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Original Article

Nano-Enabled Stem Cell Therapy Ameliorates Premature Ovarian Failure in Rats via Hormonal and Follicular Regeneration

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ABSTRACT

Background: Premature ovarian failure (POF), clinically referred to as primary ovarian insufficiency (POI), represents a loss of ovarian function occurring before the age of 40 and remains a major cause of infertility in young women. Existing therapeutic interventions provide limited benefit, highlighting the need for innovative regenerative strategies. Stem cell-based therapies combined with bioengineered nanomaterials have recently emerged as promising approaches to restore ovarian physiology and fertility. **Materials and Methods:** Cerium oxide (CeO_2) nanoparticles were produced using an eco-friendly synthesis technique and were comprehensively characterized using SEM, EDX, FTIR, DLS, and zeta potential measurements. Unrestricted somatic stem cells (USSCs) were extracted from human umbilical cord blood. The therapeutic potential of CeO_2 nanomaterials alone and in combination with USSCs was explored in a chemotherapy-induced POF rat model. **Results:** Physicochemical analysis confirmed spherical CeO_2 nanoparticles with an average particle size of 10–30 nm and a surface charge of approximately +3 mV. In vitro assays demonstrated acceptable biocompatibility with minimal cytotoxicity up to 64 µg/mL. Notably, co-administration of CeO_2 nanoparticles and USSCs *in vivo* resulted in marked ovarian recovery, reflected by improvement in reproductive hormone levels (FSH and E_2), increased ovarian index, and superior pregnancy success compared to individual treatments. **Conclusion:** The study demonstrates that green-synthesized CeO_2 nanoparticles support stem cell-based ovarian repair and may represent a viable combined therapeutic approach for the management of premature ovarian failure.

Keywords: Premature Ovarian Failure, Regenerative Medicine, Unrestricted Derived Stem Cells.

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INTRODUCTION

Premature Ovarian Failure (POF) is defined by amenorrhea or oligomenorrhea and high level of Follicle-Stimulating Hormone (FSH) in females under the age of 40 years. POF is the loss of ovarian function, resulting in clinical symptoms including the early development of menopausal syndromes and infertility.[1] The prevalence of Primary Ovarian Insufficiency (POI) is as high as 3.7% in globally. Women suffering from POI fail to undergo the pubertal transition and experience from vasomotor symptoms, sleep disturbance, genitourinary syndrome, reduced skin elasticity, emotional irritability, elevated risks for cardiovascular disease, and infertility. Current methods of treating POI focus on enhancing quality of life through means such as adopting healthy lifestyle choices, Hormone Replacement Therapy (HRT), and inducing puberty in young patients.[2] Nonetheless, these methods are unable to stop ovarian processes such as follicular growth, ovulation, and the production of female sex hormones. Patients with POI might benefit from effective interventions aimed at reestablishing normal ovarian function.[3,4]

The use of stem cells in the treatment of POI shows promising restorative effects. Multipotent Stem Cells (MSCs) have the ability to self-renew, proliferate rapidly, and differentiate into a wide variety of cell types. Several chronic and severe conditions, such as lung injury, nephropathy, osteoarthritis, nervous system disorders, intrauterine adhesion, and POF, have been treated with them.[5,6] Differentiation into the target tissue cells, anti-apoptosis actions, and potent capacities of immunological control and inflammation inhibition are the primary mechanisms by which MSCs assist in the repair and regeneration of damaged tissues.[7-9] Extensive evidence from both preclinical and clinical studies pointed to a paracrine mechanism by which MSCs repair damaged ovaries. By secreting paracrine regeneration factors, MSCs have proven to be a promising therapy for POI

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treatment.[10-12] Unfortunately, stem cell therapy's potential clinical utility has been impeded by several inherent constraints, such as the possibility of immunogenicity and tumorigenicity. Some researchers have looked into using secretomes formed from stem cells instead of stem cells themselves.[13,14] Differentiating characteristics of human Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) include their "younger" somatic stem cell origin, severe lack of immunogenicity, minimal tumorigenicity, high proliferation speed, and lack of ethical concerns. As a result, UC-MSCs have lately emerged as a promising possibility for clinical applications. The processes underlying the regenerative effects of UCMSCs, which are now being employed in some clinical trials to treat POF, are not well understood.[15,16] In the field of cancer and reproductive medicine, nanotechnology has the potential to greatly benefit the study, diagnosis, and treatment of infertility-related illnesses without causing any harm to the patient (oncological or non-oncological).[17]

Modification and construction of structures on the molecular level, typically 100 nm in size, is at the heart of nanotechnology. Because of their size, nanoparticles can take advantage of preexisting cellular mechanisms to help them perform their intended task, just like proteins and intracellular structures. In oncology, nanoparticles have been effectively exploited to load, carry, and deliver medications, making this discipline a pioneer in the establishment of tissue-targeted nanotherapeutic delivery.[18,19] In addition, nanoparticles can be tailored to preferentially target a specific site, organ, or tumor, increasing drug efficacy while reducing systemic exposure to the drug. Targeted nano-delivery systems may have a profound effect on reproductive medicine, where unintended side effects can have dire consequences.[20-24]

The developing gamete and embryo are particularly vulnerable, so any use of nanoparticles in infertility must be carefully considered to avoid serious harm. Drugs can have far-reaching impacts on development by affecting only one gamete, but it is in these early phases that therapies like genetic editing can offer the most promise. Treatment of gynecological conditions that affect fertility is often tempered by worries about potential toxicological effects on the female reproductive system. Fertility treatments are delicate, but targeted drug delivery systems could have a major impact while reducing the need for invasive surgical procedures.[25-27] This method would be a huge step forward for the industry.

