Effect of Topical application of Stem Cells - Conditioned Medium versus their derived Exosomes in Rat model of corneal Ulcer

Original Article

Manar Fouli Gaber Ibrahim[1], Soha Abdelkawy Abdelwahab[1], Sayed Fouad El-Sheikh Ali[2], Ahmed Sayed Mahmoud Mohamed[1]

Department of [1]Histology and Cell Biology, [2]Anatomy, Faculty of Medicine, Minia, University, Egypt.

ABSTRACT

Background: Corneal ulcers are a serious vision frightening emergency. Even with accurate treatment, patients may suffer complications as perforation, scarring, cataracts, glaucoma, synechiae, and even loss of vision. Stem cells-based treatment is considered as a promising approach in corneal ulcer treatment. Now a days, alternative stem cell conditioned medium (CM) and exosomes (EXs) -based treatment for corneal ulcers are feasible approaches..

Aim: To compare the possible ameliorating effects of stem cells conditioned medium (CM) versus their derived exosomes (EXs) in corneal ulcer healing.

Materials and Methods: 40 rats were randomly allocated into five groups; the control, corneal ulcer, CM-treated, EXs-treated and recovery groups; corneas were obtained for biochemical assessment of TNFα, VEGF, MDA and GSH levels and for histological structure . Corneal damage was assessed by histopathological scoring and immunohistochemical staining.

Results: Corneal ulcer group revealed a significant increase in TNFα, VEGF and MDA levels and also revealed significant decrease in the GSH level. Histological assessment revealed structural alteration indicating severs corneal damage. Moreover a significant increase in P53 expression. Interestingly, CM and EXs administration significantly decreased TNFα, VEGF and MDA levels and increased GSH level. Additionally, there was obvious improvement in histological structure of corneas, and significant decrease in P53 expression.

Conclusion: To the best of our knowledge, this is the first study to investigate CM versus EXs in treatment of corneal ulcer. Both EXs and CM ameliorate the healing of corneal ulcers with having a superior amelioration effect to EXs. The superior effect of EXs could be attributed to their properties as anti-apoptotic, anti-inflammatory, and anti-oxidative.

Key Words: Apoptosis, corneal ulcer, exosomes, TNFα, VEGF.

Revised: 13 January 2023, **Accepted:** 9 February 2023

Corresponding Author: Manar Fouli Gaber Ibrahim, MD, Department of Histology and Cell Biology, Faculty of Medicine, Minia University, Egypt, **Tel.:** 01001226542, **E-mail:** manar.fouli@yahoo.com

ISSN: 2536-9172, June 2021, Vol. 6, No. 1

INTRODUCTION

Cornea is the anterior transparent part of the eye ball covering the pupil, iris and the anterior chamber (**Zhu, Fang *et al*. 2022**). Its shape and transparency are essential for the clear vision (**Nishida, Sugioka *et al*. 2021**).

Corneal ulcer is a serious inflammatory and infective condition involving the disruption of the epithelium with contribution of corneal stroma (**Nishida, Sugioka *et al*. 2021**). It is a medical emergency, and in the recalcitrant cases; it leads to perforation, which is a surgical ophthalmological emergency to prevent the complications that can lead to many serious ocular morbidities (**Stamate, Tătaru *et al*. 2019**).

Corneal ulcer normally heals spontaneously because of the ability of epithelium of the cornea to proliferate; but, healing of the epithelium may be minimized, even with treatments (**Bremond-Gignac, Daruich *et al*. 2019**)

Stem cells-treatment is considered the most hopeful approach in treatment of corneal ulcer (**Casaroli-Marano, Nieto-Nicolau *et al*. 2015**). Mesenchymal stem cells. (MSCs) considered superior to other types of stem cells (**Abdelwahab, Elsebay *et al*. 2021**).

MSCs produce their effects through the secretion of multiple trophic molecules in to their culture medium, nowadays known as secretome (**Teixeira and Salgado 2020**). Secretome contains many factors such as nitric oxide, transforming growth factor, interleukins-6, -10, prostaglandin E2, hepatocyte growth factor, and thrombospondin type 1 (**Prakoeswa, Natallya *et al*. 2018, Gugjoo 2022**).

This study aims to compare between therapeutic effect of EXs and CM in a model of corneal ulcer.

 DOI: 10.21608/jmh.2023.187270.1112

MATERIALS AND METHODS

The current study was carried out-according to the approved-guidelines by animal care-Ethical-Committee in the Faculty of-medicine in the Minia-University for the use of lab. Animals. (Its approval no. (541:12-2022)). 40 adult male albino rats of 8-12 weeks old were used in this study. Their weight range between 130-170 gm. Rats divided into five groups; which are; control, alkali-burn corneal ulcer model, EXs-treated, CM-treated, and recovery groups. Additionally; 10 male albino rats were used to obtain MSCs weighing 70–80 g.

2.1 Isolation of MSCs from bone marrow

Bone marrow (BM) of rats was isolated under complete aseptic conditions. Cultured stem cells were characterized by-anti-CD45 and-anti-CD44 (**Abd El Zaher, El Shawarby *et al.* 2017**).

2.2 CM preparation

CM was prepared according to our protocol (**Abdelwahab, Elsebay *et al.* 2021**)

2.3 EXs preparation

EXs were prepared according to (**Ouyang, Han *et al.* 2018**). Isolated EXs were characterized by transmission-electron-microscopy (TEM). EXs concentrations determined according to (**Bradford 1976**).

2.4 Western blotting EXs characterization

CD63 and CD81 against β-actin were assessed using western blot as previously described (**Kumar, Gupta *et al.* 2015**).

Was done according to the manufacturer instruction. Briefly, homogenized tissues mixed with solution of protease inhibitors was added to the gel buffer in equal ratio. The mixes were boiled for eight minutes. 40µl the samples loaded on SDS-polyacrylamide gel. Proteins were transfered to nitrocellulose membranes. Membranes incubated in 5 % milk protein for three hours to block non-specific immunoglobulin binding. Primary antibody 1: 300 added and kept at room temperature for 2 hours. Bands detected by alkaline-phosphatase kits and developed with a chromogen

2.5 PKH26-labelling-of-EXs

EXS were labeled-for detection of their-homing by Fluorescent-PKH26 dye (**Ibrahim and Allam 2022**).

Corneal-ulcer-model

Corneal ulcers were induced in the-rats by NaOH (ElGomhoria-chemicals company, Cairo, Egypt). Rats were-anesthetized by IM-injection of 0.5mg/ kg ketamine, then a filter paper of 5mm diameter-soaked in 1 M NaOH was put on the middle of-corneas of the right eyes-only for 60 seconds then was rinsed with 30 ml of distilled-water. The left eyes were left without any burn to enable the animals to move, eat and drink (**Bermudez, Sendon-Lago *et al.* 2015**).

2.6 Fluorescein test:

The orange dye of the fluorescein used with the blue light to detect the corneal ulcer by direct application of fluorescein sticks in the outer layer of the eye. Touch the surface of eye by a piece of blotting paper that contains the dye. Apply the blue light directly on the eye, so any corneal defects will be stained by the fluorescein dye and take the green color under the blue light, by using this technique we can determine the occurrence of corneal ulcer (**Jassim, Naeem *et al.* 2020**).

2.7 Study groups

Forty rats were randomly assigned into 5 groups 8 rats in each group as follows:

1. Control group (Group I): The corneas of right eyes were treated only with normal saline.

2. Corneal ulcer group (Group II): the ulcers were induced using alkali burn as described above. Rats were sacrificed a day later for assessing the corneas at acute phase of ulceration (**Abdelwahab, El-Hameed *et al*. 2017**).

