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Randomized Clinical Study of Nano-Cerium Oxide and its Efficacy in Sciatic Nerve Regeneration: A Histopathological Evaluation

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Peripheral nerve damage is a frequent clinical condition. peripheral nerve injuries are variable, and these injuries are mostly caused by trauma. Blunt trauma has many types, including contusion, laceration, stretching, traction, penetrating and perforating injuries, abnormal sleeping positions, external pressure, internal compression, ischemia. Cerium oxide nanoparticles are used widely in the materials field. Besides their material applications. Objective: this study was examining how cerium oxide nanoparticles impact axonal regeneration of the sciatic nerve in rats.
Materials and Methods: 24 adult animals used, weighing average (M±SD: 230 ± 20 g). The animals were separated into two group. The first group (control) was left without treatment, The second group (Nano cerium Oxide) was treated orally with 50 mg of Nano cerium oxide daily for 7 days. Right Sciatic nerve was completely transected in all animals. The result evaluated at 4th, 8th and 12th weeks post-operative.

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Results: The histopathological results, indicated that the high effective result in the second group, then the control group. The control group has a significant nerve damage and some regenerative signs over time. While there are indications of severe damage particularly at 4 and 8 weeks, then presence of almost normal nerve fibers at 12 weeks suggests partial recovery.
Conclusion: Nano Cerium Oxide accelerated the regeneration of the peripheral nerves These findings suggest that this nanoparticle could can be seen as a novel therapy for promoting the regeneration of the nervous system.

Keywords: Nervous system; peripheral nerve injury; Nano cerium oxide; nerve tissue regeneration.

1. INTRODUCTION

Peripheral nerve damage can occur from different causes like pulling, compressing, lack of blood supply, cutting, and fractures of long bones, resulting in axonotmesis or, more severely, neurotmesis. (Allodi, et al. 2012). Damage to the peripheral nerve leads to secondary muscle wasting, resulting in different degrees of impairment. When the sciatic nerve is harmed, degeneration happens beyond the injury site through Wallerian degeneration and also prior to the injury site through retrograde degeneration, affecting the relevant neurons (Navarro et al., 2007). Damage to the main nerve pathway starts in the most sensitive area of the nerve, the end of the longest one, and then moves towards the center (neuropathy dependent on length). While motor issues are frequent, sensory issues are the most common (Ali et al., 2023). Although the peripheral nervous system has the ability to regenerate after serious injuries, the regeneration process is usually insufficient and the recovery of function is not complete (Kim et al., 2011, Ghayour et al., 2016a, 2016b). Surgery is the primary treatment for severe or complete nerve damage. New methods have been created to fix damaged nerves through innovative techniques like allograft, autograft, and new materials science and engineering methods. (Gu et al., 2011). However, non-surgical methods have also been created to aid in nerve regeneration, either as the main treatment for axonotmesis or as a supplementary option following surgical repair. Therapies using nanoparticles like Nano cerium oxide have been suggested to have beneficial impacts on nerve regeneration and functional improvement. It was also stated that growth factors and transplanting neural stem cells have significant neuroprotective effects (Kokai et al., 2011, Shi et al., 2024). Nanoparticles have been integrated into medical uses, presenting a noninvasive therapeutic approach utilized in multiple areas. It proves successful in reducing pain and aiding in the healing process of conditions like tendinopathies, osteoarthritis, rheumatoid arthritis, wound recovery, and nerve damage (Rahimi et al., 2023). Nanoparticles (NPs) are incorporated into numerous commercial goods and services The destiny, possible harm, and functions of new applications in biomedicine remain uncertain movement of molecules within cells in biology (Ahmed et al., 2020). Nanoparticles have diverse uses such as catalysts, drug delivery, antibacterial purposes, electronics, and optoelectronics (Calvache-Muñoz et al., 2017). These nanoparticles show promising medical applications in treating oxidative stress-related diseases, such as neurodegenerative diseases. The (CONPs) are redox-active rare earth nanomaterials (Ban et al., 2022) (Soluki et al., 2020).

