Possible Effect of Green Tea Extract on Trans-differentiation of Adipose Stem Cells in Induced Diabetes Type I Keratopathy Rat Model

Original Article

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ABSTRACT

Background and Objectives: Diabetic corneal morphological changes are frequent. Green tea extract (GTE) is a natural product which was proved to have antioxidant and anti-inflammatory effects. Adipose mesenchymal stem cells (AMSCs) are commonly used as cellular therapies. The current work aimed to study and compare the effect of AMSCs therapy individually or combined with GTE in keratopathy complicating induced type1 diabetes (T1D) in adult male rats.

Methods and Results: 38 male rats were randomly divided into: In *Vitro* Study Group: 2 rats for isolation, culture, and label of AMSCs. Control Rats (Group A): consisted of 8 animals.

Induction of diabetes: 28 rats were induced to be diabetic by a single intraperitoneal (IP) injection of 50mg/Kg of streptozotocin (STZ), then after 4 weeks of induction of diabetes they were subdivided into the following groups. Diabetic group (group B): seven animals were left untreated. GTE Group (Group C): seven animals that were given GTE 50 mg/kg daily orally. AMSCs Group (Group D): seven animals that were given intravenous (IV) injection of 1x106 cells (AMSCs). GTE and AMSCs Group (Group E): seven animals that were given a combination of AMSCs injection and GTE. Animals of all groups were sacrificed after 8 weeks from start of experiment. Corneal dimensions, serological, biochemical, morphological, and quantitative studies were performed. Group B revealed inflammatory and degenerative changes in the various layers that regressed by various therapies.

Conclusions: T1D induced corneal inflammatory and degenerative morphological changes. Both GTE and AMSCS proved amelioration of morphological and quantitative parameters, the amelioration was more remarkable in response to AMSCs therapy. Combined GTE and AMSCs therapy guaranteed the most remarkable effect.

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Key Words: Cornea, diabetes, GTE, immunohistochemistry, MSCs.

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INTRODUCTION

Diabetic corneal alterations including edema and recurrent erosions are frequent. The corneal epithelium, corneal nerves and to a lesser extent the endothelium are commonly affected in type 1 diabetes (T1D)[1]. Limbal stem cell deficiency was also documented in autoimmune disorders as T1D[2].

Green tea extract (GTE) was reported to be an antioxidant, where GTE dosages used in recent studies have protective effects from the adverse effects of oxidation[3]. The expression of the proinflammatory cytokine inflammation was ameliorated by oral consumption of GTE as documented by Fang *et al*[4].

Recent therapeutic agents, including cell therapies, are under trial nowadays[5]. MSCs are widely distributed among multiple tissues and organs of the body. In cell transplantation, MSC are frequently used. Adipose tissue, which is easily obtained, is considered a preferred source for MSCS when compared to bone marrow or umbilical cord[6]. A recent study showed direct therapeutic benefits of antioxidants on stemness of corneal epithelial cells and documented recent modalities as MSCs therapy to be more effective in treatment[7].

The current work aimed to study and compare the effect of therapy of AMSCs individually or combined with GTE in keratopathy complicating induced type1 diabetes (T1D) in adult male rats.

MATERIALS AND METHODS

Medications

- Streptozotocin (STZ): 1gram vial (Sigma, United States). The determined dose was dissolved in citrate buffer.
- Green Tea Extract (GTE): (Technomad, El Obour City, Egypt). 200 milligram (mg) tablets. The determined dose was dissolved in saline.

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Animals

Thirty-eight male albino rats aged 3 months and weighed 200 grams. They were kept in Kasr Al-Ainy Animal House in hygienic stainless-steel cages. The animal room was clean and well ventilated. The animals were allowed for free water and food ad libitum. All treatments were performed according to Cairo University Institutional Animal Care and Use Committee.

The animals were divided randomly into

In-*Vitro* Study (Donation) Group: included 2 rats from which AMSCs were isolated, cultured, phenotyped and labeled.