3. EXs-treated group (Group III): 1 hour after ulcer induction; each rat was treated topically with exosomes (250µg/100µl PBS) in four divided doses with 24 hours interval (**Samaeekia, Rabiee *et al.* 2018, Ibrahim and Allam 2022, Li, Wang *et al.* 2022**).

4. CM-treated group (Group IV): 1 hour after ulcer induction; each rat was treated topically (one drop) with 0.5 ml CM divided into four doses per day for three days (**Bermudez, Sendon-Lago *et al.* 2015, Park, Heo *et al.* 2020**).

5. Recovery group (Group V): Corneal ulcers were induced in the right-eyes. Rats were left without treatment and sacrified at the-end of the experiment to assess-the spontaneous recovery.

All groups received local antibiotic, as levofloxacin eye drops, administered twice per day throughout the duration of the experiment (**Ebrahim, Mohammed *et al.* 2017**). Rats of group I, III, IV, V were sacrificed 7days after-alkali burn.

At the end of the experimental duration, anesthetize the rats with intramuscular injection of 0.5 mg/kg of ketamine, then the right eyes were carefully enucleated and the excised, corneas were processed for the biochemical, the histological and the immunohistochemical analysis. Samples from 4 rats of each group were used for histological study and samples from 4 rats were used for biochemical study.

2.8 Biochemical studies

Frozen corneas were homogenized for biochemical assessment of the levels of tumor-necrosis-factor-Alpha (TNFα) (Elabscieence,. Cat. No. E-EL-R0019), vascular endothelial growth factor-A (VEGF-A) (Elabscience,. Cat. No. E-ELR2603), malondialdehyde (MDA) (ThermoFisher-Scientific, Cat. No. MA5-27560), and glutathione (GSH) (ThermoFisher-.Scientific, Cat. No. EIAGSHC) (**El-Salam, Faruk *et al.* 2019**).

2.9 Histological studies

a. Light microscopic study

For light microscope studies, samples were fixed in 10% formalin solution and processed for paraffin embedding. Serial sections (5–7 μm) were used to be stained with the hematoxylin and eosin (H&E) stain.

Histopathological scoring

Semi-quantitative scoring for the lesions in the cornea were assessed including the epithelial erosion, vacuolation, and the-stromal pyknotic-nuclei (**Razi, Mosleh *et al.* 2021**).

For immunohistochemical staining, the primary antibody (anti-P53 antibody) (A18803, 1:100; Abclonal, UK) were used according to the manufacture instruction manuals. Briefly, the corneal sections were firstly deparaffinized then rehydrated. Block the activity of endogenous peroxidase by 0.01% hydrogen peroxide at 37 ◦C for 10–15 min. Perform the antigen-retrieval-process in the EDTA buffer for 20 min in a microwave. The sections were then-incubated in primary-antibody (P53) overnight at 4 ◦C. Next, the sections were incubated for 20 min in the secondary antibody HRP (horseradish peroxidase), then, sections were washed out and they were incubated for 15 min with diaminobenzidine (DAB). Finally, these sections were washed-by the PBS solution and counterstained-with a hematoxylin and dehydrated-by ascending-grades-of-alcohol. Then, sections were cleared and mounted. Positive and negative control was done (**Buchwalow and Böcker 2010**). The reaction for P53 is nuclear and cytoplasmic.

b. Fluorescence microscope. For assessing EXs homing using PKH26-labeled.

c. For characterization of Exs in the PBS by Transmission electron microscope.

2.10 Image-capture

EXs labeled with PKH-26 were photographed by fluorescence-microscope. TEM image of Exs was taken by the transmission-electron microscope. Light microscopic images were taken by BX51-microscope and connected to the computer programmed-with software LC--microapplication.

2.11 Morphometric measurements

P53 area percentage was assessed by the image-analysis--software (**Ibrahim and Allam 2022**) in a randomly chosen eight fields from each animal in every group at-magnification ×400.

STATISTICAL ANALYSIS

All the statistical analysis done by the one-way ANOVA method and the post-hoc-test. All statistical analyses were done on IBM personal computer using statistical software "SPSS for Windows" Version 19, and results were expressed as the mean ± standard-error (SE). The value-of $P < 0.05$ was considered-significant.

RESULTS

3.1 Biochemical results

The study revealed a significant ($P < 0.05$)-increase in TNFα, VEGF and MDA levels in corneas in the corneal-ulcer compared-to treated groups. Contrarily, groups treated with EXs or CM revealed a-significant-($P<0.05$) decrease in the previous parameters compared-to corneal ulcer group.

GSH indicated a significant-decrease in corneal tissue in corneal ulcer compared to the other groups. Recovery group showed-insignificant ($P>0.05$) difference-compared to control rats. CM-treated and EXs-treated groups showed-a-significant ($P<0.05$) increase in GSH levels compared to all other groups. Rats treated with EXs approved a significant ($P<0.05$), increase compared to rats with CM. (Figure1).

3.2 characterization of exosomes

I. Western blot results

EXs were strongly positive to CD 81 and CD 63 (Figure 2a).

II. TEM study

Identified EXs isolated from stem cells-CM by ultracentrifugation were determined (Figure 2b).

3.3 Fluorescein labelling of Exs:

The corneas in corneal ulcer group revealed fluorescein dye labeled ulcer (Figure 2c).

3.4 Histological results:

I. Fluorescent microscopic results

Rats treated with-EXs revealed several PKH26-labeled immunofluorescent EXs in corneal tissues (Figure 2d).

II. Light microscopic results

1. Hematoxylin and Eosin (H&E) results

Corneas of control group (Figure 3a) showed stratified squamous-non-keratinized-epithelium-resting on the basal lamina and bowman's-layer that appeared as acellular layer. Stroma containing keratocytes within acidophilic parallel regular collagen lamella were observed. Endothelial-cell-nuclei were seen in a single layer beneath-the Descemet's-membrane. The corneal ulcer group (Figure 3b-e) revealed complete epithelial loss. Epithelial cells hyperplasia was noticed. The anterior stroma showed vascular congestion, inflammatory cells infiltration, disorganized separated collagen fibers, and disorganized keratocytes.

CM-treated group (Figure 4 a, b)revealed apparent increase in thickness of epithelial cells with irregular surface, less separation of collagen, and vascular congestion. EX-treated group (Figure c) showed nearly restoration of normal structure of cornea; stratified squamous-non-keratinized-epithelium-resting-on the basal lamina; bowman's layer; parallel regular lamella of collagen in stroma with keratocytes in between; endothelial cell nuclei; and descemet's membrane. Minimal separation of collagen fibers was noticed. The recovery group (Figure 4d) revealed vacuolated epithelial cells, inflammatory cells infiltration, extravasated RBCs, and widely separated collagen fibers.

Interestingly; corneal ulcer and recovery group revealed a significant ($P<0.05$) increase in epithelial cell erosion, vacuolation, and stromal pyknotic nuclei compared to the other groups. While; treatment with EXs showed a significant ($P<0.05$)-decrease compared to non-treated-or rats treated with CM (Table I, II).

2. Immunohistochemistry for anti-P53 antibody

Negative immune reaction of P53 was appeared in a negative control slide (Figure 5a). Positive immune reaction of P53 in the form of brown cytoplasmic and nuclear reaction appeared in hepatic ischemic reperfusion model (Figure 5b).