Objective: The objective of this study was to examine how cerium oxide nanoparticles impact axonal regeneration of the sciatic nerve in rats.

2. MATERIALS AND METHODS

Study area: All animal Prepared by the Animal House of the College of Veterinary Medicine, University of Basrah They were housed in suitable cages with favorable conditions at the animal facility of the College of Veterinary Medicine at the University of Basrah. They were fed commercial pellets and given water while being kept in their designated cages for 15 days prior to the examination.

Experimental groups: Twenty-fur adult females healthy Wistar rats were included in this study. having bodyweight average (M±SD: 230 ± 20) grams. They were housed in suitable cages with favorable conditions at the animal facility of the College of Veterinary Medicine at the University of Basrah of Iraq. They were fed commercial pellets and given water while being kept in their designated cages for 15 days prior to the examination, Right Sciatic nerve was completely transected in all animals then instant coaptated was done. Then it was divided equally and

randomly to two groups (n=12 of each). First group, Control group (CG) (n=12) that was left without any extra interferences after coaptating the nerve. Second group, Nano-Cerium Oxide group (n = 12), the rats will have treated with Nanoparticles given orally at dose (50 mg/kg) every day for 1 week after surgery.

Preparation of cerium oxide nanoparticles: Cerium oxide nanoparticles were individually created and then aseptically thinned as listed:

- 300mg of Ceo2 nanoparticles was measured using a delicate balance.
- 3ml of distilled water was used to dilute the 300mg of Ceo2 nanoparticles
- 0.5ml of the diluted solution was orally administered to each rat.
- The procedure was replicated for a total of seven days the Nano group

Surgical procedure: The animals will be anesthetized based on a protocol by injection of 10 mg/kg Xylazine HCL (2%) and 75 mg/kg ketamine HCL (10%) (Helal et al., 2022). All animals in this study will have their right limb selected. The region between the middle of the abdomen and the back of the hind limbs was clipped and shaved then washed thoroughly with distal water; followed by a 2-3 minutes' surgical scrub of the area using diluted liquid soap and then an antisepsis (ethanol alcohol 70%) was applied on the whole clipping area and finally, the site of the incision was applied with 2.5% tincture iodine (Helal et al., 2022). A sterile towel covered the distal end of the right limb (the target limb) and secured with towel clips; the surgical area was then draped for the surgery. By locating the sciatic nerve through feeling the greater trochanter of the femur. and stifles as landmarks, an incision was determined at the posterior-lateral thigh about 1 cm caudo-lateral to the greater trochanter, about one-third of the femur, at the distal level. A three cm incision length was made on the proximal half of the distance between the stifle joint and the trochanter major. The biceps femoris and The semitendinosus muscles were blindly dissected with the dull tips of Mayo scissors to reveal the nerve (Bannai, 2015). After placing the sciatic nerve on a damp wooden surface, a scalpel was used to make a complete cut. (using blade 15 sizes) was proximally made at about 1-1.5 cm to the site of the bifurcation of the nerve (Fig. 1). The coaptate of the two extremities will be created utilizing a basic interrupted suture technique (3 stitches) using 8-0 Nylon suture material, the suturing involves only the epineurium in the area between the distal and proximal segment. A regular closure was performed on the muscles and skin (Helal, and Hussein, 2022)

The animals were then given Meloxicam (0.25 mg/kg BW) as analgesic and (2 mg/kg BW) Ceftiofur HCL once daily for 3 days and 5 days' post-surgery for Meloxicam and Ceftiofur HCL respectively (Helal, and Hussein, 2022).

At the end of the experiment, we euthanized all groups at periods of 4, 8, and 12 weeks by giving a over dose of anesthesia to the rats.