Group A: (Control Group): All control rats were injected once with citrate buffer IP, then they were divided into 4 subgroups that were euthanized with groups B, C, D and E respectively.

- Subgroup 1: 2 rats left for 8 weeks without treatment
- Subgroup 2: 2 rats, 4 weeks after citrate buffer IP injection, were given 50 mg/kg green tea every day for another 4 weeks by oral gavage.
- Subgroup 3: 2 rats, 4 weeks after citrate buffer IP injection, were injected IV in tail vein with 0.5 ml phosphate buffered saline (PBS) twice on 2 successive days and sacrificed after another 4 weeks.
- Subgroup 4: 2 rats, 4 weeks after citrate buffer IP injection, were given PBS as for the 3rd subgroup and GTE as for 2nd subgroup & sacrificed after another 4 weeks.

Induction of diabetes: Diabetes was induced by a single IP injection of 50 mg/kg[8] STZ, dissolved in 0.5 ml citrate buffer. Estimation of blood glucose level was done 3 days following induction. A fasting blood sugar level higher than 200 mg/dl was considered +ve result[9].

After induction of diabetes animals were then divided into the following groups

Group B: (Diabetic Group): Consisted of seven animals that were left untreated.

Group C: (GTE Group): Four weeks later, GTE was given, each rat 50 mg/kg[10] dissolved in 0.5 ml saline every day by oral gavage using a gastric tube for 4 weeks[11].

Group D: (AMSCs Group): Four weeks later, 500000 of cultured rat AMSCs in 0.5 ml PBS suspension[12], was injected IV in the tail vein twice on 2 successive days[13].

Group E: (GTE and AMSCs Group): Four weeks later, 500000 of cultured rat AMSCs in 0.5 ml PBS[12], was injected IV in the tail vein twice on 2 successive days. In addition, GTE was given as in group C.

Groups A, B, C, D and E were sacrificed 8 weeks from start of the experiment. Sample collection and expansion of rat AMSCs[14,15]

After sacrifice of rats of donation group, abdominal adipose tissues were washed with saline, collected, and incubated in Dulbecco's modified Eagle's medium (DMEM), (GIBCO/BRL). Resuspension in a medium supplemented with 1% penicillin-streptomycin at 37° C in 5% humidified CO2 for 14 days. Large colonies formation is followed by washing with PBS and adding 0.25% trypsin in 1mmol EDTA for 5 minutes. After centrifugation, serum-supplemented medium was used for resuspension. After incubation in culture flask, trypsinization, and counting of adherent colonies was exerted on day 14 (3rd passage culture).

Phenotyping of AMSCs

Trypsinized cells were adjusted to 1×106 cells/ml by hemocytometer. Ten µl of CD34 and CD44 monoclonal antibodies (Lab Vision, USA) were used. After 60 minutes incubation, washing with 2% fraction crystallizable solution in PBS was performed. Resuspension of cells in 500µl PBS was followed by centrifugation for 5 minutes. Percentage of cells was determined using CYTOMICS Flow Cytometer 500 (Beckman coulter, USA) and analyzed using CXP Software version 2.2[13]. Cytochemical analysis was established[16].

Labeling of AMSCs with feridex[17]

375 nanogram/ml polylysine was added before incubation. Then 25 micrograms of Feridex (Berlex Labs) were used for labeling in the culture for 1 day.

Blood Sugar Estimation

Venous blood samples were obtained at the end of the experiment for serum glucose level measurement.

Sacrifice of rat

The rats were euthanized by cervical dislocation[18]. Eye specimens were collected to perform the following studies:

Biochemical study

In the left eye specimens of each rat of groups A, B, C, D and E, the cornea was surgically dissected[19]. One ml normal saline was used for homogenate formation by using (Ortoalresa homogenizer, Spain). Centrifugation for 15 minutes was followed. The supernatant was kept in Eppendorf tubes at - 20°C. Catalase and malondialdehyde (MDA)[20] were estimated by using colorimetric method kits (Biodiagnostic, Egypt).