Using anti-P53 antibody; the control rats (Figure 6a) revealed negative-cytoplasmic immunoreaction for p53 in corneal epithelial, endothelial cells, and keratocytes. While the corneal-ulcer group (Figure 6b) indicated strong positive-cytoplasmic or nuclear and cytoplasmic expression in corneal-epithelial cells and positive expression in keratocytes and endothelial cells. CM-treated group (Figure 6c) revealed moderate positive-cytoplasmic expression in the epithelial cells and positive-expression in endothelial cells and negative in keratocytes. EX-treated-group (Figure 6d) showed a minimal positive-cytoplasmic expression in corneal epithelial cells and negative-expression in keratocytes and endothelial cells. The recovery group (Figure 6e) showed more-positive cytoplasmic or nuclear and cytoplasmic-expression in corneal epithelial and in endothelial cells. Morphometrical analysis of p53 surface area fraction showed a negative expression in keratocytes and a significant ($P <0.05$) upregulation in the corneal-ulcer and recovery groups compared to all other groups. Contrarily, EXs and CM-treated groups showed a significant decrease in P53 expression compared to corneal ulcer group. Interestingly, P53 immunoreactivity in the EXs-treated group showed a significant decrease compared with the CM-treated group (Figure 6f).

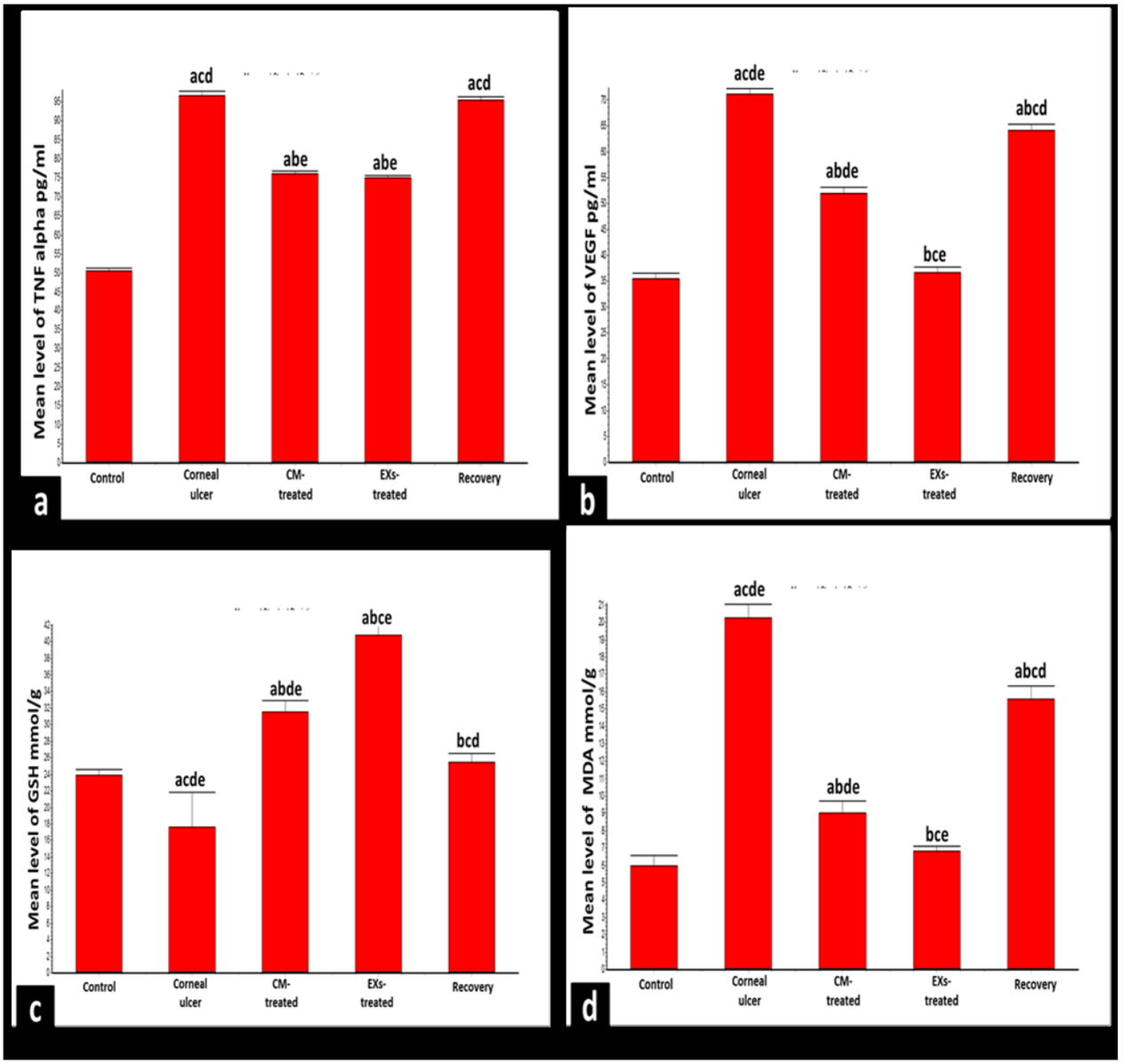

Fig. 1: (a) Mean level of TNF alpha in corneal tissue among studied groups. (b) Mean level of VEGF in corneal tissue. (c) Mean level of GSH in corneal tissue. (d) Mean level of MDA in corneal tissue. Significant:- a versus control group, b versus corneal ulcer group, c versus CM-treated group, d versus EXs-treated group, and e versus recovery group.