Neuro histopathological examination: Three centimeters of Sciatic nerve (1.5 cm above and 1.5 cm below of the nerve transected) were taken at 4, 8, and 12 post-surgery for neuro histopathological examination secondly The specimen was immersed in 10% buffered formalin for 24 hours, then trimmed to size, placed in a special cassette, washed with tap water, sliced using a microtome, and treated with ethanol and xylene to eliminate formalin. The astronauts then place the tissue in paraffin wax for preservation. than the paraffin templates sectioning by the microtome and fixation the small spaceman to the glass slid and staining the section by using Hematoxylin and Eosin stain (H&E) (Hussein et al., 2014).

Analysis of Histopatholgical examination using different scoring system: Using Gibson-Corley et al.'s principles (Gibson-Corley et al., 2013) and based on model of Savastano et al., (2014). we designed various scoring systems to evaluate histopathological changes and compare them between groups. In the current study, we discovered a link between the designed scoring system and the obvious histopathological changes in different groups over time, including vaccination, degeneration, edema, inflammatory cells, cell nuclei morphology, blood vessel congestion, Schwann cells, axis sheath, macrophages, and debris at 4 weeks, 8 weeks, and 12 weeks (Table 1).

We used a descriptive scoring method to confirm research findings, facilitate direct comparison of histopathological alterations between control and experimental groups (Schafer, K.A., et al., 2018), as well as between treatment groups, and ease the interpretation of our histological data (Meyerholz, D.K. and A.P. Beck, 2018). Histopathological characteristics such as

vacuolation, degeneration, and edema were examined for each group at particular time points (4 weeks, 8 weeks, and 12 weeks). Each parameter score in this scoring system is classified as mild, moderate, or severe according to the grouping criteria. The dominant category of individual criteria determines the total severity for each group at any given time point (Table 3).

