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Article

A Histopathological Evaluation of the Effects of Cerium Oxide Nanoparticle on Corneal Ulcer Healing 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-four 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 (10 rabbits), (C) Treated group (10 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≥ 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≤ 0.05). **Conclusion:** The Nano cerium oxide enhancing and accelerated the regeneration of cornea injury and enhanced of the corneal wound healing because have anti-inflammatory, antibacterial, and antioxidant properties.

Keywords: Nanoparticles, Cerium Oxide, Rabbits, Cornea anatomy

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1. Introduction

Corneal injuries may arise from foreign bodies, ill-fitting contact lenses, bacterial or viral infections, exposure to bleach, acids, or alkaline substances, as well as ultraviolet radiation from sunlight, tanning beds, or reflective surfaces, potentially resulting in temporary or permanent damage and visual impairment. The cornea functions as the eye's primary refractive surface, crucial for transmitting and concentrating light onto the retina. Owing to its anterior location, it is vulnerable to injuries, infections, and various inflammatory conditions, with corneal ulcer being among the most severe [1].

A corneal injury constitutes a disruption in the continuity of the corneal epithelium and may arise from various factors, including trauma, eyelid disorders (such as distichiasis, entropion, and trichiasis), diminished tear production or exposure due to anesthesia, undiagnosed or inadequately managed keratoconjunctivitis sicca, self-inflicted trauma, orbital pathologies, or experimental induction. Surgical intervention

may be necessary for approximately 30 to 40% of corneal ulcers, contingent upon the severity of the infection at presentation [2].

Cerium oxide nanoparticles exhibit many biological properties, enabling their extensive use in biomedical applications. CeO2 nanoparticles have shown efficacy in improving wound healing by mitigating inflammation, diminishing oxidative stress responses, lowering infection risks, and facilitating angiogenesis throughout the healing process. These attributes render CeO2 nanoparticles a compelling candidate for wound healing applications [3]. CeO2 nanoparticles can both facilitate and impede angiogenesis. [4]. CeO2 nanoparticles can cause vasoconstriction, activate thrombin, and promote platelet aggregation [5].

CeO2 nanoparticles exhibit remarkable antioxidant characteristics that mitigate oxidative stress and inflammatory reactions, hence facilitating the wound healing process. CeO2 nanoparticles have antibacterial capabilities by inhibiting wound infections and bacterial growth, hence reducing infection risks [6]. The principal antibacterial mechanism of CeO2 nanoparticles is direct engagement with the bacterial membrane. Initially, positively charged nanoparticles adhere to the negatively charged membranes of both Gram-negative and Gram-positive bacteria, leading to their adhesion to the bacterial surface [7].

2. Materials and Methods

Animals

Twenty-four adult rabbits, each one-year-old and weighing around (1.5±0.5 Kg), were acquired. All animals were kept at room temperature and had unrestricted access to food and drinking water. All experimental techniques were conducted in compliance with 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 (10) 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=10), the animals received distal water topical drops after one-day post-injury daily and persist for seven days. The group three (treated group) (n=10), were the animals treated by Cerium oxide is locally applied at the injury site of cornea daily with the concentration of (5μg/ml) for one week.

preparation of cerium oxide nanoparticle

Nanoparticle cerium oxide were prepared and diluted as following according to [8]:

To prepare cerium oxide nanoparticles at a concentration of 5 μg/ml, dissolve 0.5 mg of cerium oxide nanoparticles in 100 ml of distilled water, ensuring thorough mixing in a flask. This results in a solution where each 1 ml contains 5 μg/ml of cerium oxide nanoparticles. 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 (Islam *et al.,*2020):

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 via intramuscular injection of 10 mg/kg xylazine and 25 mg/kg ketamine hydrochloride [9]. The experiment was conducted on the right eye of each specimen. The corneal injury was caused by an alkaline burn, as previously described. A circular filter paper disk, 10 mm in diameter, saturated with 4% sodium hydroxide (NaOH; 4 μl, 1 mol/l), was placed to the cornea of the right eye for 20 seconds. Subsequently, the disk was removed, and the cornea was rinsed with sterile distilled water for 60 seconds figure (1).