Cerium oxide Nanoparticles (CeNPs, nanoceria) have a diverse range of applications, including chemical mechanical polishing/planarization, corrosion protection, solar cells, fuel oxidation catalysis, and automobile exhaust treatment.[28-31] Beyond these industrial uses, CeNPs exhibit remarkable bio-relevant properties, making them highly promising for biomedical applications. They mimic the enzymatic activities of Superoxide Dismutase (SOD), catalase, peroxidase, oxidase, and phosphatase, enabling them to scavenge Reactive Oxygen Species (ROS) such as hydroxyl radicals, nitric oxide radicals, and peroxynitrite. These antioxidant and catalytic properties are primarily attributed to their unique ability to absorb and release oxygen, as well as their efficient redox cycling between Ce^{3+} and Ce^{4+} states on the nanoparticle surface. Owing to these bio-relevant activities, CeNPs have emerged as potential pharmacological agents, drug delivery vehicles, and bioscaffolds in regenerative medicine.[32-35] However, concerns regarding their biocompatibility and toxicity have prompted the exploration of bio-directed synthesis approaches. The use of natural matrices as stabilizing agents in CeNP synthesis has gained considerable attention as an eco-friendly and biologically safe alternative to conventional methods. Green synthesis techniques not only enhance the biocompatibility of CeNPs but also provide a more sustainable, cost-effective and safer approach to nanoparticle fabrication, making them particularly valuable for pharmaceutical and biomedical applications.[36-39]

Among green synthesis methods, plant-mediated synthesis (phytosynthesis), fungus-mediated synthesis, polymer-mediated synthesis, and nutrient-mediated synthesis are widely used. In plant-mediated synthesis, plant extracts act as natural stabilizing and capping agents, promoting nanoparticle formation without the need for toxic chemicals.[40-42] Given these advantages, we employed a green chemistry approach to synthesize CeNPs with desirable chemical, physical, and biological properties. The synthesized CeNPs were extensively characterized and subsequently applied in combination with Umbilical Cord-derived Mesenchymal Stem Cells (UC-MSCs) as a novel therapeutic strategy for treating Premature Ovarian Failure (POF). By leveraging the synergistic effects of CeNPs and UC-MSCs, this study aims to enhance ovarian tissue regeneration and restore ovarian function.

MATERIALS AND METHODS

Reagents

In the current study, chemical and reagents were obtained from Sigma (St. Louis MO, USA), unless otherwise specified. The biological experiments-related substances and kites were obtained from Fisher Scientific (Pittsburgh, PA) and ICN (Costa Mesa, CA) if their origins are not mentioned in the text.

Biogenic synthesis of CeO_2 NPs

The green synthesis was applied to synthesize Cerium oxide NPs. Briefly, *Avena sativa* L. was washed with both regular and distilled water, then dried, milled, and stored. The annual grass *Avena sativa* L. (Poaceae) grows tall, straight culms anywhere from 40 to 180 cm in height. It has a linear hilum and is covered in hairs all around. For 4 hr at 80°C, we boiled the powdered samples (150 g) in a pot of boiling distilled water while stirring constantly. After filtering, the extract was placed in a refrigerator at 4°C. In this case, the extract served double duty as a reducing and capping agent. After keeping an aqueous cerium nitrate solution (1 mM) at 80°C with vigorous stirring for 3 hr, the white precipitate turned

yellow, indicating the generation of Cerium oxide NPs when 10 mL of *S. acuta* extract was added drop by drop to the mixture. NPs were washed for 10 min in distilled water, centrifuged for 15 min at 10,000 rpm, dried for 1 hr at 80°C, then calcinated for 2 hr to eliminate residuals. Cerium oxide nanoparticles were obtained by further grinding this calcinated solid into a powder.

Characterizations

The synthesized biogenic Cerium oxide nanoparticles were characterized using SEM (for morphological evaluation, Energy Dispersive Spectroscopy (for semi-quantitative elemental analysis), XRD (for crystallinity assessment), FTIR (for surface functional groups) DLS (for hydrodynamic size measurement) and Zetasizer (for zeta potential measurement).

Isolation and expansion of USSCs from fresh CB

After giving birth to healthy infants, cord blood was harvested in 50 mL falcon tubes without anticoagulant. All participants gave their written informed consent and the collection was approved by the university's ethics council. After waiting 2-4 hr, the blood was centrifuged at 1,000 g for 30 min at 20°C. The sera were extracted, pooled, and processed using 0.22 µm filters. Finally, aliquots of the combined serum were frozen at 20°C for later use. After obtaining permission from the ethics committee at Damascus University and obtaining signed informed consent from all subjects, three umbilical cords were retrieved from healthy pregnant women undergoing cesarean births. The materials were preserved in Phosphate-Buffered Saline (PBS), transported in PBS, cleaned with PBS, and then divided into 5-cm^2 sections in the laboratory. Blood arteries were severed and the sections were slit lengthwise. The sections were then grown in DMEM with 100 U/mL penicillin and 100 g/mL streptomycin in 25 cm^2 flasks. Uninterrupted for 7 days, flasks were incubated at 37°C in a humidified environment containing 5% CO_2. The medium was changed for the first time after one week in the incubator, and subsequent changes occurred every three to four days. Adherent cells were cultured for another 21 days after UC explants were removed, after which they were detached using 0.05% trypsin-EDTA. Following trypan blue counting, the cells were subcultured. The morphology of USSCs was examined using an inverted microscope. Cells at passage 3 were grown at a density of 1,000 cells/well in a 12 well plate containing DMEM media supplemented with either 10% PL or 10% CBS or 10% FBS. The media were changed twice a week. The cells were stained with 0.1% crystal violet solution after two weeks.