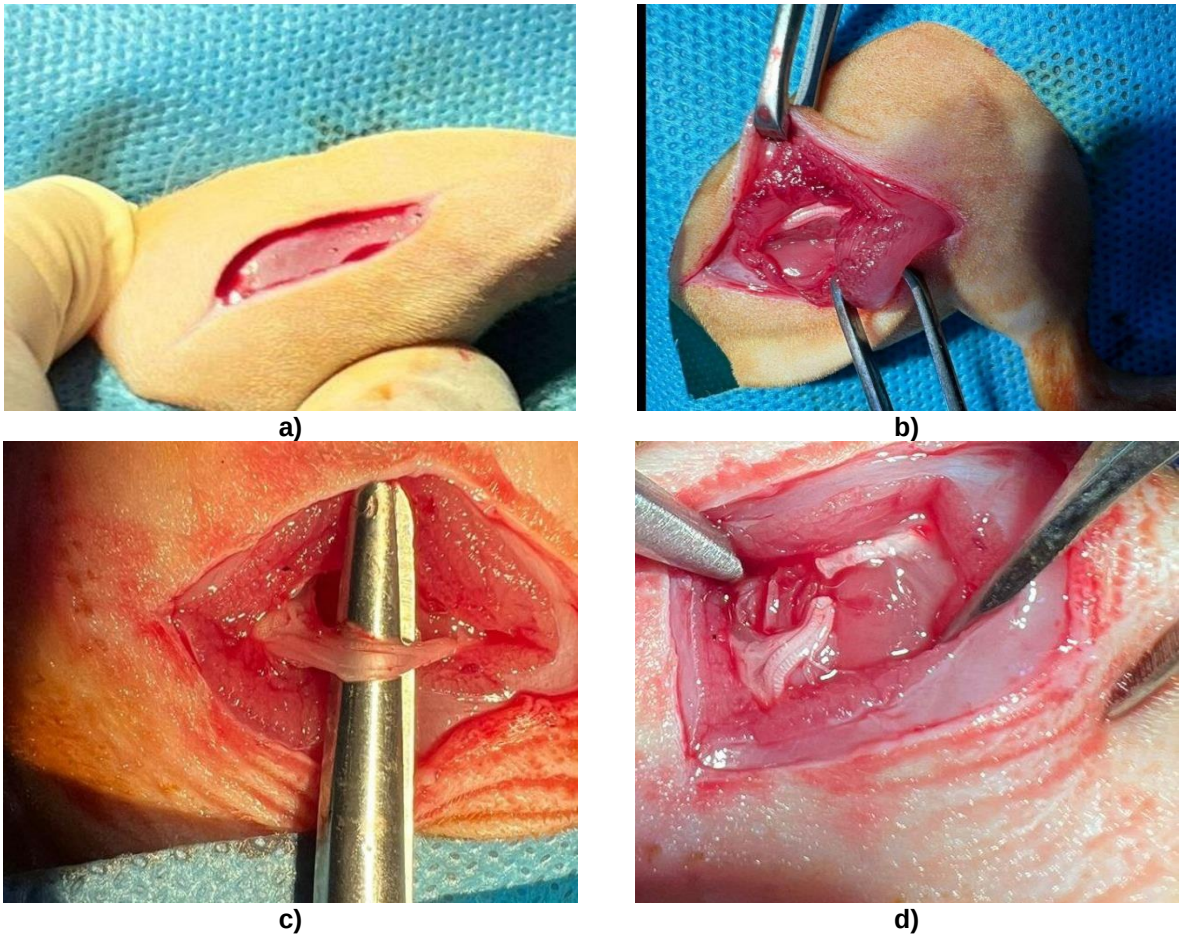

Fig.1 (a-d) showed the surgical site, showed exposure of sciatic nerve, sciatic nerve a complete transect

Table 1. Comparing the groups over time and emphasizing pathological abnormalities in the sciatic nerve using the comprehensive scoring system

Parameter	Time period		
	4 Weeks	8 Weeks	12 Weeks
Positive Control			
Vacuolation	2	3	3
Degeneration of nerve fibers	2	2	3
Edema	2	3	3
Inflammatory Cells	2	2	3
Cell Nuclei abnormality	1	1	2
Congestion of Blood Vessels	2	2	2
Status of Schwann Cells	2	2	2
Condition of Axon Sheath	2	3	3
Presence of Macrophages/Debris	2	3	3
Total	**17**	**21**	**24**

Table 2. List of Nano-treated materials

Nano-treated			
Vacuolation	2	3	1
Degeneration of nerve fibers	2	3	3
Edema	2	2	2
Inflammatory Cells	2	2	2
Cell Nuclei abnormality	1	1	2
Congestion of Blood Vessels	1	1	1
Status of Schwann Cells	1	1	1
Condition of Axon Sheath	1	2	1
Presence of Macrophages/ Debris	1	1	1
Total	**13**	**16**	**13**

0: No lesion visible; 1: Few (<30%) lesions present.; 2: Moderate (30-70%) lesions presence; 3: Many (>70%) lesions visible

3. RESULTS

The results of histopathological Changes: After we performed the euthanasia of all laboratory rats at different period. The description of the histological examination of sciatic nerve slices obtained across a range of time periods (4, 8, and 12 weeks) from distinct experimental groups. These groups include positive control, and Nano-treated. Hematoxylin and Eosin (H&E) staining is used to offer observations at various magnifications for each of these groups. The thorough analysis of every category according to the provided descriptions:

The histopathological observation of Control Group at period of 4 weeks showed Presence of small vacuolated foci with larger multilocular vacuoles containing less intense eosinophilic material and edema (Fig. 2). Furthermore, distributed arrangement of fibers, degenerated nerve fibers, swollen or missing axons, vacuolation, and various degrees of edema (Fig. 2). While, at period of 8 weeks the histopathological changes characterized by edema, infiltration of inflammatory cells, multilocular vacuolation, distorted cell nuclei, extravassated RBCs (Fig. 3). Moreover, Disarrangement of fibers, atrophied nerve fibers, congestion, and edema (Fig. 3). On the other hand, after 12 weeks the microscopical examination of the pathological changes at this group indicated presence of disarrangement of fibers, degenerated cells, expanded axon sheaths harboring debris, normal Schwann cells and axons, severe engorgement of blood vessels, severe degeneration, and 2 necrosis of nerve fibers, inflammatory cells, and congestion of blood vessels (Fig. 4).

