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Histopathological Study Of Nano Cerium Oxide On Healing Of Corneal Ulcer In Rabbits

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ABSTRACT

Background: injury in the cornea in animals tend to heal delay due to a vascular structure. Therefore, treatments that accelerate the healing of cornea needs to investigated. Object: To enhancing and evaluation of the healing process of cornea injury through clinically, fluorescent dye, and histopathological examination. Materials & methods: twenty-eight adult rabbits were used in this study. Under general anesthesia, done induce cornea injury by NaOH 0.4%. the animals were divided in to three groups: Group (A) Negative control group (4 rabbits), (B) Control group (12 rabbits), (C) Treated group (12 rabbits). The animals were examined clinical during the studied period and histopathological evaluation was performed at 1st, 2nd, and 4th week post operation. Results: in all periods of study, show significant differences in the epithelial regeneration ($P \geq 0.01$) in the Nano treated groups. While stromal edema and inflammation were significantly improved only in the fourth week, where it shows a significant difference than the other periods of the study ($P \leq 0.05$). Conclusion: The Nano cerium oxide enhancing and accelerated the regeneration of cornea injury.

KEYWORDS: *NANOPARTICLES, CERIUM OXIDE, CORNEA ANATOMY.*

INTRODUCTION

The cornea serves as the eye's principal refractive surface and is essential for transferring and focusing light onto the retina. Due to its anterior position, it is susceptible to injuries, infections, and different inflammatory disorders, with corneal ulcer being one of the most serious (Pavesio *et al.*, 1997). A corneal injury is a break in the continuity of the corneal epithelium and may occur for a variety of reasons like trauma, eyelid diseases (distichiasis, entropion, and trichiasis), decreased tear production or exposure secondary to anesthesia, undiagnosed or poorly controlled keratoconjunctivitis sicca, self-trauma, orbital diseases or experimentally induced (Gelatt *et al.*, 2013).

Cerium oxide nanoparticles demonstrate diverse biological characteristics, facilitating their widespread application in biomedical fields. CeO2 nanoparticles have demonstrated the ability to enhance wound healing by alleviating inflammation, decreasing oxidative stress responses, reducing infection risks, and promoting angiogenesis during the healing process. These characteristics make CeO2 nanoparticles an attractive contender for wound healing applications (Walkey *et al.*, 2015; Yaser *et al*., 2025). CeO2 NPs can both promote and inhibit the new blood vessels. (Cheng *et al*., 2021). CeO2 NPs can also induce vasoconstriction, activate thrombin, and facilitate platelet aggregation. (Das *et al.*, 2007.). CeO2 NPs demonstrate excellent antioxidant properties, which aid in alleviating oxidative stress and inflammatory responses, thus promoting the wound healing process. (Cheng *et al*., 2021). CeO2 nanoparticles demonstrate antibacterial properties by impeding wound infections and bacterial proliferation, thereby diminishing infection risks. The primary antibacterial mechanism of CeO2 nanoparticles entails direct interaction with the bacterial membrane. Initially, positively charged nanoparticles cling to the negatively charged membranes of both Gram-negative and Gram-positive bacteria, resulting in their attachment to the bacterial surface (Babenko *et al*., 2012; Yasser *et al*., 2025)

MATERIALS AND METHODS

Animals

Twenty-Eight adult rabbits which are year old and weighing about (1.5±0.5 Kg) were purchased. All animals were maintained at temperature of room and had free access to a diet and drinking water. All of the experimental procedures were performed in accordance with the Basrah University, Veterinary Medicine College guidelines for the welfare of experimental animals. Animal experimentation ethics approval MUCH/AEC/HS/2012/14.

Experimental design

All the rabbits are divided into three groups, each group containing (12) rabbits randomly; Group one ..

(Negative control group) (n=4 rabbits), standard This group serves as an indicator for comparison with all study groups, standard health cornea for comparison with other groups. Group two (Positive control group) (n=12), the animals received distal water topical drops after one-day post-injury daily and persist for seven days. The group three (treated group) (n=12), were the animals treated by Cerium oxide is locally applied at the injury site of cornea daily with the concentration of (10µg/ml) for one week.

preparation of cerium oxide nanoparticle

Nanoparticle cerium oxide were prepared and diluted as following according to (Malyla, 2023):

To prepare cerium oxide nanoparticle at concentration (5µg/ml) Take 0.5mg of cerium oxide nanoparticle were diluted with 100 ml of distal water then mixed well in flask, and then became each (1) ml contain on (5 µg/ml) of cerium oxide nanoparticle. And prepare cerium oxide nanoparticle at concentration (10µg/ml) take (1mg) of cerium oxide nanoparticle were diluted with 100 ml of distal water, and then became each (1) ml contain on (10µg/ml) of cerium oxide nanoparticle, this material was prepared at the Research Center in the College of Veterinary Medicine.

preparation of NaOH

NaOH prepare according the following formula according to (Mane,2011):

100×volume of solution in (ml) /W/V% = weight of solute in (g)

Dissolved (4g) of NaOH in 100 ml of distal water at the research center in the College of Veterinary Medicine and close the container tightly.

Method of inducing of Corneal Injuries

Rabbits were anesthetized by intramuscular injection of 10 mg/kg xylazine and 25 mg/kg ketamine HCl (Hashim, 2014, Hashim et al., 2021 and Akter et al., 2023). The experiment was carried out on the right eye of each animal. The Corneal injury was performed by alkaline burn as described by (Gronkiewicz et al., 2016). A round 10 mm diameter circular filter paper disk soaked in 4% sodium hydroxide (NAOH;4 µl,1mol/l) and applied to cornea of right eye for 20 seconds then the disc was removed and the cornea rinsed with sterile distal water for 60 seconds figure (1).