Experimental Animals

The animals (female Sprague Dawley (SD) rats, two months-old and weighing 200-220 g) were received and housed in a standard animal facility with free access to water and were fed a standard pellet diet. The laboratory conditions were standard (12 hr light/dark cycle; 25°C, relative humidity of 45-55%) during the experiments. The animal model (premature ovarian failure) was induced based on the approved protocol.[43,44] Two weeks after inducing the model, the animals were randomly divided into control groups, and test 1 (treated with Cerium oxide NPs), test 2 (treated with the cells), and test 3 (treated with the combination of Cerium oxide NPs and the cells). Rats were given ketamine (45 mg/kg body weight) through intraperitoneal administration to induce anesthesia before UCMSCs were transplanted. Less than 30 min were spent storing UCMSCs (passage 2 cells) at 4°C in PBS before they were transplanted. For the *in situ* injection group, UCMSCs (25L, at a density of 2×106 /mL) were implanted directly through both ovaries of POF rats using a proper device (micro injector).

BW and ovary weight

All of the animals were weighed again after 30 days. After taking blood samples and killing the rats by cervical dislocation, the ovaries were quickly removed, weighed, frozen at 80°C, and dissected. Blood was centrifuged at 1,200 g for 10 min to separate the serum.

Fertility evaluation

We experimented on three separate groups of female rats to determine the treatment's impact on fertility. 10 male and 10 female rats from each group were housed together in a mating cage. Success in mating was determined by the presence of a white suppository at the vaginal opening, which was detected by smearing the vaginal mucus and observing it under an optical microscope (U-III Multi-point Sensor System; Nikon Corporation, Tokyo, Japan). The number of pregnant animals was used as a proxy for the success of the mating.

Serum parameter analysis

Serum parameters were measured by drawing blood from anesthetized animals by a retroorbital puncture. After 1h of incubation at room temperature, samples were centrifuged at 4000 r/min for 10 min to collect the supernatant. Commercial kits (Bühlmann Laboratories AG, Switzerland) were used to detect plasma concentrations of estradiol (E2), Follicle-Stimulating Hormone (FSH); A fully automated clinical chemistry analyzer was used for all biochemical analyses (Type 7170A; Hitachi Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

Various approaches have been proposed and applied for the synthesis of nanoparticles that can be categorized as physical, chemical, and more recently, green synthesis methods. Physical and chemical methods are gradually being replaced by green synthesis methods because of issues related to the consumption of large amounts of energy, the release of toxic and harmful chemicals, and the use of complex equipment and synthesis conditions.[45-47]

Green synthesis has many advantages compared to chemical and physical methods: it is non-toxic, pollution-free, environmentally friendly, economical, and more sustainable. In this concept, the primary and secondary metabolites found in plant extracts serve as reducing and capping agents, respectively, to produce nanoparticles that are both stable and tunable in terms of shape, size, crystallinity, and morphology. Various biosystems have been utilized to synthesize biogenic nanoparticles. Oat dietary fibre (-glucan) is an important component of *Avena sativa*, which is also a rich source of protein, vital minerals, lipids, and avenanthramides (an indole alkaloid), gramine, flavonoids, flavonolignans, triterpenoid saponins, sterols, and tocols. Oats have a long history of use and have been studied for their potential stimulant, antispasmodic, anticancer, diuretic, and neurotonic properties. Antioxidant, anti-inflammatory, wound-healing, immunomodulatory, anti-diabetic, anti-cholesterolemic, and other pharmacological actions are all present in oat. Oat has a wide variety of biological actions, making it a promising medicinal agent.

Morphological analysis

In order to analyze a wide variety of materials at high magnifications and generate high resolution images, SEM analysis is a potent analytical technique. SEM works by exposing a sample to a concentrated beam of electrons from an electron gun and then detecting the high-energy electrons emitted from the sample's surface. The SEM objective lens concentrates the electron beam into a pinpoint on the surface of the sample. The results of SEM imaging are presented in Figure 1 with different magnifications (12000X in Figure 1A and 24000X in Figure 1B). The size measurement showed that the biogenic Cerium oxide nanoparticles have a size in the range of 10-30 nm.

Elemental analysis

Chemical characterization and elemental analysis of materials are made possible by energy-dispersive X-ray spectroscopy (EDS, abbr. EDX, or XEDS), an analytical technique. When a sample is excited by a strong enough source of energy (like the electron beam of an electron microscope), the electrons in its core and shell will be ejected, releasing some of the energy that was absorbed. When an outer-shell electron with a higher energy level replaces one that has left, the atom emits an X-ray with a unique spectrum due to the energy gap. By doing so, the energetically excited contents of a specific volume of sample can be analyzed for their constituent parts. Element identification is accomplished by locating the peaks in the spectrum, while signal strength is proportional to element concentration. We conducted the EDX analysis to semi-quantitatively characterize and evaluate the presence of Ce and O elements in the as-synthesized biogenic Cerium oxide nanoparticles. As shown in Figures 2 and 3, the presence of the elements is obvious in Cerium oxide nanoparticles.

Hydrodynamic size and zeta potential

Dynamic Light Scattering (DLS) provides data on the agglomeration condition of nanoparticles in solution by measuring their hydrodynamic diameter. Focusing a light beam on the sample and measuring the resulting changes in scattering intensity due to the Brownian motion of the object is what is known as DLS. The DLS analysis showed that as-synthesized Cerium oxide nanoparticles have a hydrodynamic size of around 50 nm and a zeta potential of around 3 mV.