The histopathological alterations that has been showed in Nano-treated group after 4 weeks of treatment included observation of almost normal nerve fiber, congestion of blood vessels, thickening of the perineurium layer, few necrotic nerve fibers, edema, and severe infiltration of inflammatory cells (Fig. 4). While, vacuolation, dilated axon sheaths containing debris, congested vessel, infiltration of macrophages, and fragmentation of axons and/or myelin have been recorded in Nano-treated group after 8 weeks (Fig. 5). However, 3 the microscopical changes that were indicated after 12 weeks included normal nerve fibers, vacuolation, and mild congestion of blood vessels (Figs. 6 ,7)

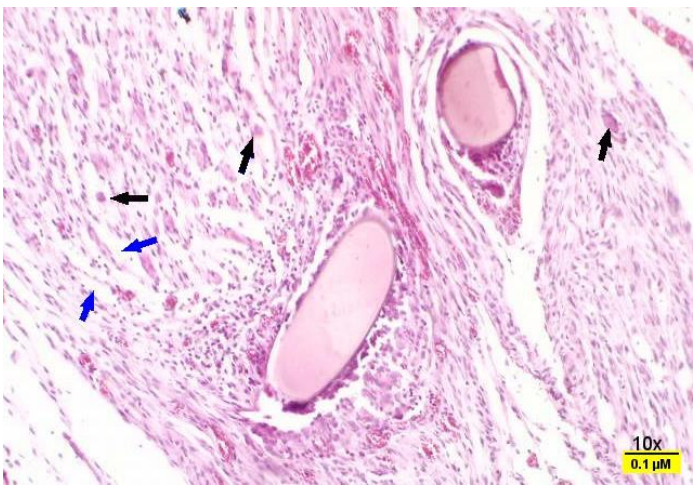

Fig. 2. control-4 weeks. Photomicrographs of sciatic nerve the negative control showed distributed of the arrangement of fibers, degenerated nerve fibers, and they had swollen (black arrows) or missing axons (blue arrows) with various degrees of edema. H&E, 10x

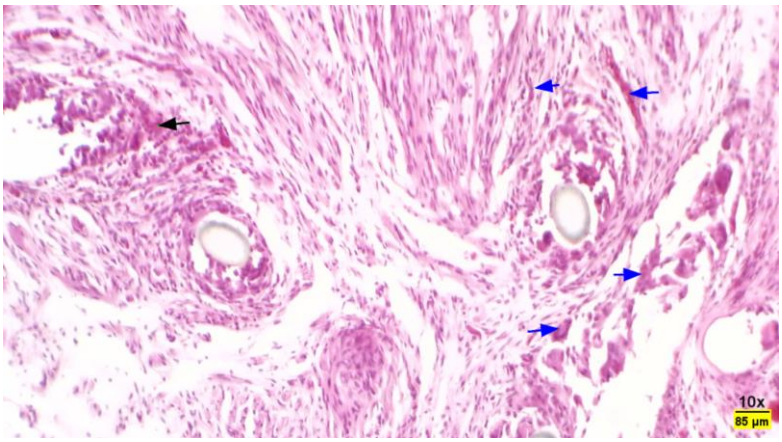

Fig. 3. control- 8 weeks. Photomicrographs of sciatic nerve the ALA group showed severe disarrangement and degeneration of fibers (blue arrows), notable degeneration of Schwan cells, edema, and infiltration of inflammatory cells (black arrow). H&E, 10x