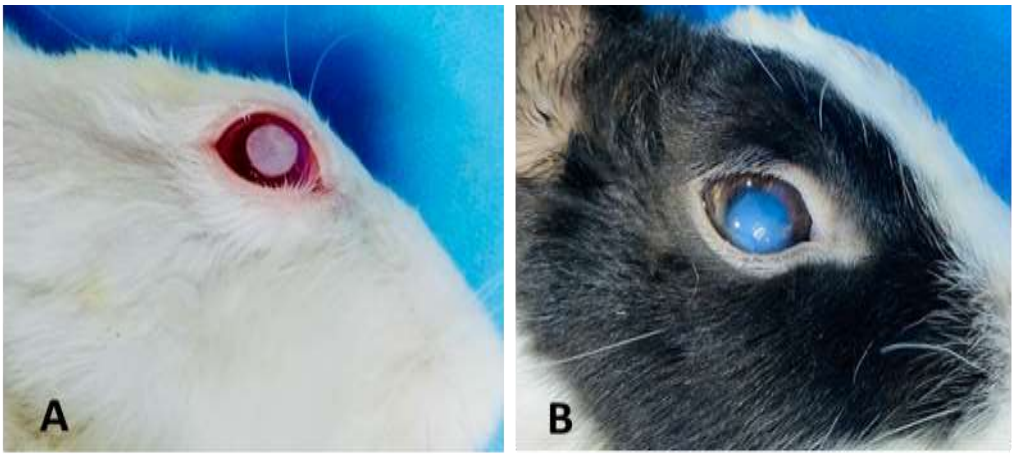

Figure (1): Photographic images, (A) showed induced cornea injury by NaOH. (B) Showed cornea injury and opacity.

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) [10].

Statistical analysis

A Standard error were included in the results. The 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 ($P \leq 0.05$) [11].

3. Results and Discussion

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 and 3).

Cornea of the induction 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 subepithelial region, all these changes were seen in the first week after induction of the corneal injury, figures (4-7), table (1). Mild regeneration of the superficial epithelium, score (1) with marked stromal edema, score (2) was seen in the cerium- oxide (5µg/ml) treated group in this period; figure (8-9), table (1).

In the period of two weeks the following changes were detected in the induction and other 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 (10 and 11), table (2). The cerium- oxide (5µg/ml) treated group shows well regenerated bowman's membrane and superficial epithelium where there were about one to two layers with moderate edema and inflammation in the stroma under the bowman's membrane in the

site of induction, score (3), figures (12 and 13). Descemet's membrane and endothelial cells still normal.

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 (14and 15) with marked edema in the stroma, score (2), table (3). Descemet's membrane shows marked disintegration with interrupted areas of endothelial sloughing. The cerium- oxide (5µg/ml) treated group shows well regenerated bowman's membrane and superficial epithelium, score (4) in which there were about two to three layers with normal looking stroma with no evidence of edema or inflammation, score (0) in the site of induction, figures (16 and 17), table (3). Descemet's membrane and endothelial cells are normal.

Regarding induction group, statistical analysis shows that there was significant improvement in regeneration of superficial epithelium in the fourth week (P≥ 0.05).

Regarding stromal edema and inflammation, this group shows no significant difference in all periods of study (P<0.05), table (4)

Cerium oxide nanoparticle(5µg/ml) treated group shows significant improvement in epithelial regeneration (P≥ 0.01) in all periods of the study. Stromal edema and inflammation were significantly reducing only in the fourth week period were it shows significant differences than the other periods (P≥ 0.05), table (5).

All periods of study show significant differences in the epithelial regeneration (P≥ 0.01) in the 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≥ 0.05), table (3).

In conclusion cerium oxide treated group shows a superior results regarding improvement of epithelial regeneration, then induction group particularly in the fourth week after induction of the corneal injury (P≥ 0.05).

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	100A	4	0 B	0	21± 2.11C	1
Stromal edema	0 A	0	60± 0.3 B	3	43± 1.71 B	2
Stromal inflammation	0 A	0	27± 1.7 B	2	24± 0.6 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.
Group1: refers to negative control group.
Group 2: refers to induction group.
Group 3: refers to cerium oxide treated group.

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

Parameter	Group1		Group4		Group5	
	V	S	V	S	V	S
Epithelial regeneration	100A	4	7 B	1	57± 2.36C	3
Stromal edema	0 A	0	47± 0.22 B	2	41± 2.7 B	2
Stromal inflammation	0 A	0	19± 1.31 A	1	29± 2.88 B	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.
Group1: refers to negative control group.
Group 4: refers to induction group in the 2nd week.
Group 5: refers to cerium oxide treated group in the 2nd week.

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

Parameter	Group1		Group6		Group7	
	V	S	V	S	V	S
Epithelial regeneration	100A	4	56± 0.7 B	3	87± 0.3A	4
Stromal edema	0 A	0	40± 0.3 B	2	0± 0.93 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 cerium oxide treated group in the 4th week.

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

Parameter	Group2		Group4		Group6	
	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.

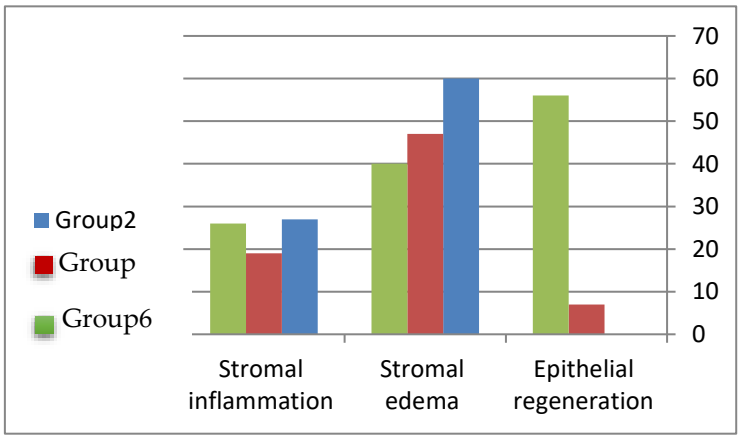

Figure (18) shows the comparison of improvement in the induction group among all periods of study

Table (5) shows the comparison of cerium oxide (5µg/ml) treated group among the periods of study.