Functional groups results

The Fourier Transform Infrared Spectroscopy (FTIR) method uses infrared light to scan samples, allowing for the characterization of organic, inorganic, and polymeric components. When the

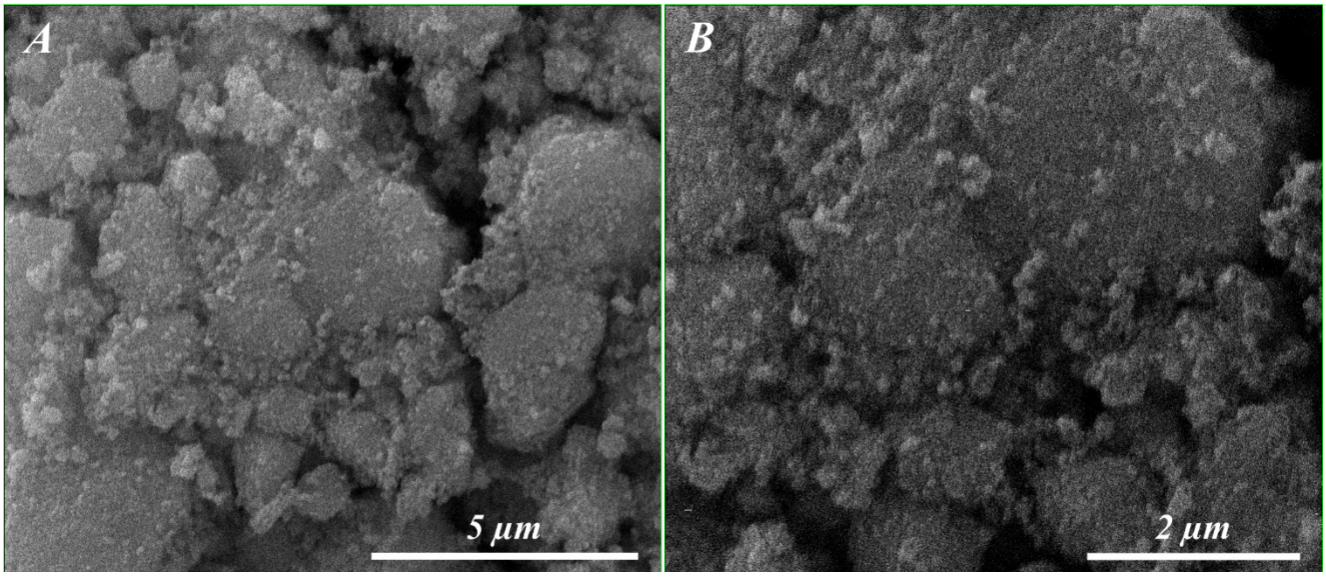

Figure 1: SEM micrographics of the biogenic Cerium oxide nanoparticles with different magnification (12000X in Figure 1A and 24000X in Figure 1B).

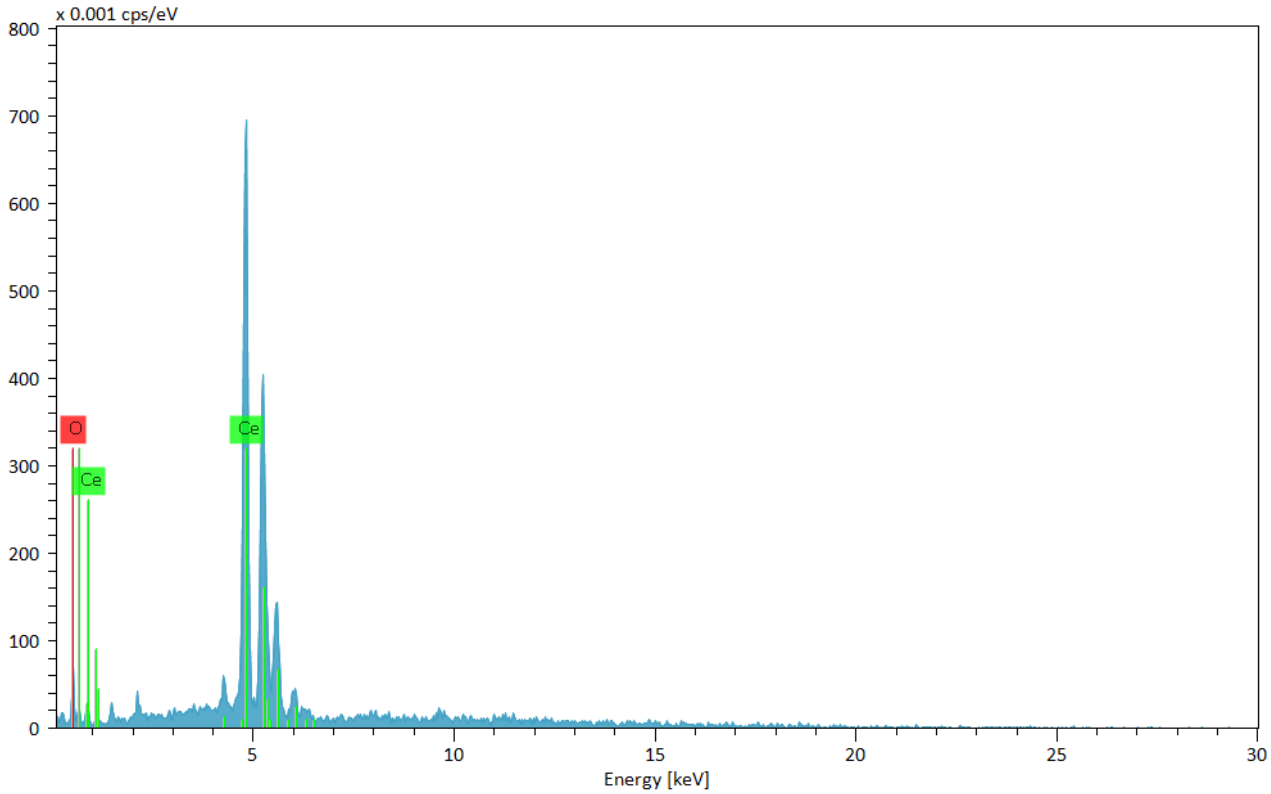

Figure 2: Energy dispersive X-ray (EDX) point analysis of as-synthesized biogenic Cerium oxide nanoparticles.

usual pattern of absorption bands shifts, it's easy to see that the material's composition has changed. The Fourier Transform Infrared Spectroscopy (FTIR) is a valuable tool for analyzing a wide variety of materials and processes, including the detection of pollutants, the location of additives, and the detection of decomposition and oxidation. As shown in Figure 4, the obtained extract exhibited the characteristic peaks of the extract and the resultant biogenic Cerium oxide nanoparticles have the same functional groups.