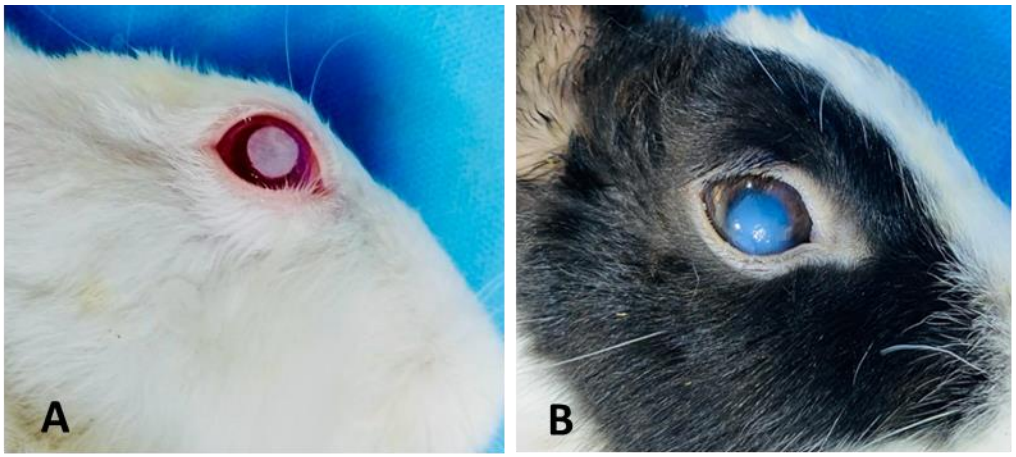


Figure (1): Photographic images, (A) showed induced cornea injury by NaOH. (B) Showed cornea injury and opacity.

HISTOPATHOLOGICAL EVALUATION

Sample of the cornea after the protocol were removed and were processed in automatic tissue processor unit for light microscopy followed by procedures like fixating, dehydrating, embedding, and cutting using microtome (SLEE 9911). These sections were made from wound were stained with hematoxylin-eosin (HE – basic staining) (Jasim et al.,2025).

STATISTICAL ANALYSIS

Standard errors were included in the results. A statistical software package was used to perform One-Way ANOVA with multiple comparison tests on the data (SPSS for windows version 22, USA). The significance level for the differences was set at ($P \leq 0.05$) (Abdulrazak et al., 2018).

RESULTS

In the negative control group, the cornea is within normal appearance. It consists of non-keratinized stratified squamous epithelium of about 5 layers. A thick acellular layer underlies the corneal epithelium called the Bowman's membrane composed of type I collagen fibers. A thick layer of stroma consists of type I collagen fibers interspersed by fibroblasts is located deep to the epithelium consisting most of the corneal thickness. Down to the stroma there is the Descemet's membrane which represents the basement membrane of the corneal epithelium. The posterior surface of the cornea is lined by single layer of simple squamous to simple cuboidal epithelium represents the corneal endothelium, figures (2,3 and 4).

In the one-week post-corneal injury, the Cornea of positive control group, shows complete sloughing of the epithelium with complete exposure of the stroma, score (0). The stroma showed marked edema, score (3) with dis-cohesion of the fibers from each other because of accumulation of edematous fluid, hemorrhage and necrosis of the fibroblasts as well as intensive inflammation in the sub epithelial region, all these changes were seen in the first week after induction of the corneal injury, figures (5-9), table (1). Cerium oxide nanoparticle (10µg/ml) treated group of the same period shows mild degree of epithelial regeneration, score (1) with marked edema in the stroma, score (2); figures (10and 11), table (1). Inflammation was seen in the stroma under the Bowman's membrane in all groups in this period, it shows score (1) for the 10% cerium oxide treated group, table (1).

The two weeks, the following changes were detected in the induction and treated groups; induction group shows no obvious regeneration of the superficial epithelium yet, score (1) in addition to inflammatory infiltration in the stroma score (1), while Descemet's membrane and the endothelial cells were normal, figures (12 and 13), table (2). Mild stromal inflammation and edema were seen in the Cerium oxide nanoparticle (10µg/ml) treated group in this period, score (1), figure (14), table (2). With normal Descemet's membrane and endothelial cells, figures (15).

The fourth week period, shows the following; mild regeneration of the bowman's membrane and superficial epithelium which were about one to two layers in the positive control group, score (3), figures (16 and 17) with marked edema in the stroma, score (2), table (3). Descemet's membrane shows marked disintegration with interrupted areas of endothelial sloughing, figure (16). findings were seen in the Cerium oxide nanoparticle (10µg/ml) treated group there was full thickness restoration of the superficial epithelium which range from 4-5 layers' thickness, score (4) in this period with no evidence of edema or inflammation, score (0), figure (18), table (3). Normal Descemet's membrane and endothelial cells, figures (19).

Regarding induction group, statistical analysis shows that there was significant improvement in regeneration of superficial epithelium in the fourth week ($P \leq 0.05$). Regarding stromal edema and inflammation, this group shows no significant difference in all periods of study ($P > 0.05$), table (4)

All periods of study show significant differences in the epithelial regeneration ($P \geq 0.01$) in the Cerium oxide nanoparticle (10µg/ml) treated group. While stromal edema and inflammation were significantly improved only in the fourth week, where it shows a significant difference than the other periods of the study ($P \leq 0.05$), table (5).

In conclusion Cerium oxide nanoparticle (10µg/ml) treated group shows a superior results regarding improvement of epithelial regeneration, particularly in the fourth week after induction of the corneal injury ($P \leq 0.05$), while there was no significant difference between these groups regarding stromal edema and inflammation ($P > 0.05$), in which the degree of improvement and absence of edema and inflammation was similar, table (3).