Parameter	Group3		Group5		Group7	
	V	S	V	S	V	S
Epithelial regeneration	21± 2.11A	1	57± 2.36B	3	87± 0.3C	4
Stromal edema	43± 1.71 A	2	41± 2.7 A	2	0± 0.93 B	0
Stromal inflammation	24± 0.6 A	0	29± 2.88 A	2	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.

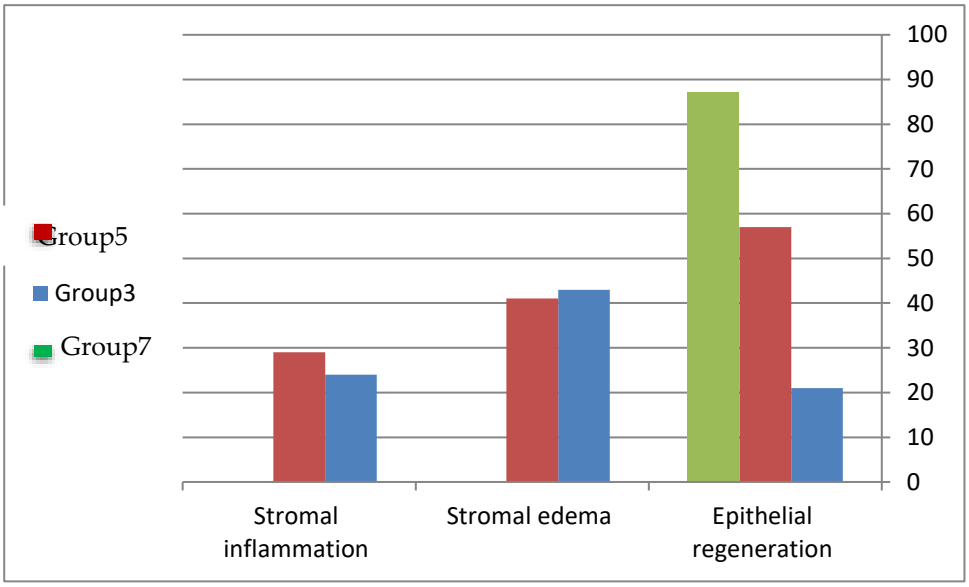

Figure (19) shows the comparison of improvement in the 5% cerium oxide treated group among all periods of study

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

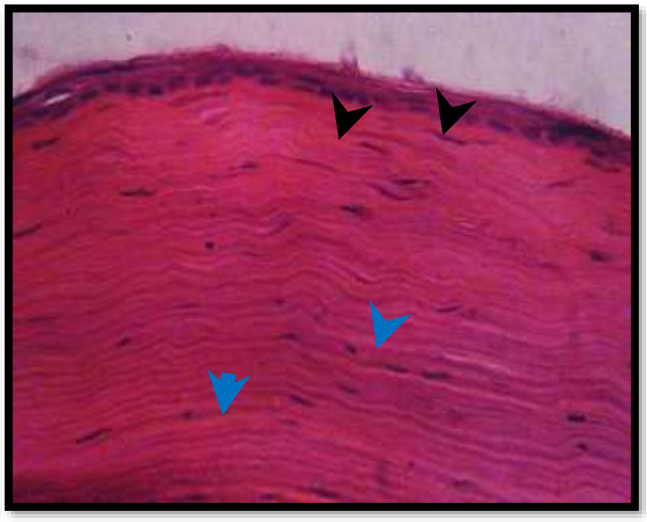

Fig (3) corneal section of negative control group shows normal superficial epithelium and bowman's membrane (black arrow), normal corneal stroma (blue arrow) in the site of induction. H&E 40X