Crystallinity findings

X-ray powder Diffraction (XRD) is a quick analytical method that can reveal information on the unit cell dimensions of a crystalline material and is therefore commonly employed for phase identification. We finely grind and homogenize the studied material to find its average bulk composition.

Toxicity assay of nanoparticles

Metabolic activity can be used as a proxy for cell viability, proliferation, and cytotoxicity, and the MTT assay is commonly employed for this purpose. This colorimetric assay depends on the ability of metabolically active cells to convert yellow tetrazolium salt into purple formazan crystals.[48] In live cells, NAD(P)H-dependent oxidoreductase enzymes convert MTT to formazan, indicating cell viability. Once the insoluble formazan crystals have been dissolved in a solubilization solution, the multi-well spectrophotometer is used to measure the absorbance of the resulting colored solution between 500 and 600 nm. A higher concentration of cells indicates a higher number of cells that are alive and actively metabolising their surroundings. The results (Figure 5) show that the synthesized Cerium oxide nanoparticles did not induce significant cell toxicity up to 64 µg/mL. The higher concentrations (128, 256, and 512 µg/mL) substantially compromised the cell viability. There is controversy over the toxicity of green synthesized Cerium oxide nanoparticles. Biosynthesis of Cerium oxide-NPs from *Salvadora persica* aqueous extract was reported by Miri *et al*. The nanoparticles produced were tested for their cytotoxic effect against a colon cancer cell line (HT-29). Even at concentrations above 800 g/mL, the manufactured materials showed no appreciable cytotoxic effect.[49] In another study, to study its cytotoxic effects, UV protection, and photocatalytic activity, Cerium oxide nanoparticles were produced using Musa sapientum peel extract. This study found that nanoparticles were non-toxic to lung (A549) cell lines at doses below 500 g/mL, as shown by the cytotoxic activity. [50]

Body and ovarian weights

To verify the availability of the subsequent research data, we first compared the body weight of each animal after POF induction; the results showed no significant changes in rat weight. What we found (Figure 6) across all experimental groups, rat body weight was significantly higher than in the control group. The results

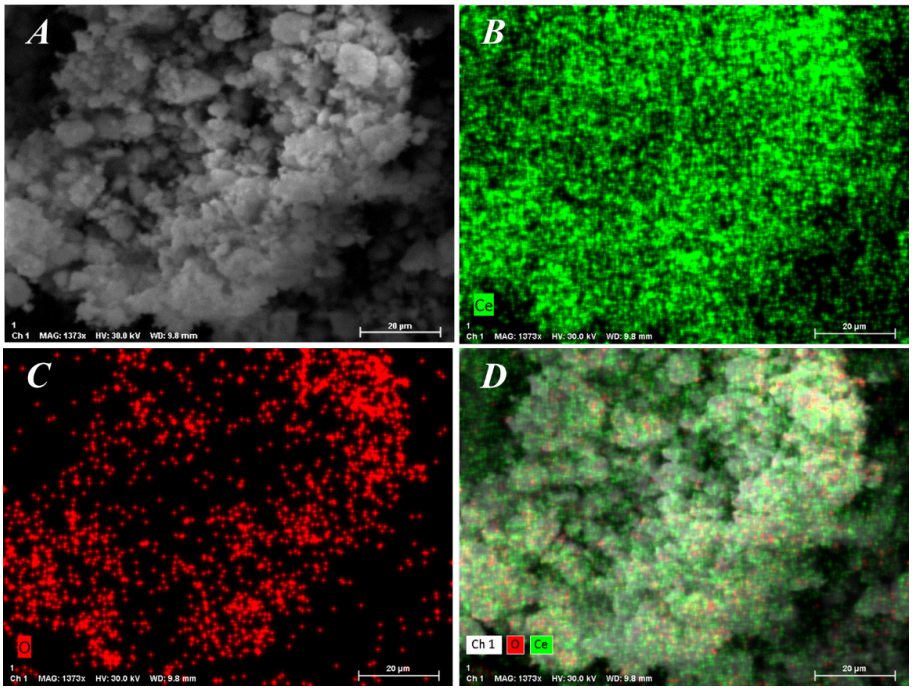

Figure 3: Energy dispersive X-ray (EDX) mapping analysis of as-synthesized biogenic Cerium oxide nanoparticles.