Table (1) shows the scoring of the corneal changes in all study groups at the first week

Parameter	Group1		Group2		Group3	
	V	S	V	S	V	S
Epithelial regeneration	100 A	4	0 B	0	20±0.45 C	1
Stromal edema	0 A	0	60± 0.3 B	3	40± 0.5 B	2
Stromal inflammation	0 A	0	27± 1.7 B	2	23± 0.92 B	1

- V: refer to the Value.
- S: refer to the score.
- The values in the table expressed as a mean ± standard error (SE)
- Capital letters refer to the statistical status between the rows of the table; difference in the letters refers to the

statistical differences.

- Group1: refers to negative control group.
- Group 2: refers to induction group.
- Group 3: refers to treated group Cerium oxide nanoparticle (10µg/ml) at1st week.

Table (2) shows the scoring of the corneal changes in all study groups at the second week.

Parameter	Group1		Group 4		Group 5	
	V	S	V	S	V	S
Epithelial regeneration	100 A	4	7 B	1	72±0.11 D	3
Stromal edema	0 A	0	47± 0.22 B	2	28± 0.72 C	2
Stromal inflammation	0 A	0	19± 1.31 A	1	21 ± 0.03 B	1

- V: refer to the Value.
- S: refer to the score.
- The values in the table expressed as a mean ± standard error (SE)
- Capital letters refer to the statistical status between the rows of the table; difference in the letters refers to the statistical differences.
- Group1: refers to negative control group.
- Group 4: refers to induction group in the 2nd week.
- Group 5: refers to treated group Cerium oxide nanoparticle (10µg/ml) in the 2nd week.

Table (3) shows the scoring of the corneal changes in all study groups at the fourth week.

Parameter	Group1		Group 6		Group 7	
	V	S	V	S	V	S
Epithelial regeneration	100 A	4	56± 0.7 B	3	100±0.1 C	4
Stromal edema	0 A	0	40± 0.3 B	2	0± 0.02 A	0
Stromal inflammation	0 A	0	26± 0.97 B	2	0 A	0

V: refer to the Value.

S: refer to the score.

The values in the table expressed as a mean ± standard error (SE)

Capital letters refer to the statistical status between the rows of the table; difference in the letters refers to the statistical differences.

Group1: refers to negative control group.

Group 6: refers to induction group in the 4th week.

Group 7: refers to treated group Cerium oxide nanoparticle (10µg/ml) in the 4thweek.

Table (4) shows the comparison between induction group among the periods of study

Parameter	Group2		Group 4		Group 6	
	V	S	V	S	V	S
Epithelial regeneration	0 A	0	7A	1	56± 0.7 B	3
Stromal edema	60± 0.3 A	3	47± 0.22 A	2	40± 0.3 A	2
Stromal inflammation	27± 1.7 A	2	19± 1.31 A	1	26± 0.97 A	2

V: refer to the Value.

S: refer to the score.

The values in the table expressed as a mean ± standard error (SE)

Capital letters refer to the statistical status between the rows of the table; difference in the letters refers to the statistical differences.

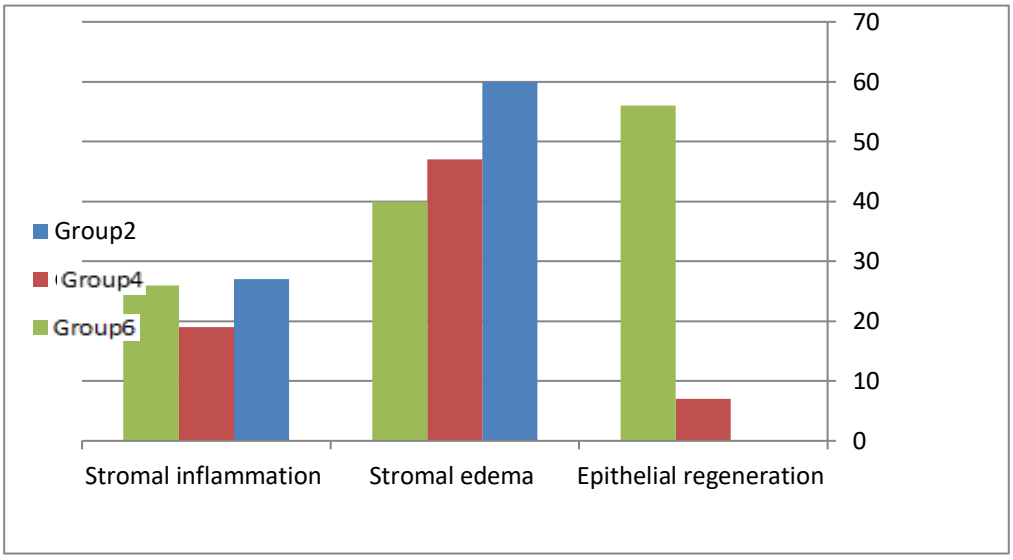


Figure (20) shows the comparison of improvement in the induction group among all periods of study

Table (5) shows the comparison of Cerium oxide nanoparticle (10µg/ml) treated group among the periods of study