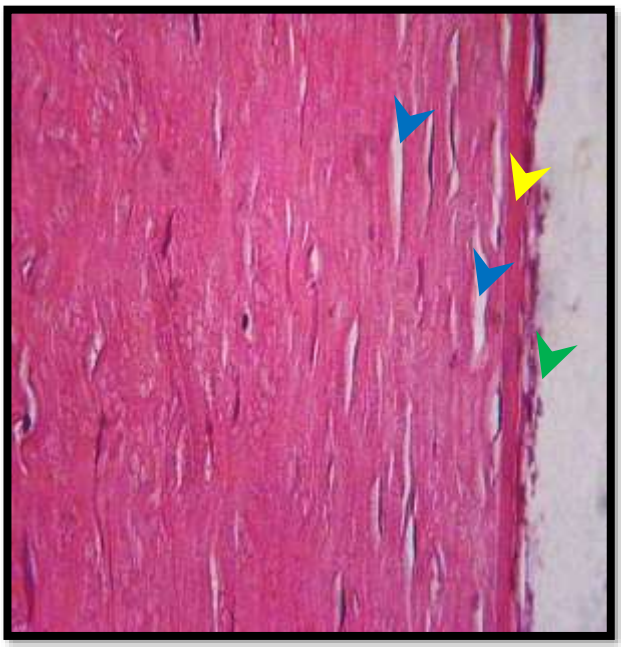

Fig (4) corneal section of positive control group shows edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 40 X

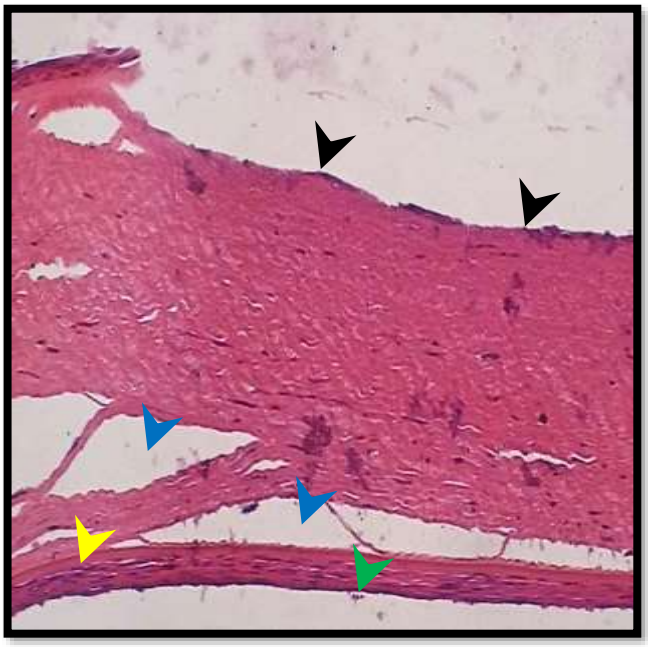

Fig (5) corneal section of positive control group shows total sloughing of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of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

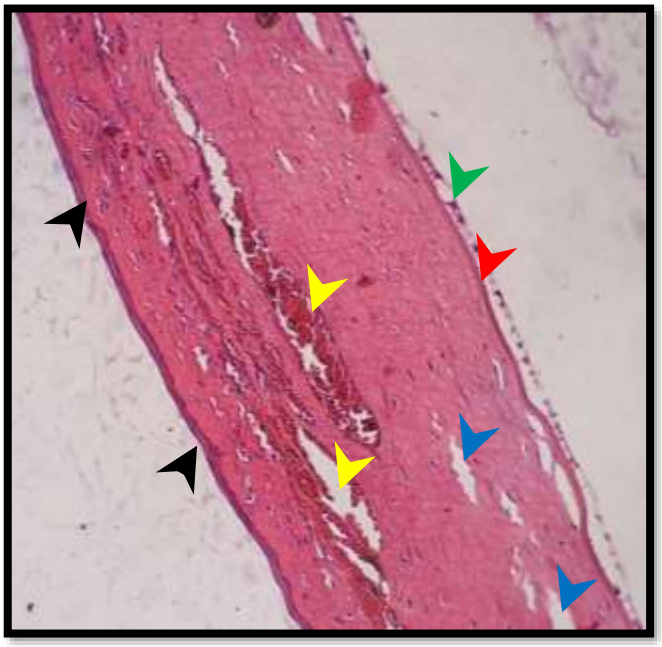

Fig (6) corneal section of positive control group shows normal superficial epithelium and the bowman's membrane (black arrow), edema (blue arrow) and congestion (yellow arrow) of the corneal stroma, intact Descemet's membrane (red arrow) and endothelial cells (green arrow) adjacent to the site of induction. H&E 10 X