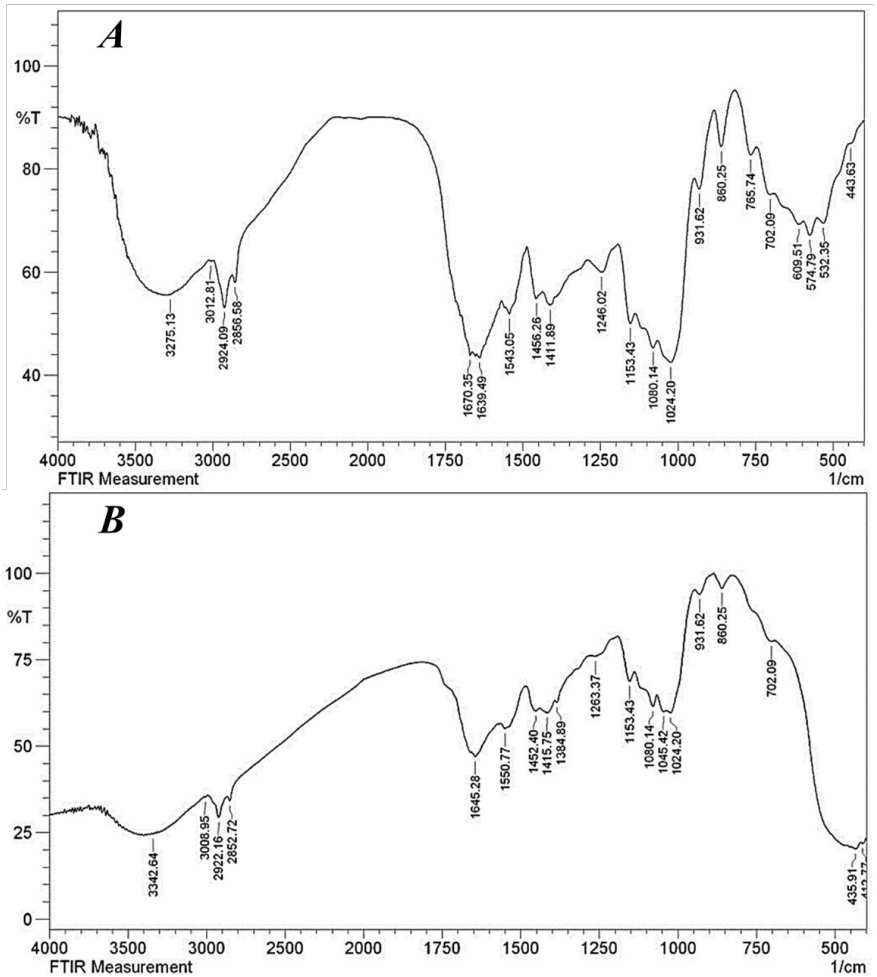

Figure 4: FTIR spectra of (A) the extract and (B) as-synthesized biogenic Cerium oxide nanoparticles.

revealed that the POF has an adverse effect on the healthiness of rats. Moreover, the combination therapy (treated with the combination of Cerium oxide NPs and the cells) increased the weight of animals.

To assess the effects of the treatment on ovarian function, we evaluated the ovarian index after the treatment. The results (Figure 7) showed that the ovarian index of POI rats was significantly lower than that of control rats (p 0.001), showing substantial atrophy of the ovaries in the POI rats. However, ovarian indices in tests 2 and 3 were much higher than in POI rats, indicating that Cerium oxide nanoparticles and the cells mitigate ovarian atrophy and prevent the progression of POI.

Fertility outcome

It is reported that the POF has a direct effect on the pregnancy of patients. Accordingly, we evaluated the effects of the treatments on the pregnancy of animals (Figure 8). In this experiment, all the animals were mated with healthy adult male rats. The results showed that the treatment using NPs, cells, and a combination of NPs and cells improved the fertility of animals. The highest pregnancy outcome was obtained under treatment with the combination of NPs and cells.

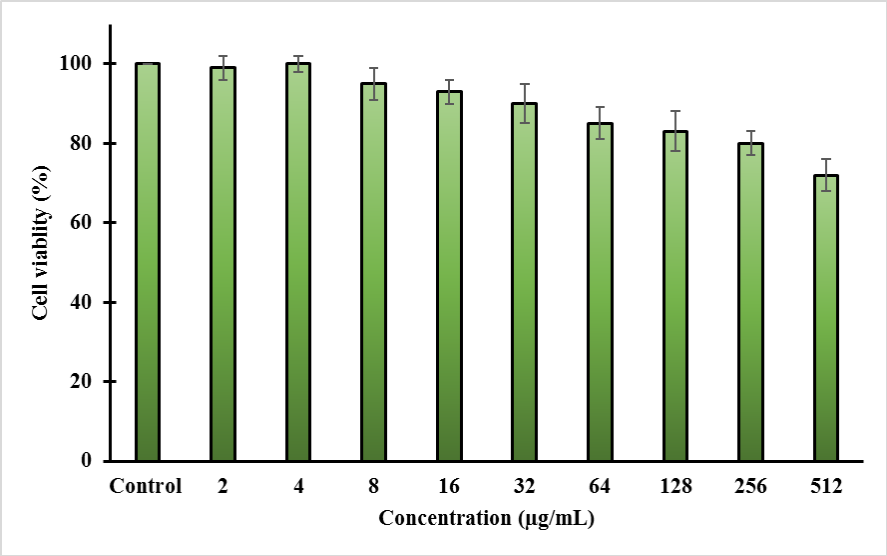

Figure 5: Cell viability of the biosynthesized Cerium oxide nanoparticles.

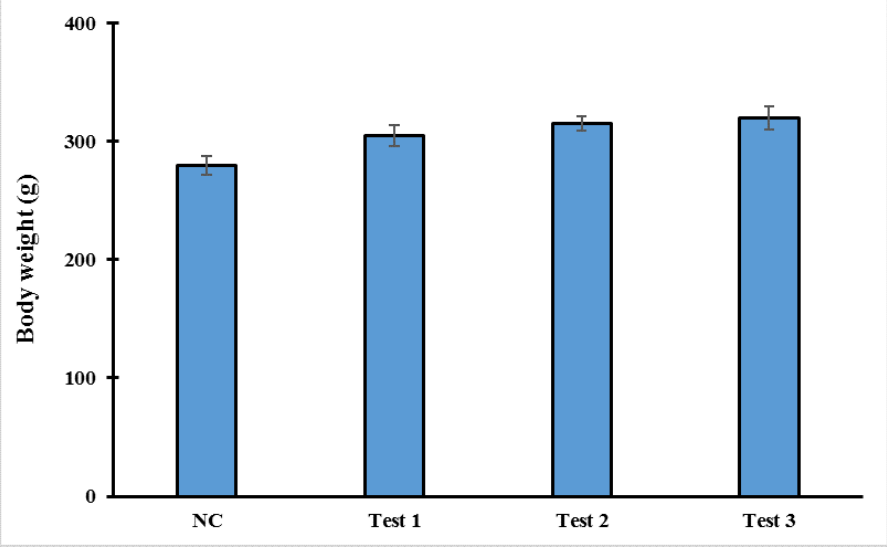

Figure 6: The body weight changes during the 4 weeks after treatments.