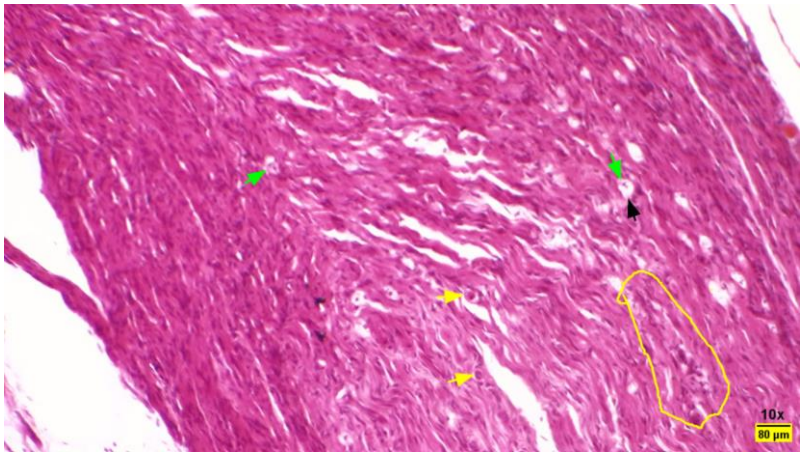

Fig. 4. control-12 weeks. Photomicrographs of sciatic nerve the positive control showed disarrangement of fibers, degenerated cells, with notable observation of expanded axon sheaths (black arrows) harboring debris (green arrows), normal Schwan cells and axons were seen (yellow arrows), also group of inflammatory cell (yellow circle). H&E, 10x

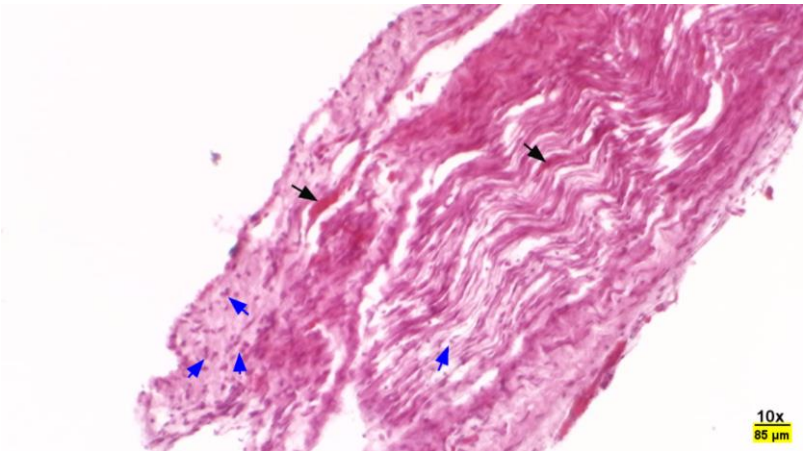

Fig. 5. 4 weeks. Sections from the Sciatic nerves of the nano-treated group revealed several necrotic nerve fiber (black arrows), edema, and severe infiltration of inflammatory cells (blue arrows). H&E, 10x

Table 3. Comparing the groups over time and emphasizing pathological abnormalities in the sciatic nerve using the comprehensive scoring system

Parameter	Time Point									
	Vacuolation	Degeneration	Edema	Inflammatory Cells	Cell Nuclei	Blood Vessels	Schwann Cells	Axon Sheath	Macrophages /Debris	Total Group Score
Positive Control										
4 Weeks	Moderate	Moderate	Mild	Mild	Mild	Mild	Mild	Mild	Mild	Mild/Moderate
8 Weeks	Moderate	Moderate	Moderate	Mild	Mild	Mild	Mild	Moderate	Mild	Moderate
12 Weeks	Moderate	Moderate	Mild	Mild	Mild	Mild	Mild	Moderate	Mild	Mild/Moderate
Nano-treated										
4 Weeks	Mild	Moderate	Moderate	Moderate	Mild	Mild	Mild	Mild	Mild	Mild/Moderate
8 Weeks	Moderate	Moderate	Moderate	Moderate	Moderate	Mild	Mild	Moderate	Moderate	Moderate
12 Weeks	Mild	Moderate	Mild	Mild	Moderate	Mild	Mild	Mild	Mild	Mild