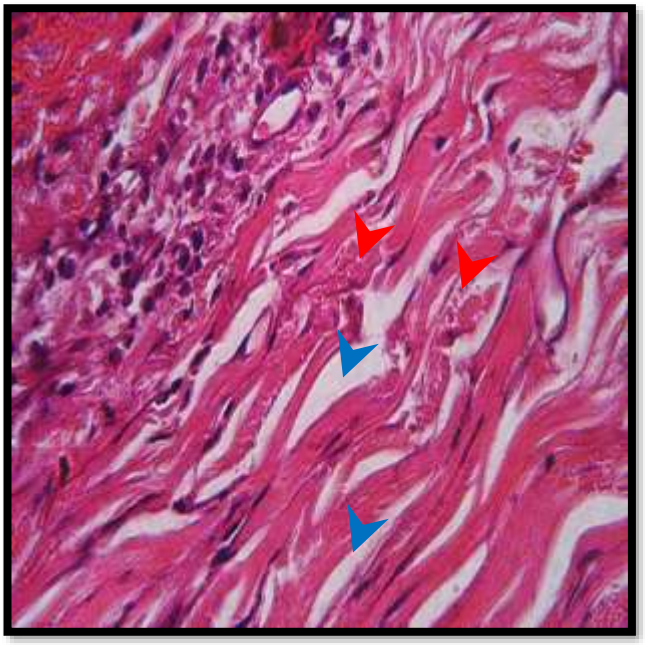

Fig (7) corneal section of positive control group shows inflammation (black arrow) marked edema and disintegration of the corneal stroma (blue arrow), necrotic fibroblasts (red arrow) in the site of induction. H&E 40 X

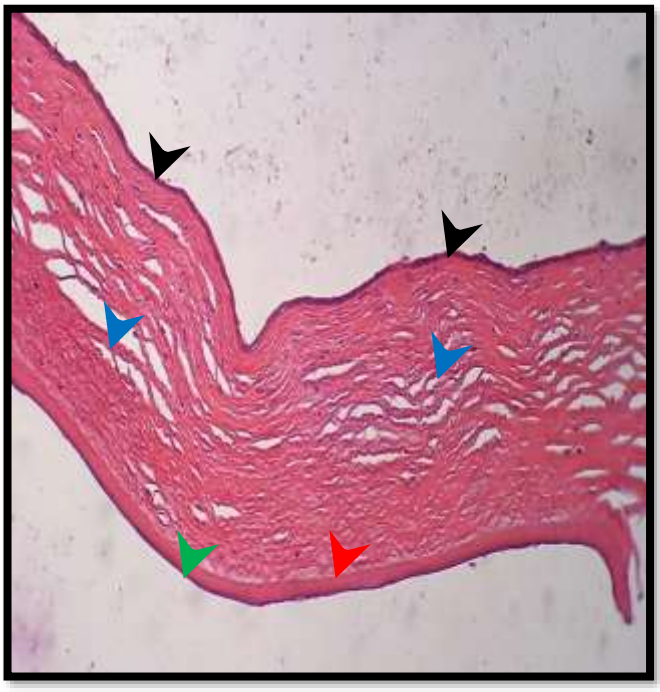

Fig (8) corneal section of 5% Nano-cerium oxide treated group after 1 week shows mild regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10 X

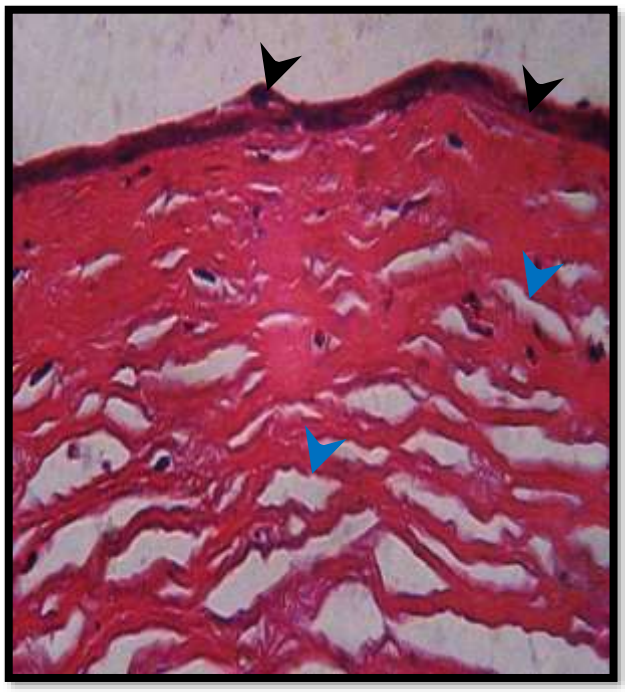

Fig (9) corneal section of 5% Nano-cerium oxide treated group after 1 week shows mild regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), in the site of induction. H&E 40 X