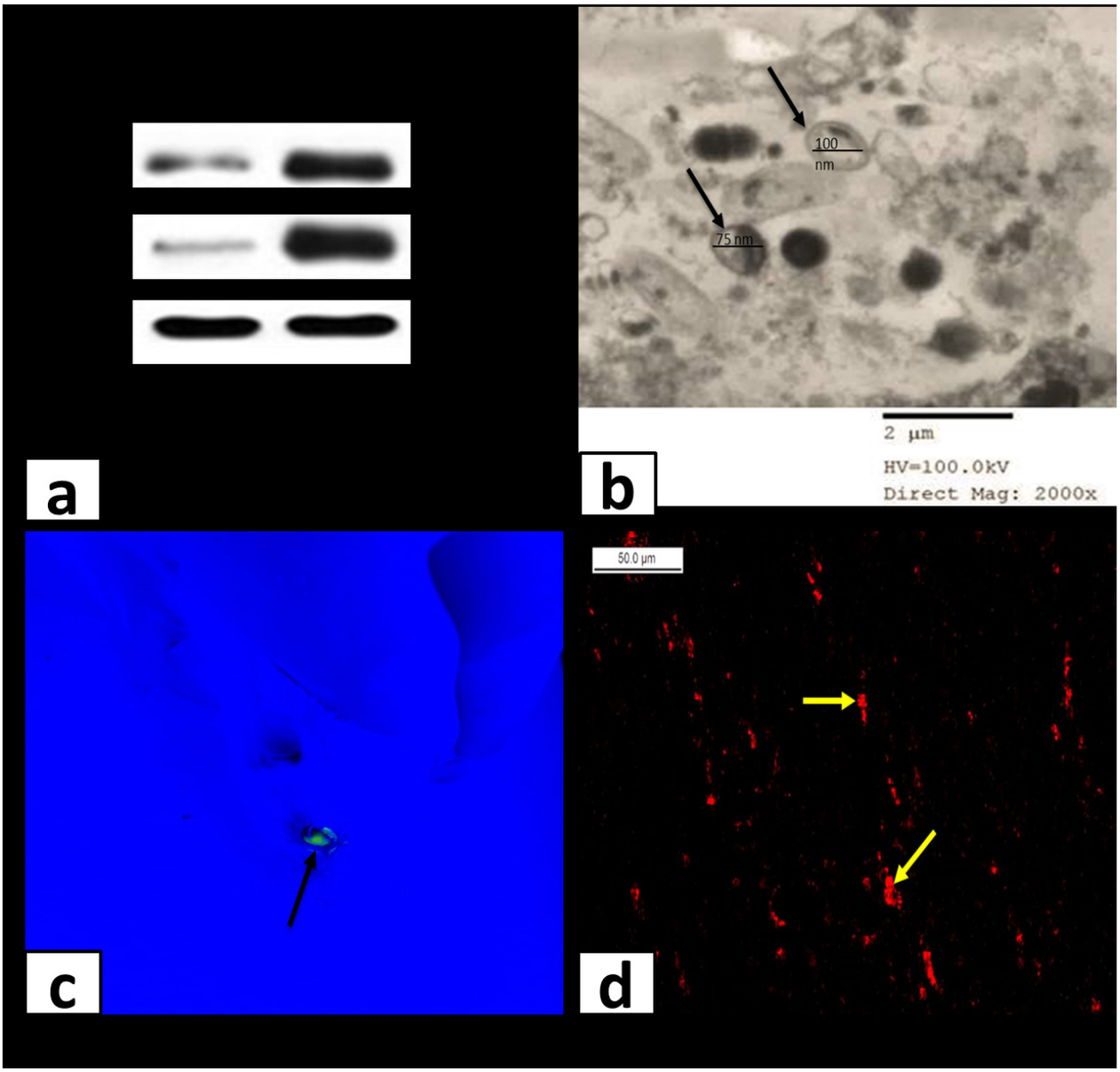

Fig. 2: Representative images of (a) Exosomes characterization by western blot. Exosomes are strongly positive for CD 81 and CD 63. (b) Electron microscopic image of identified exosomes isolated from MSCs-CM using ultracentrifugation (arrows). (TEMX2000). (c) Cornea in corneal ulcer group showing fluorescein dye labelled ulcer (arrow); n = 8. (d) Transverse section in cornea of EXs-treated group showing many Exs-labelled with PKH26 (arrows); n = 8 (FluorescentX1000).

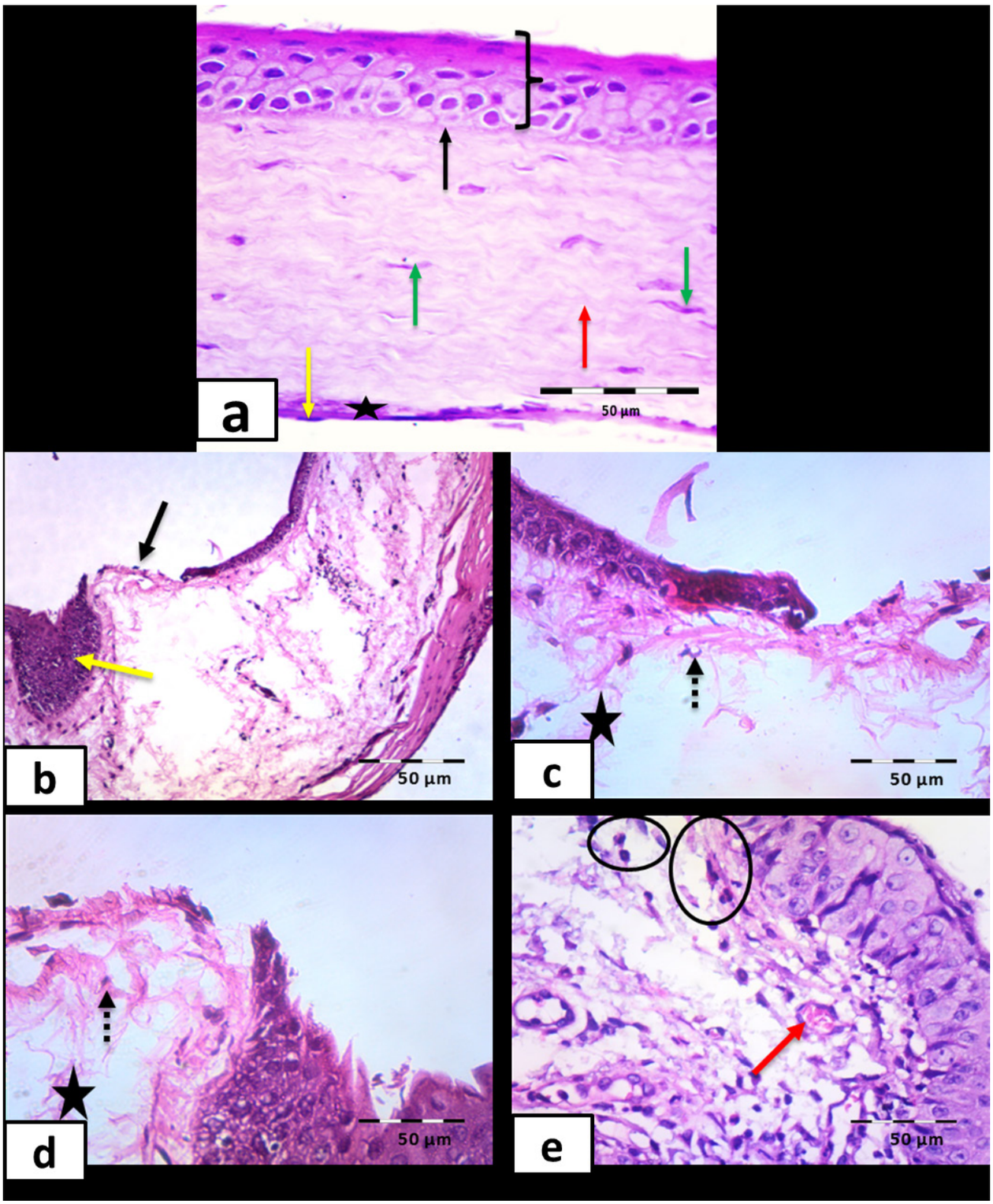

Fig. 3: Representative photomicrographs of sections in the corneas of: (a) control group: showing stratified squamous non keratinized epithelium resting on basal lamina ({). Bowman's layer appears as acellular layer (black arrow). Stroma contains keratocytes (green arrows) within acidophilic parallel regular lamella of collagen (red arrow). Endothelial cell nuclei are seen in a single layer (yellow arrow) beneath the Descemet's membrane (black star). (b, c, d, e) corneal-ulcer group showing complete loss of epithelium (black arrow) with hyperplasia of adjacent area (yellow arrow).The anterior stroma shows vascular congestion (red arrow), and inflammatory cells infiltration (circles). Disorganized separated collagen fibers (stars) and disorganized keratocytes (dotted arrows). (n=8) (H&E x 400, scale bar=50 µm).