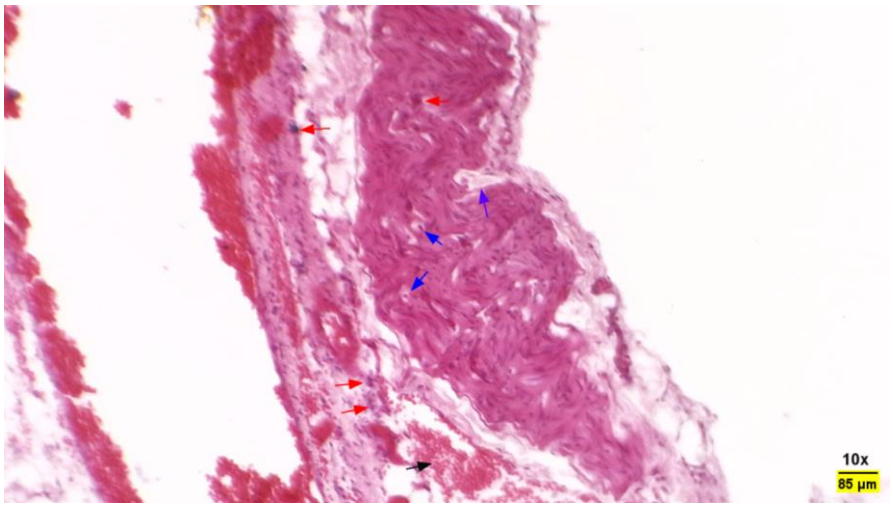

Fig. 6. 8 weeks. Sections of Sciatic nerve of nano-treated group showed vacuolation and dilated axon sheaths (blue arrows) containing debris (fragmented axons and/or myelin), congested vessel (black arrow), and infiltration of macrophages (red arrows). H&E, 10x

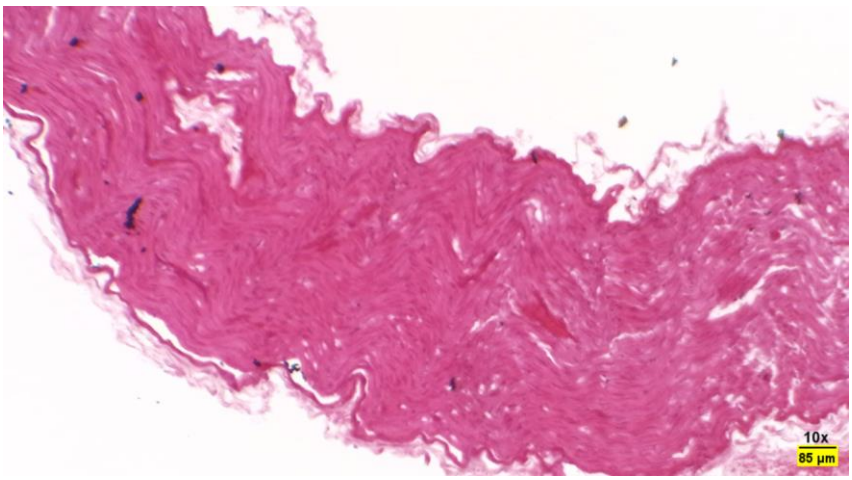

Fig. 7. 12 weeks. Sections from the Sciatic nerves of the nano-treated group revealed almost normal nerve fiber, vacuolation, and congestion of blood vessels. H&E, 10x.

Histopathological scoring index: The positive control group experienced a mild rise in vacuolation from 4 to 8 weeks, followed by a severe increase at 12 weeks. Degeneration occurs progressively over time, showing progressive nerve tissue deterioration. Edema, inflammatory cells, blood vessels, Schwann cell state, axon sheath condition, and the presence of macrophages and debris all had reasonably constant scores throughout time, with modest changes that tend to rise. while The NANO-treated group has moderate scores for degeneration, inflammatory cells, macrophages, and debris for the first four weeks, then these scores rise to the range of moderate to severe changes, indicating severe histopathological changes, and then fall at 12 weeks, indicating some improvement.