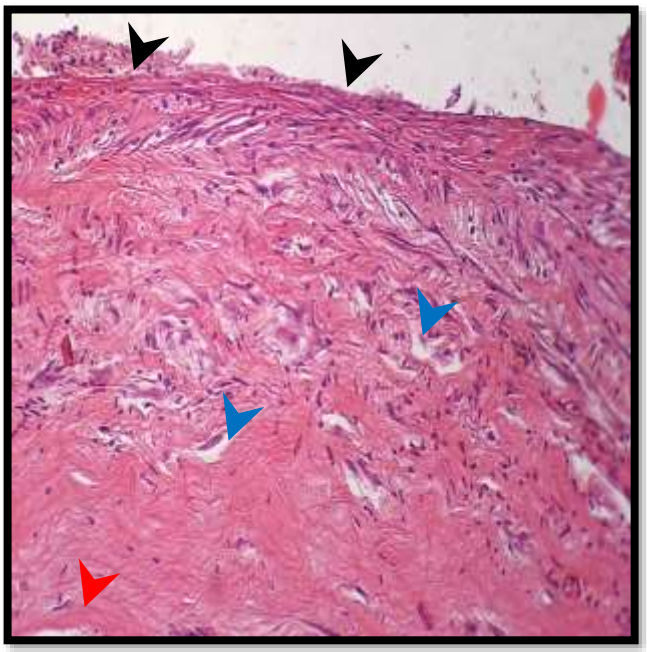

Fig (10) corneal section of positive control group after 2 week shows total sloughing of the superficial epithelium and the bowman's membrane (black arrow), marked inflammation in the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) in the site of induction. H&E 10 X

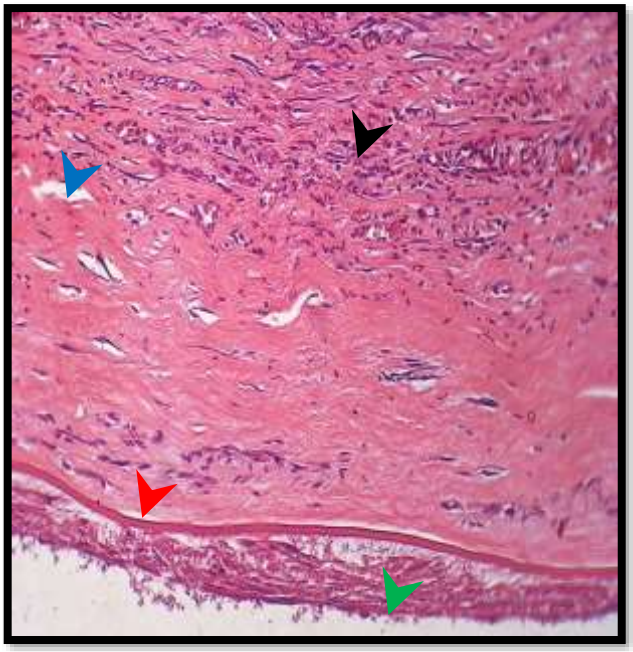

Fig (11) corneal section of positive control group after 2 week shows marked inflammation in the corneal stroma (black arrow), edema in the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10 X

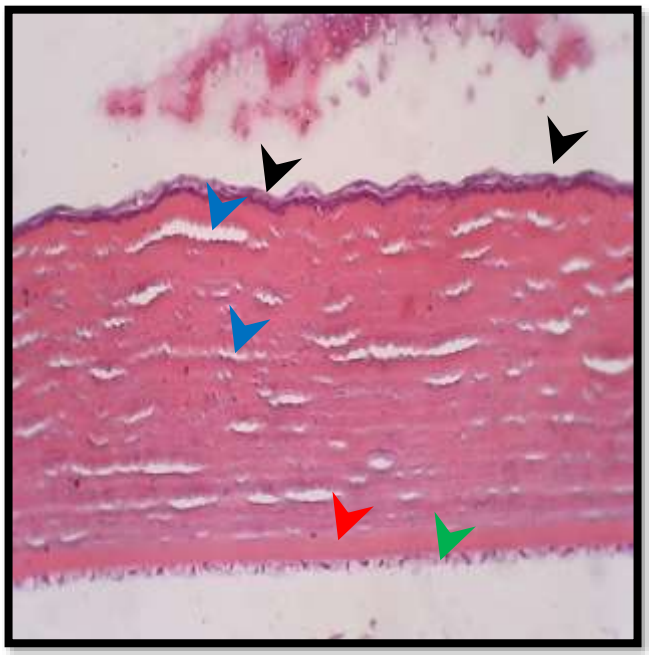

Fig (12) corneal section of 5% Nano-cerium oxide treated group after 2 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), intact Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 4 X

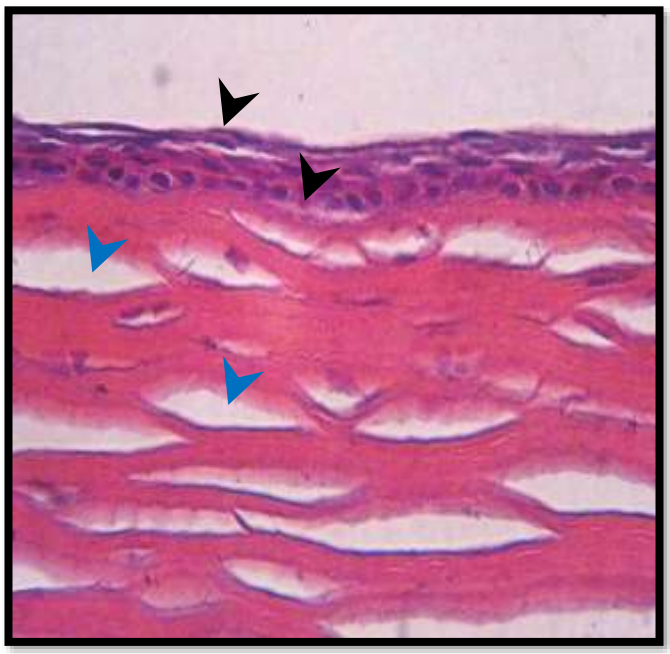

Fig (13) corneal section of 5% Nano-cerium oxide treated group after 2 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), in the site of induction. H&E 40 X