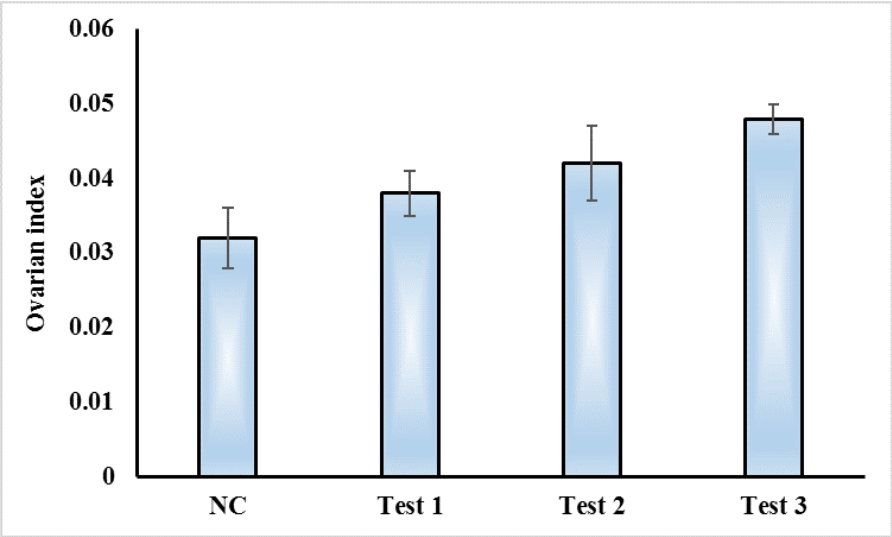

Figure 7: The ovarian index of animals after the treatment.

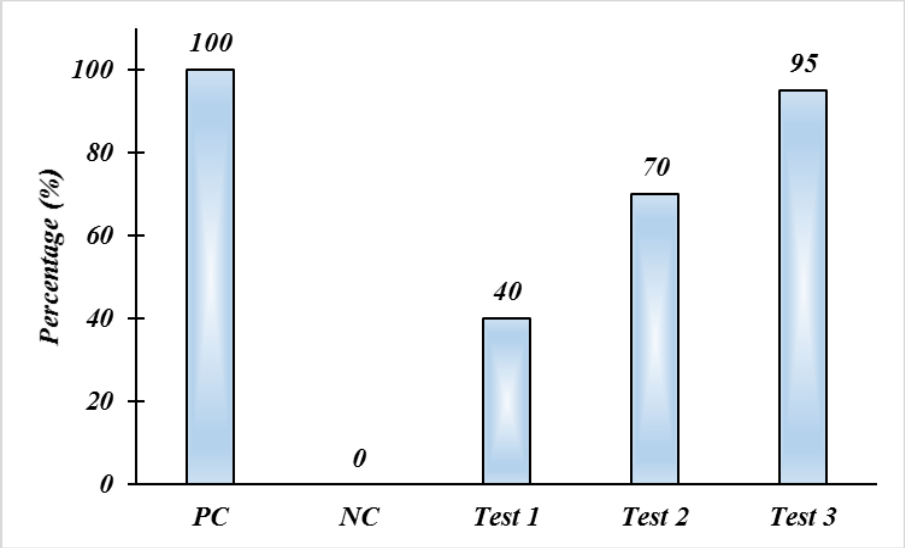

Figure 8: The percentage of pregnancy of rats under treatment with NPs (Test 1), Cells (Test 2), and combination of NPs and cells (Test 3). PC: positive control (healthy rat) and NC: negative control (treated with normal saline).

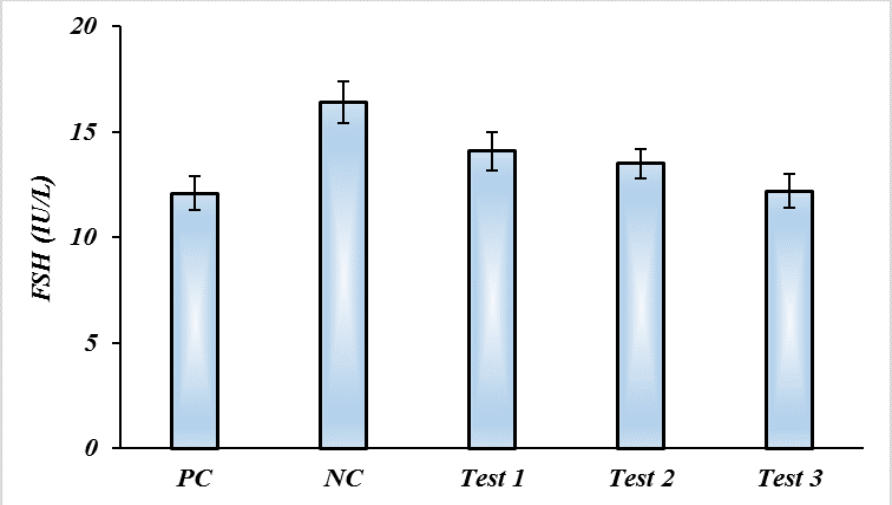

Figure 9: Quantitative analysis of serum FSH level. NPs (Test 1), Cells (Test 2), and combination of NPs and cells (Test 3). PC: positive control (healthy rat) and NC: negative control (treated with normal saline).