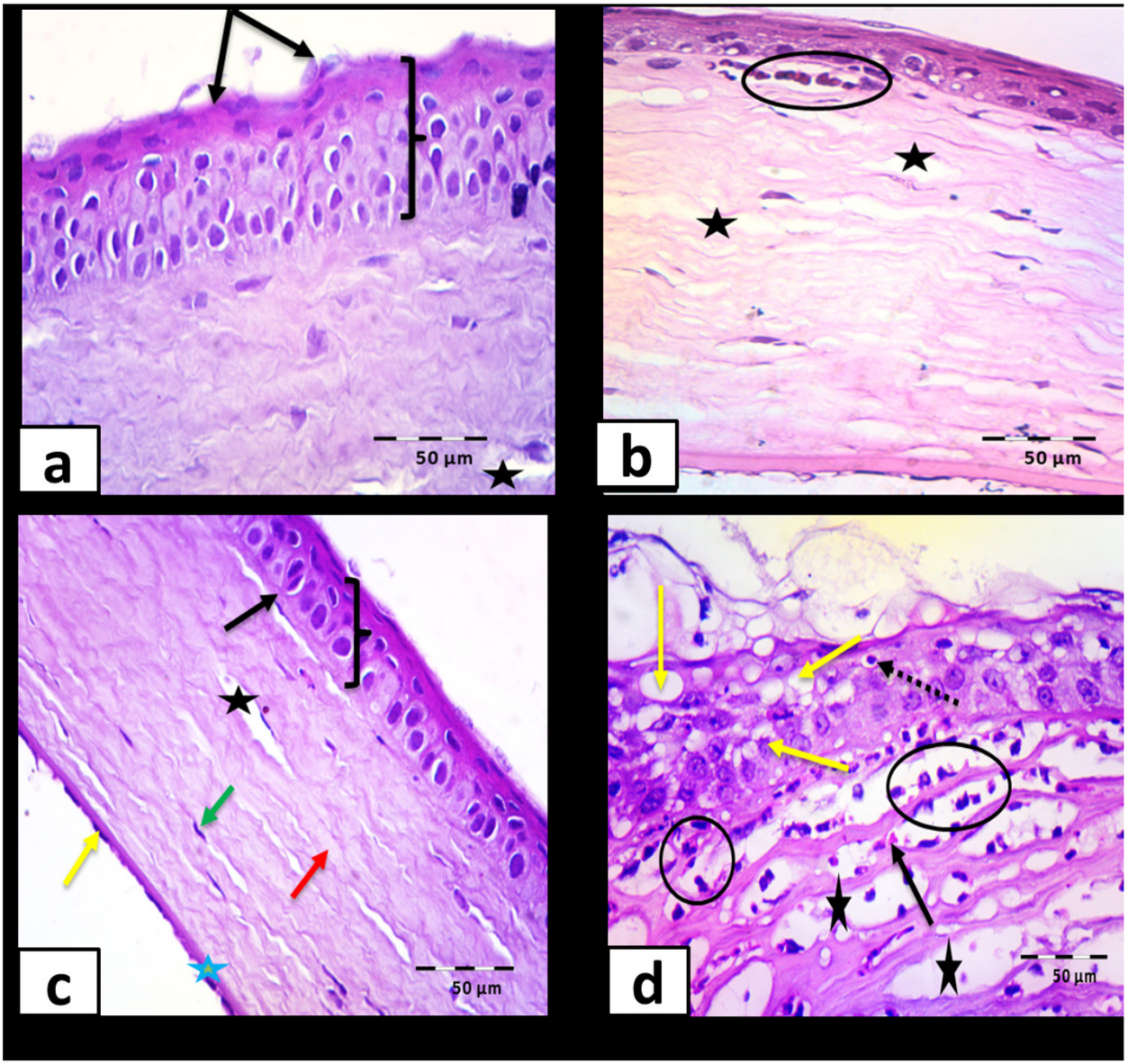

Fig. 4: Representative photomicrographs of sections in the corneas in (a, b) CM-treated group revealing apparent increase in thickness of epithelial cells (}) with irregular surface (black arrows), less separation of collagen (black stars), and vascular congestion (circle). (c) EXs-treated group showing nearly restoration of normal structure of cornea; stratified squamous non keratinized epithelium resting on basal lamina (}), bowman's layer (black arrow), parallel regular lamella of collagen in stroma (red arrow) with keratocytes (green arrow) in between, endothelial cell nuclei (yellow arrow), and descemet's membrane (blue star). Notice presence of minimal separation of collagen (black star). (d) Recovery group showing vacuolated epithelial cells (yellow arrows), cells with darkly stained nucleus (doted arrow), inflammatory cells infiltration (circles), extravasated RBCs (black arrow), and widely separated collagen fibers (stars). (n=8).(H&E x 400, scale bar=50 µm).

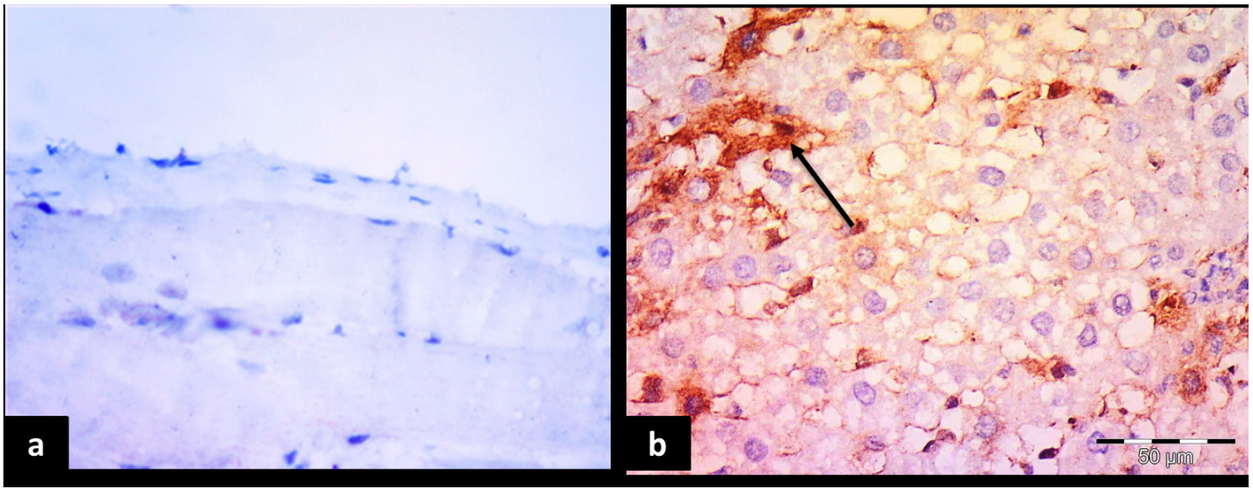

Fig. 5: Representative photomicrographs of (a) a corneal section in an adult male albino rat showing negative immune reaction of P53 in a negative control slide. (b) a section in the liver in ischemic reperfusion model in adult male albino rat showing positive immune reaction of P53 in the form of brown cytoplasmic and nuclear reaction (arrow).