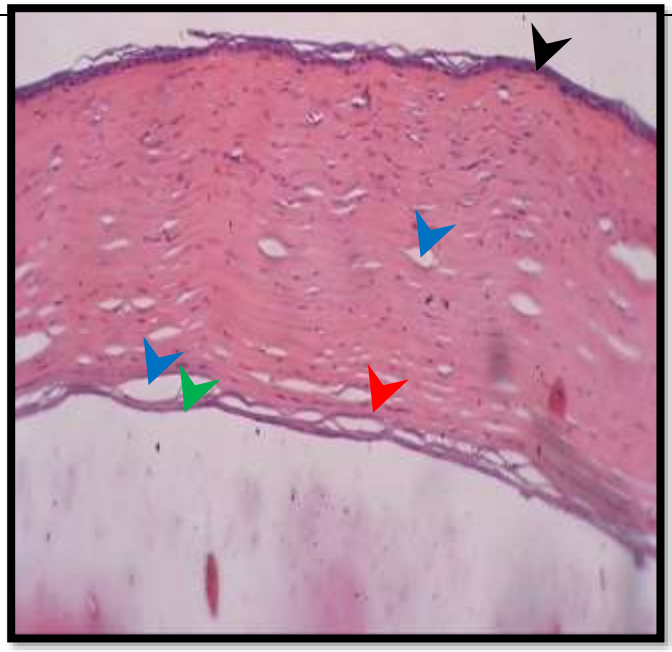

Fig (14) corneal section of positive control group after 4 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), disintegrated Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of

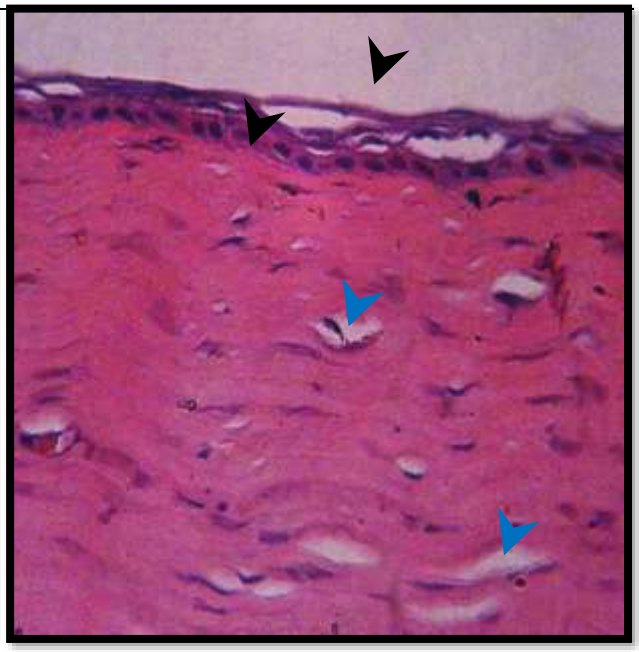

Fig (15) corneal section of positive control group after 4 weeks shows moderate regeneration of the superficial epithelium and the bowman's membrane (black arrow), marked edema and disintegration of the corneal stroma (blue arrow), in the site of induction. H&E 40 X

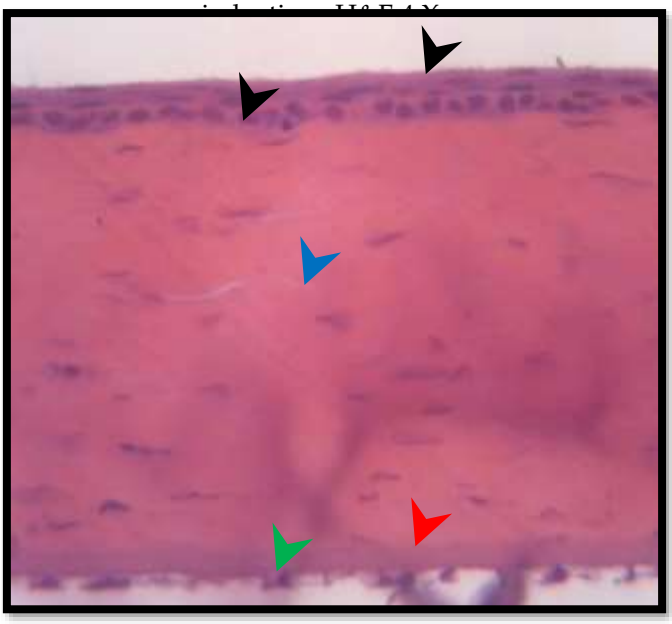

Fig (16) corneal section of 5% Nano-cerium oxide treated group after 4 weeks shows normal superficial epithelium and bowman's membrane (blue arrow), normal corneal stroma (blue arrow), normal Descemet's membrane (red arrow) and endothelial cells (green arrow) in the site of induction. H&E 10X