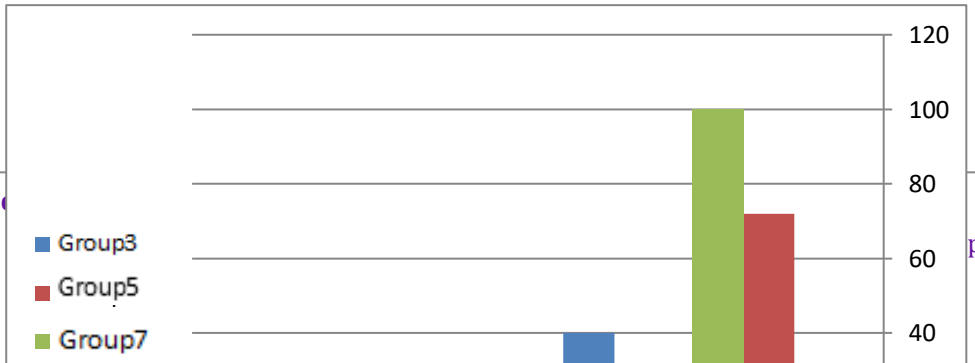
Parameter	Group 3		Group 5		Group 7	
	V	S	V	S	V	S
Epithelial regeneration	20±0.45 A	1	72±0.11 B	3	100±0.1 C	4
Stromal edema	40± 0.5 A	2	28± 0.72 A	2	0± 0.02 B	0
Stromal inflammation	23± 0.92 A	1	21 ± 0.03 A	1	0 B	0

V: refer to the Value.

S: refer to the score.

The values in the table expressed as a mean ± standard error (SE)

Capital letters refer to the statistical status between the rows of the table; difference in the letters refers to the statistical differences



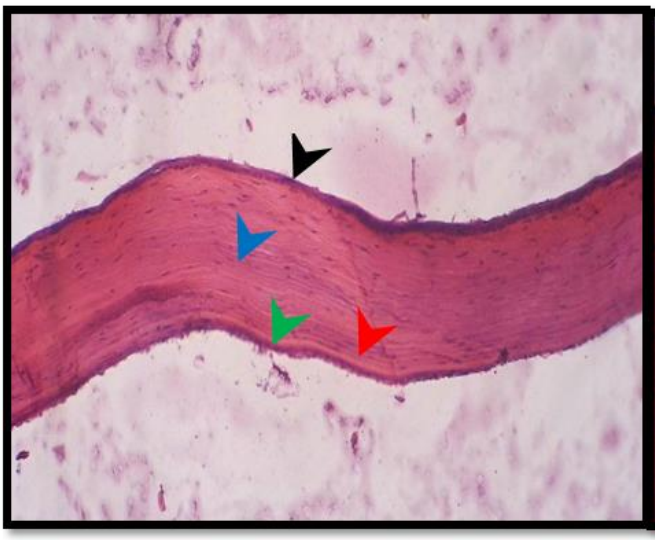


Fig (2) corneal section of negative control group shows normal superficial epithelium and bowman's membrane (blue arrow), normal corneal stroma (blue arrow), normal Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 4X

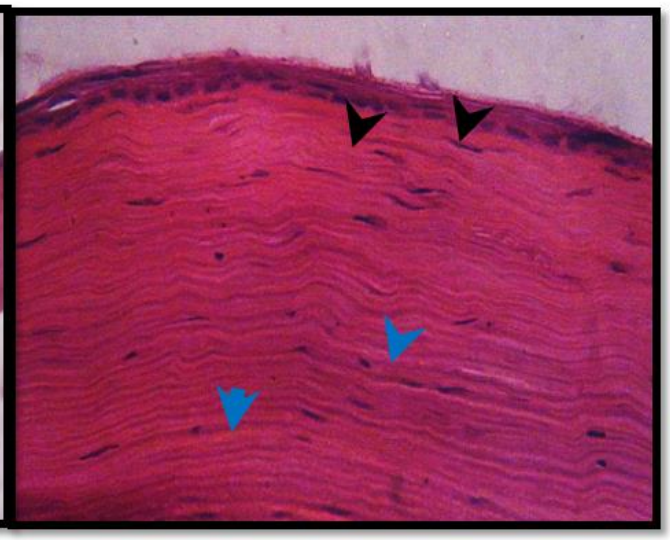


Fig (3) corneal section of negative control group shows normal superficial epithelium and bowman's membrane (black arrow), normal corneal stroma (blue arrow) in the site of induction. H&E 40X

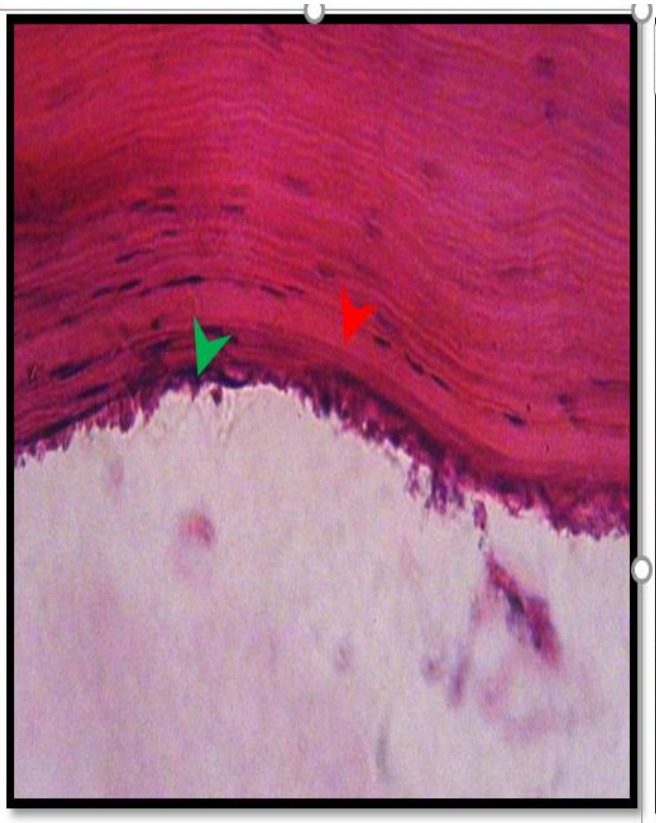


Fig (4) corneal section of negative control group shows normal corneal stroma (blue arrow), normal Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 40X