The results of this scoring system were consistent with the comprehensive scoring system. The positive control group shows moderate alterations with a higher overall severity than the other groups, In contrast, the nano-treated group exhibits moderate changes at 8 weeks, with some improvement to minor changes by 12 weeks.

4. DISCUSSION

The effects of Nano Cerium on rat sciatic nerve regeneration after transection were assessed in

this study. The findings showed that orally administering 50 mg of Nano Cerium oxide for 7 days is successful in enhancing the regeneration of the sciatic nerve, as confirmed by histopathological evaluation. The sciatic nerve controls movement and feeling in the lower extremities. Damage to the sciatic nerve may lead to secondary muscle wasting, causing different levels of disability. While it is possible for the peripheral nerve to repair itself, the process of regeneration is sluggish. Microsurgical techniques have been improved (Gu, et al., 2011). Damage to peripheral nerves causes Wallerian degeneration downstream of the injury, resulting in the loss of axons and deterioration of the myelin sheath, and retrograde degeneration upstream of the injury, leading to the breakdown of nerve cell bodies (Navarro et al., 2007). The existence of Schwann cells that support growth in the pathways of the distal nerve stumps, along with the regenerative potential of motor and sensory nerves (Gordon, et al., 2009).

Nano cerium oxide has been extensively used in medical settings to aid in the regeneration of nerves. Research has indicated that Schwann cells, which are the main glial cells in the peripheral nervous system, release neurotrophic factors that support the regrowth of the peripheral nerve. Nanoparticles have the ability to promote the growth of Schwann cells in a lab setting. In vivo studies have shown that histological improvements can enhance the regeneration of rat sciatic nerve injury, leading to an improvement in functional recovery (Shi, et al., 2024).

The present study assessed histopathological changes by Nano cerium oxide including the histopathological results, showed that the best response was in second group, then the control group. The control group demonstrates a mixed response with both significant nerve damage and some regenerative signs over time. While there are indications of severe damage particularly at 4 and 8 weeks, then presence of almost normal nerve fibers at 12 weeks suggests partial recovery. This variability suggests that the control group may have some potential benefits, but it is less consistent and effective compared to the Nano-treated group, which shows more consistent and robust signs of nerve regeneration and normal histology. A previous study by (Shi, et al., 2024) Utilized Nano cerium oxide in cases of complete transection of peripheral nerves resulted in noticeable structural alterations, including increased nerve fiber thickness, improved myelin layer regularity, and more distinct Ranvier nodes. Bae et al examined how rat sciatic nerves were affected by low doses of Nano cerium oxide, finding more myelinated axons and fewer degenerated axons in the treated group compared to the untreated control group. Apart from alterations in myelination, an elevated quantity of Schwann cells were also detected following the administration of nanoparticles (Rahimi et al., 2023), Proposing the enhancement of peripheral nerve regeneration by Schwann cells secreting diverse neurotrophic factors. Nanoparticles play a significant role in boosting nerve repair, going beyond just protecting neurons to actively promoting the growth of axons and myelin. In this part, we present the interaction of nanoparticles with the complex processes of guided neurite regeneration and myelin sheath reconstruction.

5. CONCLUSION

The study showed that CONPs can help repair nerve tissue in rats with Sciatic Nerve injury by stimulating macrophage activity. The high antioxidant capacity of CONPs contributes to faster healing in a rat sciatic nerve transection model. Therefore, our results suggest the potential pharmaceutical use of CONPs for regenerating peripheral nerve injuries.

ETHICAL APPROVAL

According to the approval. (Number16/37/2024) through the local committee of the animal care and use at the College of Veterinary Medicine, University of Basrah of Iraq.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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