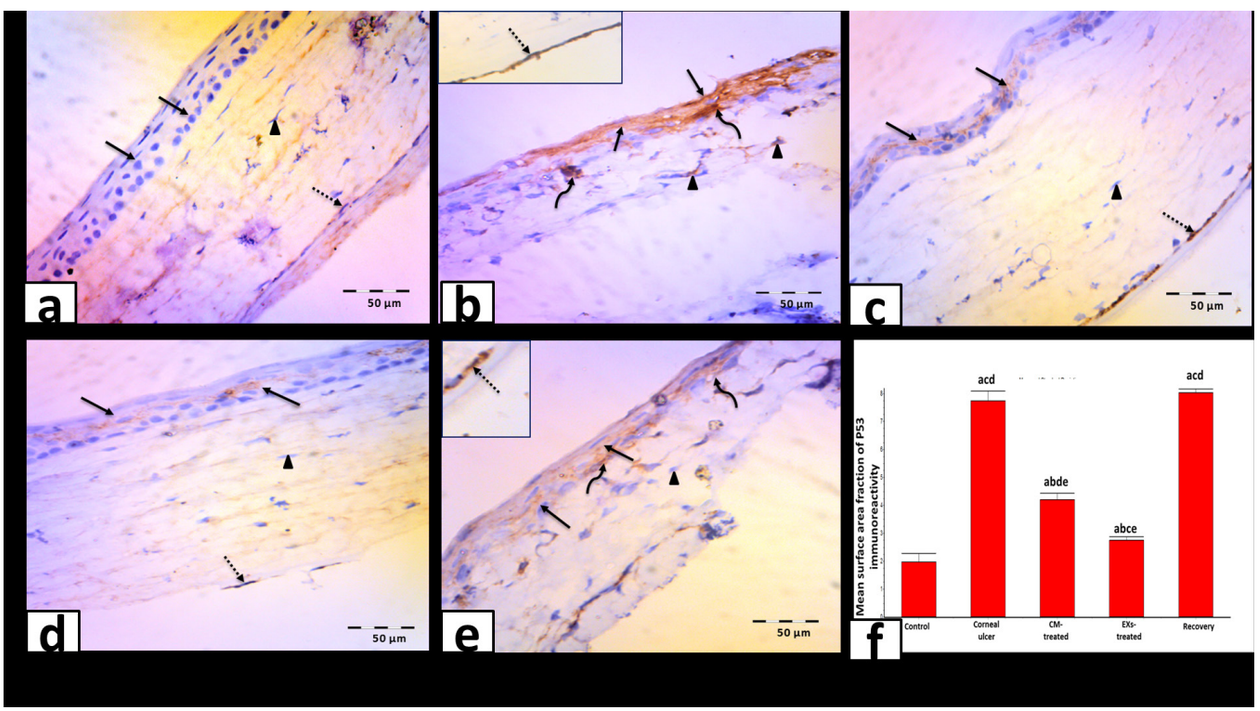

Fig. 6: Representative photomicrographs of corneal sections immunolabelled for P53 in (a) control group showing negative cytoplasmic immunoreaction for p53 in the corneal epithelial cells (arrows), endothelial cells (dotted arrow) and keratocytes (head arrow). (b) corneal-ulcer group: showing strong positive cytoplasmic (arrows) or nuclear and cytoplasmic (curved arrows) expression in corneal epithelial cells. Notice positive expression in keratocytes (arrow heads) and endothelial cells (dotted arrow). (c) CM-treated group: showing moderate positive cytoplasmic (arrows) expression in corneal epithelial cells and positive expression in endothelial cells (dotted arrow). Notice negative expression in keratocytes (head arrow). (d) EXs-treated group: showing minimal positive cytoplasmic (arrows) expression in corneal epithelial cells and negative expression in endothelial cells (dotted arrow) and keratocytes (head arrow). (e) Recovery group: showing more positive cytoplasmic (arrows) or nuclear and cytoplasmic (curved arrows) expression in corneal epithelial cells, Notice positive expression in keratocytes (arrowhead) and endothelial cells (dotted arrow). (n=8) (immunohistochemistry for P53 x 400 , scale bar=50 µm). (f) Mean surface area fraction of P53 immunoreactivity in the studied groups. Significant: . a versus control group, b versus corneal ulcer group, c versus CM-treated group, d versus EXs-treated group, and e versus recovery group.

Table I: Histopathological scoring for epithelial erosion and vacuolation in corneal tissue in the studied groups. (0, 0.5, 1,. 2,. and 3 for-normal; very-slight; slight; moderate; and severe-lesions).

Epithelial erosion and vacuolation						
score	0	0.5	1	2	3	mean
Control group	7	1	0	0	0	0.13^{bcde}
Corneal ulcer group	0	0	1	4	3	2.25^{acde}
CM-treated group	0	5	3	0	0	0.69^{abde}
EXs-treated group	2	5	1	0	0	0.44^{abce}
Recovery group	0	0	1	6	1	2^{abcd}

n=8, $P < 0.05$ is significant. a versus control group, b versus corneal ulcer group, c versus CM-treated group, d versus EXs-treated group, and e versus recovery group.

Table II: Histopathological scoring pyknotic nuclei in stroma in corneal tissue in the studied groups. (0, 1,. and 2 for normal; slight; and moderate-lesions).

score	0	1	2	mean
Control group	8	0	0	0.00^{bcde}
Corneal ulcer group	0	2	6	1.75^{acde}
CM-treated group	4	4	0	0.5^{abde}
EXs-treated group	6	2	0	0.25^{abce}
Recovery group	0	4	4	1.5^{abcd}

n=8, $P < 0.05$ is significant. . a versus control group, b versus corneal ulcer group, c versus CM-treated group, d versus EXs-treated group, and e versus recovery group.

DISCUSSION

Corneal ulcer represents a frequently occurring complication among the corneal diseases. It is the second cause of the ocular morbidity around the world (**Gross, Breitenbach *et al.* 2021**). Ocular alkali burn is a vision threatening emergency which requires adequate management. they rarely spontaneously heal and may cause neovascularization, ulceration, extensive scar formation, and subsequently visual impairment (**Wan, Zhang *et al.* 2020**).

The aim of the current study is to investigate the therapeutic effects of MSCs-CM and MSCs- derived EXs in a model of alkali-induced corneal ulcer.

The current study revealed a complete epithelial loss, vascular congestion, and inflammatory cells infiltration in agreement with (**Nasser, Morcos *et al.* 2019**). Inflammatory cells infiltration was confirmed by a significant increase in the TNF-α in corneal ulcer group compared to the other groups. TNF-α is proinflammatory cytokine produced by monocytes, activated macrophages, T lymphocytes, and natural killer cells (**Al-Kinani and Al-Kaabi**).

Corneal ulcer group showed disruption of the normal regular parallel collagen bundles arrangement. Indeed, distortion of the stroma and the separation of the collagen bundles could be attributed to the increased corneal hydration (edema) that may affect the corneal transparency (**Wilson, El Haj *et al.* 2012**).

To detect progress of the healing process which occurred normally without management, the corneal sections were examined at end of experiment. Sections of recovery group showed abnormal architecture of corneal histological structure. These findings coincided with those of the former researchers who showed persistent of corneal opacities with the obvious neovascularization (**Raghuram, Hansen *et al.* 2013**).

Extravasated RBCs, congestion of blood vessels and inflammatory cells infiltration were also detected in corneal stroma in corneal ulcer group. These corneal inflammatory and vascular changes are threatening condition usually associated with infection of the ocular surfaces (**Xiao, Xie *et al.* 2012**). Corneal chemical burn caused abundant corneal neovascularization in which severe inflammation and lysis were involved (**Velevska, Duma *et al.* 2015**). These results were confirmed by a significant increase in VEGF in rats with corneal ulcers than other groups. VEGF is a protein that promotes the growth of new blood vessels (**Mehta 2011**). Neovascularization is important complication that affects acuity of vision, also it is essential for the repair, the remodeling and the regeneration of damaged tissues during the corneal wound healing (**Nasser, Morcos *et al.* 2019**). Fibroblasts and macrophages in the diseased tissue secrete VEGF, mainly in the inflamed and vascularized corneas. The VEGF receptors of (VEGFR1 and VEGFR2) were also originated in the new proliferating vascular endothelial cells in the inflamed cornea (**Jin, Zhang *et al.* 2020**).

Malondialdehyde (MDA) is the last product of the polyunsaturated fatty acids peroxidation inside cells. Free radicals cause lipid peroxidation process in the organisms. The increase in these free radicals produces excessive of MDA. MDA is a well-known as a marker of the oxidative stress (**Subramanyam, Gurunathan *et al.* 2018**). In this study; MDA showed a significant increase in the ulcer group by comparison to the treated groups, and this indicates the contribution of oxidative stress in corneal ulceration.

GSH is an antioxidant that stops reactive oxygen species (ROS) encouraged injuries to the constituents of cells (**Unsal, Dalkıran *et al*. 2020**). In the current work; GSH level showed a significant decrease in the ulcerated group compared to control and treated indicating a possible involvement of ROS in corneal ulceration.

P53 is tumor suppressor protein that plays a key role in apoptosis. P53 is normally synthesized in cytoplasm and is transported into the nucleus to produce its transcriptional effect acting as a nuclear transcription factor that activates genes involved in apoptosis (**Elwan and Kassab 2017**). P53 can initiate the apoptosis when DNA damage, hypoxia and ROS occur and these lead to the cell cycle arrest and apoptosis (**Akbari, Mard *et al*. 2017**). Thus, upregulation of p53 expression in corneal ulcer group could be attributed partly to the reduction of antioxidant activities due to ROS accumulation with induction of apoptosis (**Esquenazi and Bazan 2010**).