Fig (5) corneal section of positive control group shows total sloughing of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10 X

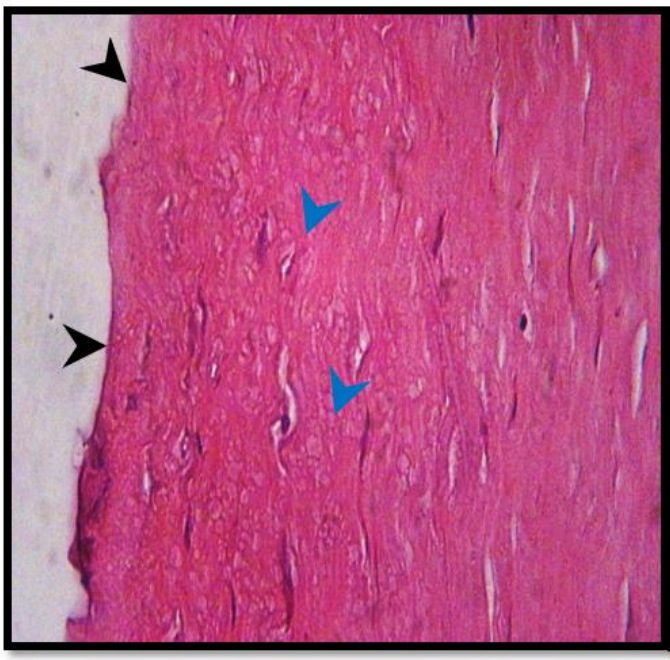


Fig (6) corneal section of positive control group shows total sloughing of the superficial epithelium and the bowman's membrane (black arrow), edema and disintegration of the corneal stroma (blue arrow), in the site of induction. H&E 40 X

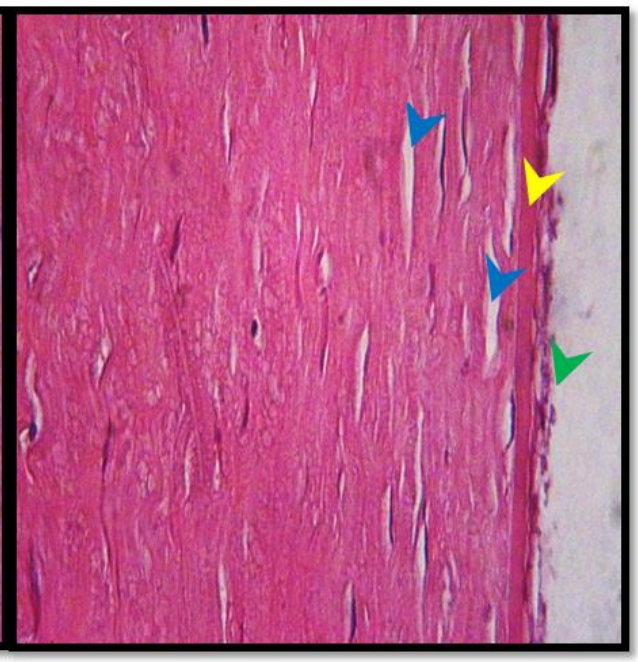


Fig (7) corneal section of positive control group shows edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 40 X

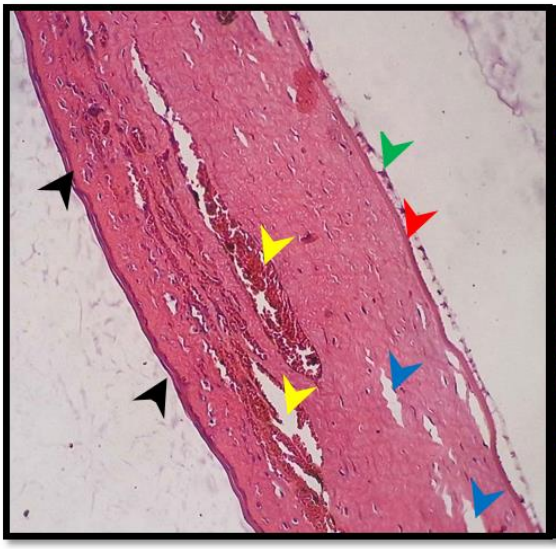


Fig (8) corneal section of positive control group shows normal superficial epithelium and the bowman's membrane (black arrow), edema (blue arrow) and congestion (yellow arrow) of the corneal stroma, intact Descemet's membrane (red arrow) and endothelial cells (green arrow) adjacent to the site of induction. H&E 10 X

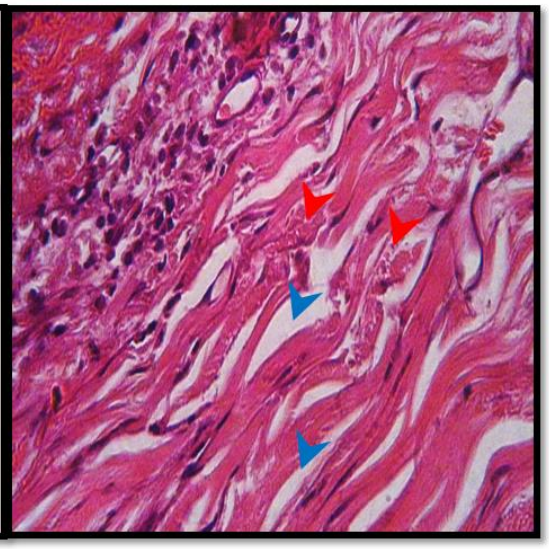


Fig (9) corneal section of positive control group shows inflammation (black arrow) marked edema and disintegration of the corneal stroma (blue arrow), necrotic fibroblasts (red arrow) in the site of induction. H&E 40 X

