. Surf Sci Rep [Internet]. 2018 Mar 1 [cited 2023 Nov 17];73(1):1–36. Available from: http://dx.doi.org/10.1016/j.surfrep.2018.02.001
8. Oxygen vacancy enhanced biomimetic superoxide dismutase activity of CeO2-Gd nanozymes. J Rare Earths [Internet]. 2021 Sep 1 [cited 2023 Nov 17];39(9):1108–16. Available from: http://dx.doi.org/10.1016/j.jre.2020.06.019
9. Abdal Dayem A, Hossain MK, Lee SB, Kim K, Saha SK, Yang GM, et al. The Role of Reactive Oxygen Species (ROS) in the Biological Activities of Metallic Nanoparticles. Int J Mol Sci [Internet]. 2017 Jan 10 [cited 2023 Nov 17];18(1):120. Available from: https://www.mdpi.com/1422-0067/18/1/120
10. Sarani M, Heydari A, Darroudi M. Green Synthesis of Nanomaterials: Cytotoxicity and Drug Delivery [Internet]. Frontiers Media SA; 2023. 107 p. Available from: https://books.google.com/books/about/Green_Synthesis_of_Nanomaterials_Cytotox.html?hl=&id=Xc_WEAAAQBAJ
11. Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles [Internet]. Elsevier; 2018. 548 p. Available from: https://play.google.com/store/books/details?id=LzV8DwAAQBAJ
12. Recent advances in biomaterials for 3D scaffolds: A review. Bioactive Materials [Internet]. 2019 Dec 1 [cited 2023 Nov 17];4:271–92. Available from: http://dx.doi.org/10.1016/j.bioactmat.2019.10.005
13. Gupta A, Sakthivel TS, Neal CJ, Koul S, Singh S, Kushima A, et al. Correction: Antioxidant properties of ALD grown nanoceria films with tunable valency. Biomater Sci [Internet]. 2019 Jul 1;7(7):3076. Available from: http://dx.doi.org/10.1039/c9bm90034a
14. Din F ud, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. IJN [Internet]. 2017 Oct 5 [cited 2023 Nov 17];12:7291–309. Available from: https://www.dovepress.com/getfile.php?fileID=38743
15. Le H, Karakasyan C, Jouenne T, Le Cerf D, Dé E. Application of Polymeric Nanocarriers for Enhancing the Bioavailability of Antibiotics at the Target Site and Overcoming Antimicrobial Resistance. NATO Adv Sci Inst Ser E Appl Sci [Internet]. 2021 Nov 12 [cited 2023 Nov 17];11(22):10695. Available from: https://www.mdpi.com/2076-3417/11/22/10695
16. Gupta A, Das S, Neal CJ, Seal S. Controlling the surface chemistry of cerium oxide nanoparticles for biological applications. J Mater Chem B Mater Biol Med [Internet]. 2016 May 11 [cited 2023 Nov 17];4(19):3195–202. Available from: https://pubs.rsc.org/en/content/articlelanding/2016/tb/c6tb00396f
17. Lin YW, Fang CH, Meng FQ, Ke CJ, Lin FH. Hyaluronic Acid Loaded with Cerium Oxide Nanoparticles as Antioxidant in Hydrogen Peroxide Induced Chondrocytes Injury: An In Vitro Osteoarthritis Model. Molecules

[Internet]. 2020 Sep 25 [cited 2023 Nov 17];25(19):4407. Available from: https://www.mdpi.com/1420-3049/25/19/4407
18. OECD. OECD Guidelines for the Testing of Chemicals, Section 2 Test No. 236: Fish Embryo Acute Toxicity (FET) Test [Internet]. OECD Publishing; 2013. 22 p. Available from: https://play.google.com/store/books/details?id=-WApAAAAQBAJ
19. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Biomed Anal [Internet]. 2016 Apr 1 [cited 2023 Nov 17];6(2):71–9. Available from: http://dx.doi.org/10.1016/j.jpha.2015.11.005
20. Srinivas A, Rao PJ, Selvam G, Goparaju A, Murthy PB, Reddy PN. Oxidative stress and inflammatory responses of rat following acute inhalation exposure to iron oxide nanoparticles. Hum Exp Toxicol [Internet]. 2012 Nov;31(11):1113–31. Available from: http://dx.doi.org/10.1177/0960327112446515
21. Gupta R, Xie H. Nanoparticles in Daily Life: Applications, Toxicity and Regulations. JEP(T) [Internet]. 2018 [cited 2023 Nov 17];37(3). Available from: https://www.dl.begellhouse.com/journals/0ff459a57a4c08d0,2f0b91ba0949bdaf,0972d8736b668842.html
22. Generation of high-porosity cerium oxide nanoparticles and their functionalization with caryophyllene oxide using supercritical carbon dioxide. J Supercrit Fluids [Internet]. 2023 May 1 [cited 2023 Nov 17];196:105901. Available from: http://dx.doi.org/10.1016/j.supflu.2023.105901