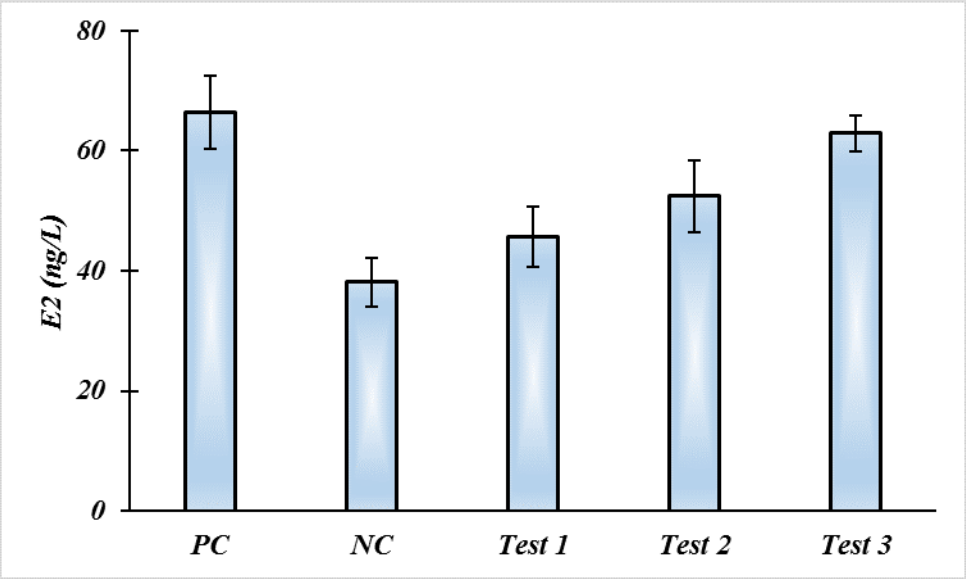

Figure 10: Quantitative analysis of serum E2 level. NPs (Test 1), Cells (Test 2), and combination of NPs and cells (Test 3). PC: positive control (healthy rat) and NC: negative control (treated with normal saline).

The treatments improved hormone secretion in POI rats

Clinical symptoms of POI include irregular or absent menstrual bleeding, elevated gonadotrophins, and low Estradiol (E2). To put it another way, ovarian insufficiency is not a binary condition but rather a spectrum of varying degrees of ovarian dysfunction or ovarian age. Premature menopause is the typical end effect of this illness, which may be temporary or progressive. The efficacy of the treatment was further evaluated using the measuring the hormone secretion in rats. The results (Figure 9) showed that the treatment using the NPs, cells, and a combination of NPs and cells improved the FSH secretion in POF rats. The combination therapy induced the highest treatment outcomes revealing the synergistic effects of NPs and cells.

Estradiol, often known as E2, is the predominant oestrogen in sexually active women who are not pregnant. Ovaries are responsible for producing the vast majority of this hormone, which plays a crucial role in reproduction (the ability to get pregnant). In addition, it is beneficial to your brain and bones. The testicles of men produce a negligible amount of oestrogen (the glands that make sperm). In POI the serum level of E2 is decreased and an elevated concentration of E2 can be indicated as the treatment efficacy. The results (Figure 10) showed that the treatment using the NPs, cells, and a combination of NPs and cells improved the E2 secretion in POF rats. The combination therapy induced the highest treatment outcomes revealing the synergistic effects of NPs and cells.

CONCLUSION

The main objective of the experiment was to develop a combination therapy based on nanotechnology and regenerative medicine for POF. We applied the biogenic/green synthesis to obtain Cerium oxide nanoparticles with desired properties, including smaller size (lower than 100 nm), uniform morphology, and decoration with proper functional groups. The results showed that the synthesized nanoparticles have the indicated characteristics. In the next step, we isolated the cells and applied them in combination with nanoparticles in the induced POF animal model. The findings revealed that the combination of the synthesized biogenic nanoparticles with the isolated cells has a synergistic effect and improved the parameters related to POF, including the animal's body weight, ovarian index, pregnancy, and fertility potential, and secretion of hormones. Based on the observations, we can suggest that the developed strategy based on nanomaterials and cell therapy can be applied as an effective treatment for POF. However, further studies are required to evaluate the fate of the applied nanoparticles in the body and also the underlying mechanism of the observed healing.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

POI: Primary ovarian insufficiency; **POF:** Premature ovarian failure; **Co:** Cerium oxide; **SD:** Sprague Dawley; **HRT:** Hormone replacement therapy; **MSCs:** Multipotent stem cells; **SOD:** Superoxide dismutase; **PBS:** Phosphate-buffered saline.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of Taian Central Hospital. Written informed consent was obtained from the patient for the publication of this report.

SUMMARY

This study investigates the use of Cerium Oxide (CeO_2) nanoparticles synthesized through a green chemistry method, combined with human Umbilical cord-derived Stem Cells (USSCs), for the treatment of Primary Ovarian Insufficiency (POI). The CeO2 nanoparticles, with sizes ranging from 10-30 nm and a zeta potential of approximately 3 mV, showed no significant toxicity up to 64 µg/mL in cell cultures. In animal studies, the combination therapy significantly improved ovarian function, increased pregnancy rates, and enhanced FSH and E2 secretion in a POF rat model. These results suggest the potential of CeO2 nanoparticles and USSCs in treating POI using regenerative medicine.

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