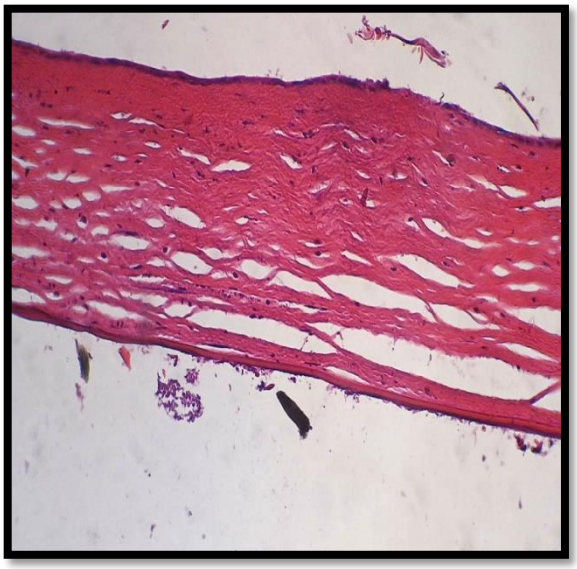


Fig (10) corneal section of Nano-cerium oxide treated group after 1 week shows mild regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10 X

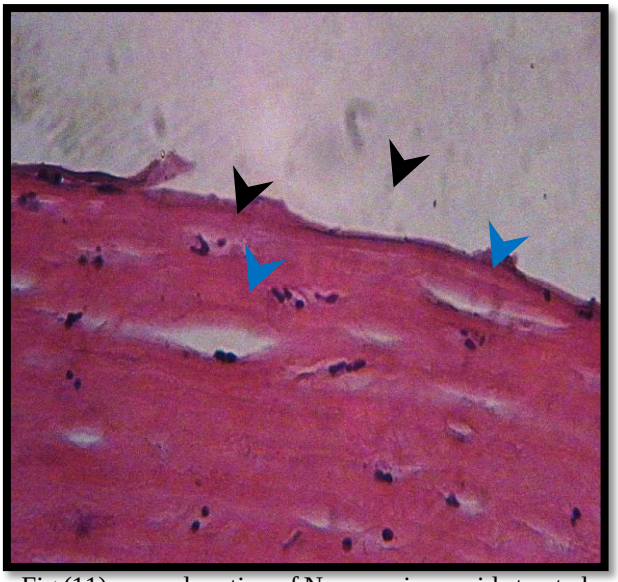


Fig (11) corneal section of Nano-cerium oxide treated group after 1 week shows mild regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow) in the site of induction. H&E 40

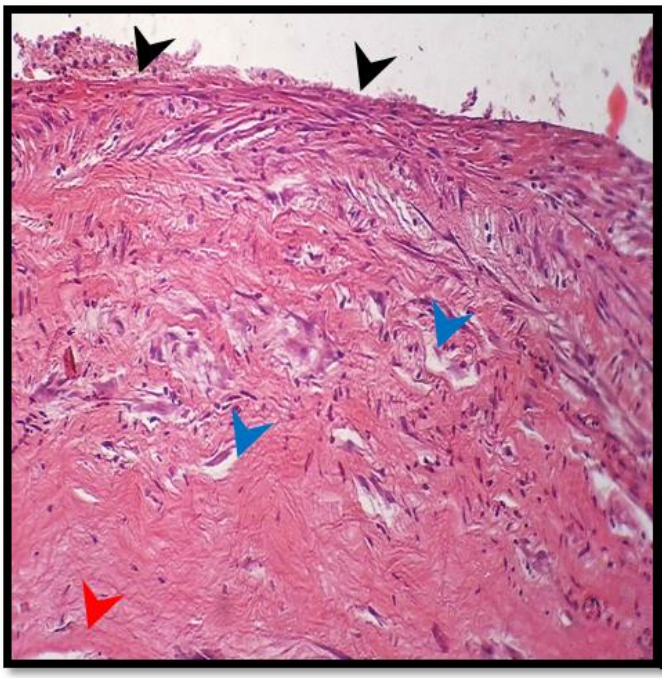


Fig (12) corneal section of positive control group after 2 week shows total sloughing of the superficial epithelium and the bowman's membrane (black arrow), marked inflammation in the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) in the site of induction. H&E 10 X

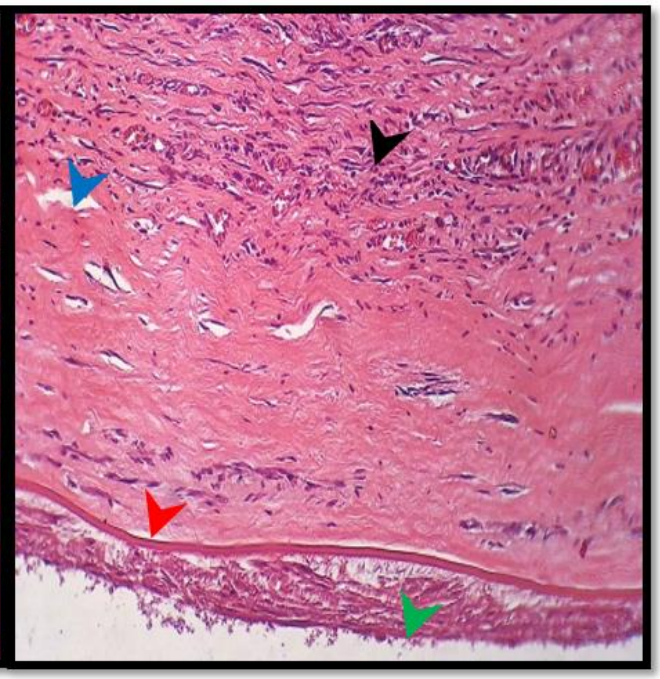


Fig (13) corneal section of positive control group after 2 week shows marked inflammation in the corneal stroma (black arrow), edema in the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10 X