In group treated with CM; obvious improvement in the histopathological findings was observed and was approved. CM contains secretome that regulates many different responses of the cell including migration, proliferation, cell survival and gene expression. Although, using CM seem to be a safe and promising (**Fui, Lok *et al.* 2019**).

Regarding group treated with EXs; there was obvious marked improvement of furthermost of the histopathological changes in corneal ulcers in line with (**Desjardins, Berthiaume *et al.* 2022**).

MSCs, their CM and Exs may down regulate VEGF in the resident environment. EXs containing the microRNA may decrease the corneal neovascularization and fibrosis. Neovascularization depletion in cornea could be produced by the increase in expression of potent anti-angiogenic factor, thrombospondin-1 (TSP-1), and decrease the inflammatory related proangiogenic factor MMP-2. (**Prakoeswa, Natallya *et al.* 2018, Gugjoo 2022**).

CM and EXs provided a guard against the toxicity of oxygen species by elevating enzymes level with antioxidant properties (**Hong, Kim *et al*. 2019, Xian, Hei *et al.* 2019**). Factors in CM can stimulate the differentiation of stem cells in the tissue. Additionally, CM can inhibit the oxidative stress via the Nrf2/ NF-κB-pathway (**Tang, Ding *et al.* 2021**).

EXs are nano-sized vesicles. They work as a facilitator of cellular interaction. EXs contain growth factors, cytokines, signaling lipids, and miRNAs. EXs also reduce risk of carcinogenesis and immunogenicity (**Yin, Wang *et al.* 2019**). EXs treatment was potent as shown by the significant decreasing in the TNFα, MDA and VEGF levels and also significant increasing in GSH level. EXs also possess anti-inflammatory effects as it increase cytokine with anti-inflammatory, and M2 macrophages (**Singla, Johnson *et al.* 2019**). EXs and CM also have anti-apoptotic effect as appeared by significant decrease in P53 surface area fraction compared to the corneal ulcer group (**Sendon-Lago, Seoane *et al.* 2019, Wang, Hou *et al.* 2020**).

CONCLUSION

In conclusion, CM, and EXs have significant therapeutic effects on corneal ulcer. Interestingly, EXS administration was superior in maintaining the cornea chiefly by its strong antioxidant, anti-apoptotic and anti-inflammatory effects.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

Abd El Zaher, F., *et al*. (2017). "Role of mesenchymal stem cells versus their conditioned medium on cisplatin-induced acute kidney injury in albino rat. A histological and immunohistochemical study." Egyptian Journal of Histology 40(1): 37-51.

Abdelwahab, S., *et al*. (2017). "Role of vitamin A in the healing process of alkali caused corneal injury of adult male albino rat: Histological and immunohistochemical study." Journal of Medical Histology 1(1): 57-68.

Abdelwahab, S., *et al*. (2021). "Comparative study between bone marrow mesenchymal stem cell and their conditioned medium in the treatment of rat model of Parkinsonism." Journal of Cellular Physiology 236(1): 440-457.

Abdelwahab, S. A., *et al*. (2021). "Cerebral and cerebellar histological changes in the rat animal model of rotenone induced parkinsonism can be ameliorated by bone marrow derived stem cell conditioned media." Journal of Chemical Neuroanatomy 111: 101892.

Akbari, G., *et al*. (2017). "The Hepatoprotective and MicroRNAs Downregulatory Effects of Crocin Following Hepatic Ischemia-Reperfusion Injury in Rats." Oxid Med Cell Longev 2017: 1702967.

Al-Kinani, R. M. H. and A. J. Al-Kaabi "Evaluation of tumor necrosis factor alpha and interlukine-11 in a sample of aborted women infected with human Cytomegalovirus."

Bermudez, M. A., *et al*. (2015). "Corneal epithelial wound healing and bactericidal effect of conditioned medium from human uterine cervical stem cells." Investigative ophthalmology & visual science 56(2): 983-992.

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." Analytical biochemistry 72(1-2): 248-254.

Bremond-Gignac, D., *et al*. (2019). "Recent innovations with drugs in clinical trials for neurotrophic keratitis and refractory corneal ulcers." Expert Opinion on Investigational Drugs 28(11): 1013-1020.

Buchwalow, I. B. and W. Böcker (2010). Immunohistochemistry: basics and methods, Springer Science & Business Media.

Casaroli-Marano, R. P., *et al*. (2015). "Potential role of induced pluripotent stem cells (IPSCs) for cell-based therapy of the ocular surface." Journal of Clinical Medicine 4(2): 318-342.

Desjardins, P., *et al*. (2022). "Impact of Exosomes Released by Different Corneal Cell Types on the Wound Healing Properties of Human Corneal Epithelial Cells." International Journal of Molecular Sciences 23(20): 12201.

Ebrahim, N., *et al*. (2017). "The potential therapeutic effect of stem cells loaded on two different vehicles (amniotic membrane and platelet rich plasma gel) in experimentally induced corneal alkali burns in rats." Egyptian Journal of Histology 40(4): 405-426.

El-Salam, A., *et al*. (2019). "Evaluation of therapeutic potentials of α-Lipoic acid versus stem cells-derived microvesicles against experimentally–induced gastric ulcer in adult Male Albino Rats (Light and Electron microscopic study)." Egyptian Journal of Histology 42(4): 915-927.

Elwan, W. M. and A. A. Kassab (2017). "The potential protective role of hesperidin against capecitabine-induced corneal toxicity in adult male albino Rat. Light and electron microscopic study." Egyptian Journal of Histology 40(2): 201-215.

Esquenazi, S. and H. E. Bazan (2010). "Role of platelet-activating factor in cell death signaling in the cornea: A review." Molecular neurobiology 42(1): 32-38.

Fui, L. W., *et al*. (2019). "Understanding the multifaceted mechanisms of diabetic wound healing and therapeutic application of stem cells conditioned medium in the healing process." Journal of tissue engineering and regenerative medicine 13(12): 2218-2233.

Gross, J., *et al*. (2021). "High-performance detection of corneal ulceration using image classification with convolutional neural networks."

Gugjoo, M. B. (2022). Mesenchymal Stem Cells Therapeutic Applications in Eye and Adnexa Ailments. Therapeutic Applications of Mesenchymal Stem Cells in Veterinary Medicine, Springer: 391-408.

Hong, H.-E., *et al*. (2019). "Antioxidant action of hypoxic conditioned media from adipose-derived stem cells in the hepatic injury of expressing higher reactive oxygen species." Annals of surgical treatment and research 97(4): 159-167.

Ibrahim, M. F. G. and F. A. F. A. Allam (2022). "Potential stem cell—Conditioned medium and their derived exosomes versus omeprazole in treatment of experimental model of gastric ulcer." Acta Histochemica 124(4): 151896.

Jassim, M. M., *et al*. (2020). "Histopathological Study of Corneal Ulcer Healing in Rabbits by the Effect of Hyaluronic Acid." Biochemical and Cellular Archives 20(2): 4635-4639.

Jin, L., *et al*. (2020). "Zeb1 promotes corneal neovascularization by regulation of vascular endothelial cell proliferation." Communications biology 3(1): 1-10.

Kumar, D., *et al*. (2015). "Biomolecular characterization of exosomes released from cancer stem cells: Possible implications for biomarker and treatment of cancer." Oncotarget 6(5): 3280.