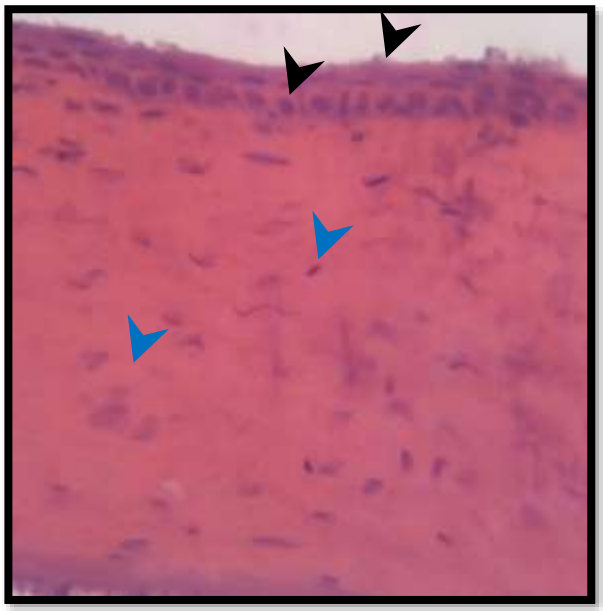

Fig (17) corneal section of 5% Nano-cerium oxide treated group after 4 weeks shows normal superficial epithelium and bowman's membrane (blue arrow), normal corneal stroma (blue arrow), in the site of induction. H&E 40X

Discussions

The present study demonstrates that treated corneal lesions in rabbits heal substantially more rapidly than untreated controls. Decreased indications of corneal opacity (haze) and scar development are seen, indicating that cerium oxide nanoparticles may improve both functional and aesthetic recovery of the ocular surface. The current investigation revealed total regeneration of the superficial epithelium, comprising 4-5 layers in thickness, over this timeframe, with no indications of edema or inflammation, and normal Descemet's membrane and endothelial cells.

The current study demonstrates that efficient corneal wound healing requires epithelial cells to move and proliferate to cover the damaged area. Cerium oxide nanoparticles may expedite this process by activating regeneration pathways, resulting in accelerated wound closure. The current study's findings validated that cerium oxide nanoparticles expedited corneal wound healing both histopathologically and statistically. Nanoparticles of cerium oxide markedly enhanced the thickness of collagen fibers in the stroma, restoring their orderly arrangement. The epithelial thickness was reinstated with a regular and palisade configuration of the basal epithelial cells.

Nanoparticles of cerium oxide can eliminate reactive oxygen species, mitigate inflammation, lower cytokine levels, and safeguard cells in both in vivo and in vitro environments. Cerium oxide nanoparticles can mitigate apoptosis and the release of inflammatory mediators by limiting oxidative stress, therefore reducing inflammation [12]. Nanoparticles of cerium oxide have been shown to enhance the rate of epithelial healing relative to control groups. This suggests that nano-CeO2 contributes not only to the alleviation of oxidative stress and inflammation but also to active tissue regeneration. The integrity of the cornea is essential for visual clarity [13].

Nanoparticles of cerium oxide may facilitate the healing of corneal lesions, and investigating novel therapies to expedite this process is a crucial element in clinical and experimental ophthalmic research. Corneal injuries are prevalent due to its location as the most anterior segment of the eye [14].

The capacity of cerium oxide nanoparticles to expedite corneal healing enhances wound repair through several processes, including angiogenesis, inflammatory management, synthesis of new tissue, and improvement of tissue remodeling. Nanoparticles of cerium oxide not only expedited wound healing at the histological and ultrastructural levels but also at the clinical level; cerium oxide nanoparticles are administered in drop form. The angiogenesis process facilitates the delivery of nutrients and progenitor cells to the wound [15].

4. Conclusion

1 The study demonstrated that cerium oxide nanoparticles can facilitate corneal healing in rabbits with corneal injuries by stimulating keratocyte activity. The elevated antioxidant activity of cerium oxide nanoparticles facilitates expedited healing of corneal injuries in rabbits. Consequently, our findings indicate the potential medicinal application of cerium oxide for the healing of corneal injuries. The elevated antioxidant capacity of cerium oxide nanoparticles facilitates expedited healing in rabbit corneal lesions. Consequently, our findings indicate the prospective medicinal application of cerium oxide nanoparticles for the repair of corneal injuries.

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Conflict of interest: According to the authors of the article, no conflicts related to interests arose during the drafting phase.

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