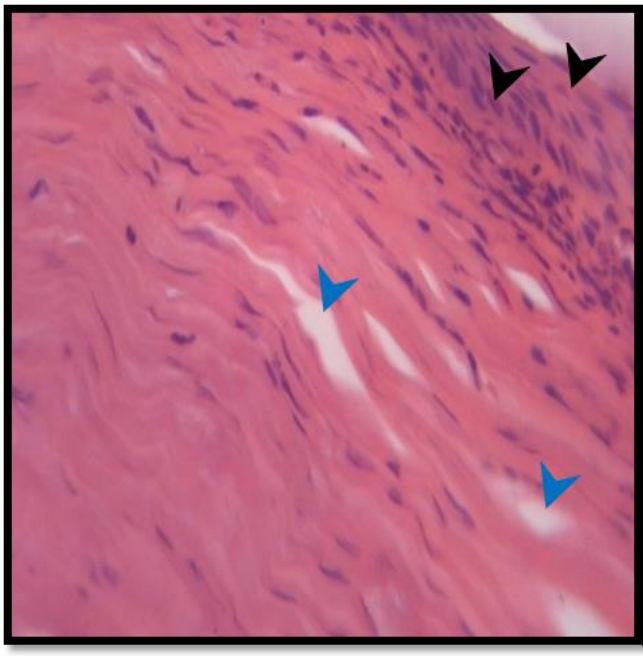


Fig (14) corneal section of Nano-cerium oxide treated group after 2 weeks shows marked regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow). H&E 40 X

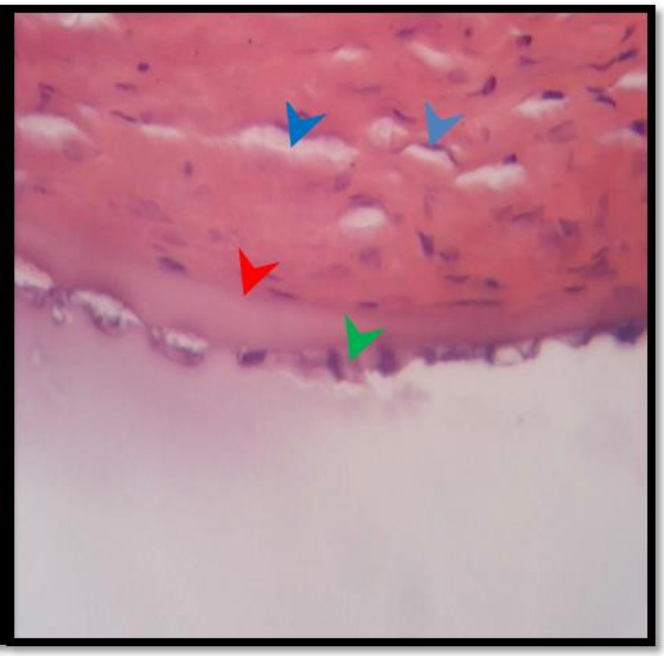


Fig (15) corneal section of Nano-cerium oxide treated group after 2 weeks shows mild edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 40 X

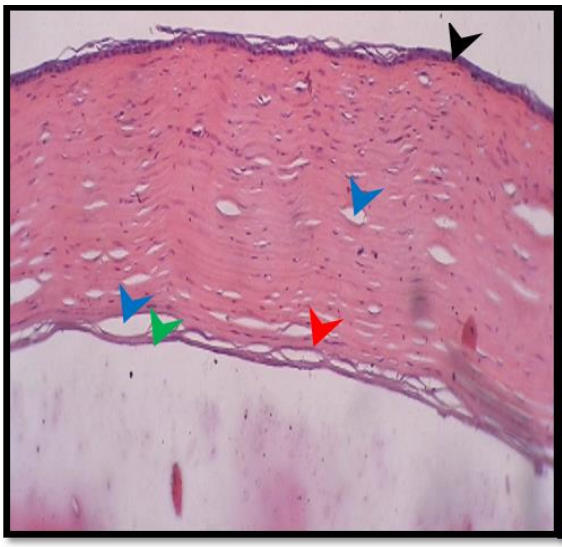


Fig (16) corneal section of positive control group after 4 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), disintegrated Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 4 X

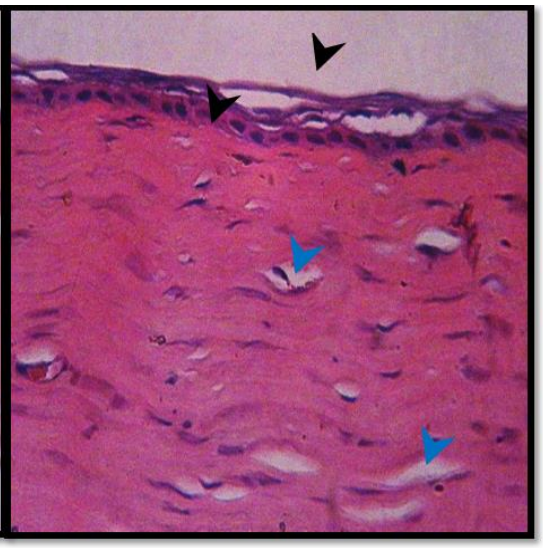


Fig (17) corneal section of positive control group after 4 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), in the site of induction. H&E 40 X