Li, X., *et al*. (2022). "Effect of Tear Derived Exosomes on Corneal Epithelial Cells During Diabetic Keraropathy."

Mehta, V. (2011). The effects of VEGF overexpression on the utero-placental circulation, UCL (University College London).

Nasser, S., *et al*. (2019). "Comparative histological study on the effect of early and late administration of adipose derived stem cells on corneal alkali burn in adult male albino rats." Egyptian Journal of Histology 42(3): 635-650.

Nishida, T., *et al*. (2021). "Pivotal Role of Corneal Fibroblasts in Progression to Corneal Ulcer in Bacterial Keratitis." International Journal of Molecular Sciences 22(16): 8979.

Ouyang, X., *et al*. (2018). "MSC-derived exosomes ameliorate erectile dysfunction by alleviation of corpus cavernosum smooth muscle apoptosis in a rat model of cavernous nerve injury." Stem cell research & therapy 9(1): 1-12.

Park, G. W., *et al*. (2020). "Topical cell-free conditioned media harvested from adipose tissue-derived stem cells promote recovery from corneal epithelial defects caused by chemical burns." Scientific reports 10(1): 1-15.

Prakoeswa, C. S., *et al*. (2018). "The efficacy of topical human amniotic membrane-mesenchymal stem cell-conditioned medium (hAMMSC-CM) and a mixture of topical hAMMSC-CM+ vitamin C and hAMMSC-CM+ vitamin E on chronic plantar ulcers in leprosy: a randomized control trial." Journal of Dermatological Treatment 29(8): 835-840.

Raghuram, A., *et al*. (2013). "Photoreceptor and postreceptor responses in congenital stationary night blindness." Investigative ophthalmology & visual science 54(7): 4648-4658.

Razi, H. M., *et al*. (2021). "Deterioration of wound healing and intense suppression of MMP-9 mRNA expression after short-term administration of different topical glucocorticoids or NSAIDs in an avian model of corneal lesions." Iranian Journal of Veterinary Research 22(3): 188.

Samaeekia, R., *et al*. (2018). "Effect of human corneal mesenchymal stromal cell-derived exosomes on corneal epithelial wound healing." Investigative ophthalmology & visual science 59(12): 5194-5200.

Sendon-Lago, J., *et al*. (2019). "Corneal regeneration by conditioned medium of human uterine cervical stem cells is mediated by TIMP-1 and TIMP-2." Experimental Eye Research 180: 110-121.

Singla, D. K., *et al*. (2019). "Exosome treatment enhances anti-inflammatory M2 macrophages and reduces inflammation-induced pyroptosis in doxorubicin-induced cardiomyopathy." Cells 8(10): 1224.

Stamate, A.-C., *et al*. (2019). "Update on surgical management of corneal ulceration and perforation." Romanian journal of ophthalmology 63(2): 166.

Subramanyam, D., *et al*. (2018). "Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries." European journal of dentistry 12(01): 067-070.

Tang, Y., *et al*. (2021). "hucMSC Conditioned Medium Ameliorate Lipopolysaccharide-Induced Acute Lung Injury by Suppressing Oxidative Stress and Inflammation via Nrf2/NF-κB Signaling Pathway." Analytical Cellular Pathology 2021.

Teixeira, F. G. and A. J. Salgado (2020). "Mesenchymal stem cells secretome: current trends and future challenges." Neural regeneration research 15(1): 75.

Unsal, V., *et al*. (2020). "The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review." Advanced pharmaceutical bulletin 10(2): 184.

Velevska, M. A., *et al*. (2015). "Evaluation of corneal changes in chemical burns with anterior segment optical coherence tomography." South-East European journal of ophthalmology.

Wan, T., *et al*. (2020). "Acetylsalicylic acid promotes corneal epithelium migration by regulating neutrophil extracellular traps in alkali burn." Frontiers in Immunology 11: 551057.

Wang, S., *et al*. (2020). "Comparison of exosomes derived from induced pluripotent stem cells and mesenchymal stem cells as therapeutic nanoparticles for treatment of corneal epithelial defects." Aging (Albany NY) 12(19): 19546.

Wilson, S. L., *et al*. (2012). "Control of scar tissue formation in the cornea: strategies in clinical and corneal tissue engineering." Journal of functional biomaterials 3(3): 642-687.

Xian, P., *et al*. (2019). "Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice." Theranostics 9(20): 5956.

Xiao, O., *et al*. (2012). "Minocycline inhibits alkali burn-induced corneal neovascularization in mice." PLoS One 7(7): e41858.

Yin, K., *et al*. (2019). "Exosomes from mesenchymal stem/stromal cells: a new therapeutic paradigm." Biomarker research 7(1): 1-8.

Zhu, Y., *et al*. (2022). "Use of ultrasound biomicroscopy to predict the outcome of anterior segment reconstruction in congenital fibrovascular pupillary membrane with secondary glaucoma." British Journal of Ophthalmology.

الملخص العربى

" تأثير التطبيق الموضعي للخلايا الجذعية الوسط المشروط مقابل - EXOSOMES المشتقة منها في نموذج الجرذ لقرحة القرنية "

تقرحات القرنية هي حالة طارئة مخيفة في الرؤية. حتى مع العلاج الدقيق ، قد يعاني المرضى من مضاعفات مثل الانثقاب ، والتندب ، وإعتام عدسة العين ، والزرق ، والتزامن ، وحتى فقدان البصر. يعتبر العلاج القائم على الخلايا الجذعية طريقة واعدة في علاج قرحة القرنية. في أيامنا هذه ، يعد العلاج القائم على الخلايا الجذعية البديلة (CM) و (Exosomes EXs) لقرحة القرنية من الأساليب الممكنة.

كان الهدف من هذه الدراسة هو مقارنة التأثيرات التحسينية الممكنة للوسط المكيف للخلايا الجذعية (CM) مقابل (Exosomes) المشتق منها (EXs) في التئام قرحة القرنية.

تم تقسيم 40 جرذًا بشكل عشوائي إلى خمس مجموعات. مجموعات التحكم ، وقرحة القرنية ، والمعالجة بالخلايا الجذعية البديلة (CM) ، والمعالجة بالخلايا الجذعية البديلة (Exosomes) ، ومجموعة الاستشفاء.

تم الحصول على القرنيات للتقييم الكيميائي الحيوي لمستويات TNFα و VEGF و MDA و GSH وللتركيب الهستولوجي النسيجي. تم تقييم الأضرار التي لحقت بالقرنية من خلال سجل الأنسجة المرضية والصبغات المناعية الهستوكيميائية.

وفي النتائج, كشفت مجموعة قرحة القرنية عن زيادة معنوية في مستويات TNFα و VEGF و MDA كما كشفت عن انخفاض معنوي في مستوى GSH. أظهر التقييم النسيجي حدوث تغيير تركيبي يشير إلى تلف شديد بالقرنية. علاوة على ذلك ، زيادة كبيرة في تعبير P53 . ومن المثير للاهتمام ، أن إعطاء CM و EXs أدي إلى انخفاض كبير في مستويات TNFα و VEGF و MDA وزيادة مستوى GSH . بالإضافة إلى ذلك ، كان هناك تحسن واضح في التركيب النسيجي للقرنيات ، وانخفاض معنوي في تعبير P53.

واستخلص من هذا البحث على حد علمنا: هذه هي الدراسة الأولى للتحقيق في CM مقابل EXs في علاج قرحة القرنية. يعمل كل من EXs و CM على تحسين التئام قرح القرنية مع تأثير تحسن كبير على EXs . يمكن أن يعزى التأثير الأعلي ل EXs إلى خصائصها كمضادات موت الخلايا المبرمج ، ومضادة للالتهابات ، ومضادة للأكسدة.