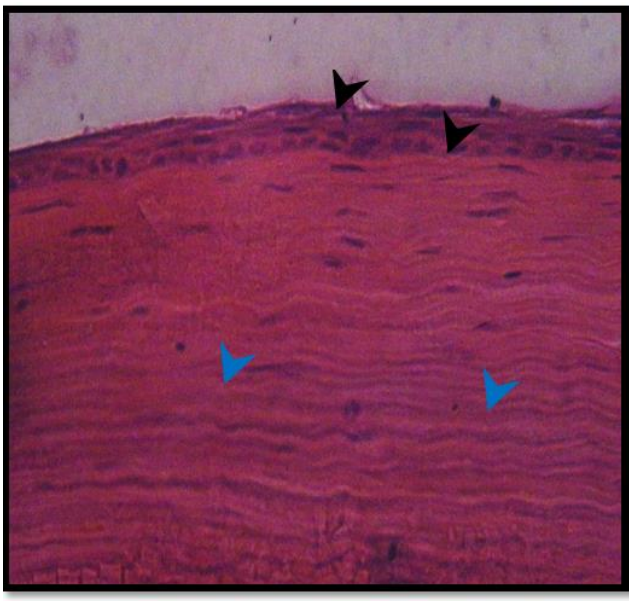


Fig (18) corneal section of Nano-cerium oxide treated group after 4 weeks shows normal superficial epithelium and bowman's membrane (blue arrow), normal corneal stroma (blue arrow) in the site of induction. H&E 40X

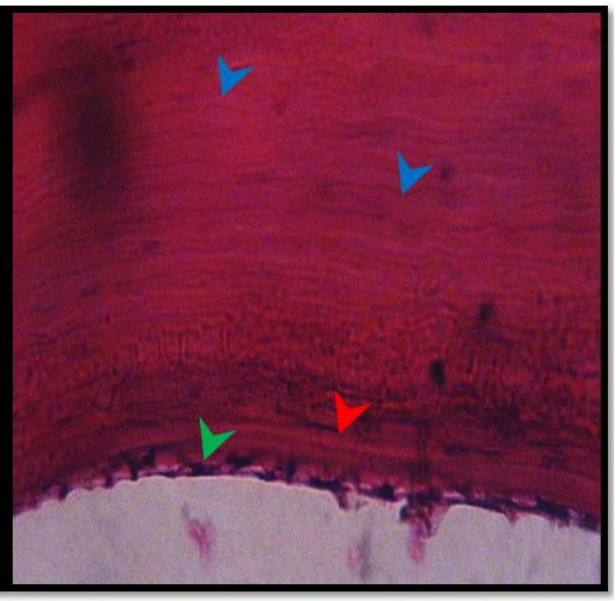


Fig (19) corneal section of Nano-cerium oxide treated group after 4 weeks shows normal corneal stroma (blue arrow), normal Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 40X

DISCUSSIONS

In the present study shown that treated corneal wounds in rabbits heal significantly faster than those in untreated controls. Reduced signs of corneal opacity (haze) and scar formation are also noted, suggesting that cerium oxide nanoparticle may enhance both functional and cosmetic recovery of the corneal surface. In the present study the observations indicated complete regeneration of the superficial epithelium, including 4-5 layers in thickness, within this period, with no signs of oedema or inflammation, and normal Descemet's membrane and endothelial cells. In the present study shown effective corneal wound healing, epithelial cells need to migrate and proliferate to cover the injured area. Cerium oxide nanoparticle may enhance this process by stimulating the regenerative pathways, leading to quicker closure of the wound. The present study results confirmed that Nanoparticles cerium oxide accelerated corneal wound healing on histopathological and statistical levels. Nanoparticles cerium oxide significantly increased thickness of collagen fibers in the stroma with restoration of their regular arrangement. The epithelial thickness had been restored with regular and palisade arrangement of the basal epithelial cells.

Nanoparticles cerium oxide possess the ability to clear reactive oxygen species, suppress inflammation, reduce cytokine levels, and protect cells both in vivo and in vitro. By inhibiting oxidative stress, cerium oxide nanoparticle can reduce apoptosis and the release of inflammatory mediators, thereby lowering inflammation (Corsi *et al*.,2023). Nanoparticles cerium oxide has been found to promote faster epithelial healing compared to control groups. This indicates that nano-CeO2 aids not just in the reduction of oxidative stress and inflammation but also in active tissue regeneration. The integrity of the cornea is crucial for visual acuity (Chen *et al*.,2024).

Nanoparticles cerium oxide can be helping healing of corneal wounds and exploring new interventions to accelerate corneal wound healing represents an important aspect in clinical and experimental eye research. Corneal wounds are very common due to its position as the most anterior part of the eye (Maccarone *et al*.,2019).

The ability of Nanoparticles cerium oxide to accelerate cornea healing that enhance the wound repair by various mechanisms as angiogenesis and inflammation regulation, as well as synthesis of new tissue and enhancement of its remodeling. Nanoparticles cerium oxide didn't only accelerate wound healing on the histopathological or ultra-structural level but also on the clinical level; Nanoparticles cerium oxide drops. This process of angiogenesis helps to deliver nutrients and progenitor cells to the wound

(Allu *et al*.,2023).

CONCLUSIONS

The study showed that nanoparticles cerium oxide can help repair cornea in the rabbits with cornea injury by stimulating keratocytes activity. The high antioxidant capacity of nanoparticles cerium oxide contributes to faster healing in rabbit's cornea injury. There for, our results suggest the potential pharmaceutical use of cerium oxide for heling of the cornea injury.

Ethical Approval

According to the approval, (61/37/2025) through the local committee of the animal care and use at the college of veterinary medicine, university of basrah, Iraq.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declares that NO generative AL technologies such as Large Language Models (ChatGPT, COPILT, ect.) 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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