Corneal Dimensions

Using Olympus microscope connected to Olympus camera, assisted image analysis system the following parameters were measured: Measurement of antero-posterior (A-P) thickness[21], assessment of thickness of epithelium and that of stroma[22], were performed in 5 different fields from 5 different sections using mean of arbitrary line in all groups.

Histological study: Fixation of the right eye specimens in 10% formol saline was done for 1 day. Dehydration in ascending grades of alcohol, clearing in xylol and paraffin embedding were performed and 5µm thick sections were subjected to:

- Hematoxylin and eosin (H&E)[23]
- Masson's trichrome stain[23]
- Prussian blue (Pb) stain (that stains ferric salts blue) to show the labeled exogenous cells with ferrum oxide[23].
- Cytokeratin (CK)3 immunostaining[24] for demonstration of cytoskeletal protein. Mouse monoclonal antibody (Ab) was supplied in the form of 7ml prediluted ready to use solution (MS-1447-R7) (Lab Vision Corporation, USA).
- Caspase3 immunostaining[25], the marker for apoptosis. Rabbit polyclonal Ab was supplied in the form of 7ml prediluted ready to use solution (RB-1197-R7) (Thermofisher, USA).
- CD44 immunostaining[26] for detecting endogenous and exogenous undifferentiated AMSCs. Monoclonal Ab was supplied in the form of 7ml prediluted ready to use solution (IW-PA1021) (Lab Vision, USA). The primary Abs were applied for 1 hour, and secondary Abs were added. Chromogen was applied and counterstaining with Mayer's hematoxylin was done.

Phenotypic Quantitation

Leica Qwin 500 LTD (Cambridge, UK) image analyzer, was used to measure the following:

- the count of Pb +ve expression on the cells and the count of CD44 immunoexpression were performed using interactive menu.
- Area% of collagen fibers, CK3 positive immunoexpression (IE) and caspase 3 positive IE were performed using binary menu.
- The measurements were done using 10 non-overlapping fields taken from 5 slides of different groups.

Quantitative polymerase chain reaction (qPCR) for tumor necrosis factor (TNF) α

Reverse Transcription was carried out using paraffin sections[27]. The TNF α primer was, 5′-oligonucleotide primer -5′ ACCACGCTCTTCTGTCTACTG -3′ and 3′-oligonucleotide primer 5′-CTTGGTGGTTTGCTACGAC -3′. The complementary DNA strand was created from RNA. The TNF DNA was set up on PCR program. β-actin was used as reference for values.

Statistical Analysis[28]

Means and standard deviations were analysed using ANOVA. Any significant difference was determined by *P-values* <0.05. Calculations were made on Statistical Package for Social Sciences software version 16.

RESULTS

General observations

In the present study, two rats died at the beginning of the experiment, two days following STZ injection, and they were compensated. Lethargy and polyuria were observed in group B that improved in groups C, D and E by the end of the present work.

Phenotyping of AMSCs

Spindle cells (AMSCs) were positive for CD44 and negative for CD34. Determination of AMSCs % revealed 97.75 % positive for CD44 (Figures 1a, b, c).

Mean serum glucose level before sacrifice indicated a significant increase in group B versus all groups and in group C versus groups A and E (Table1) (Figure 2a).

Biochemical parameters of oxidative stress

MDA mean values were in nanomole (nM/mg) and mean catalase values were in unit (U)/mg. A significant increase in mean MDA and a significant decrease in mean catalase values were found in group B compared to all other groups and also in groups C and D versus groups A and E (Table1) (Figure 2b).

Corneal dimensions (Table2, Figures 3a, b, c, d, e, f)

A significant increase in the antero-posterior and stromal thicknesses, while a significant decrease in the thickness of the epithelium was found in group B and in group C compared to groups A, D and E.

Histological phenotyping

Sections in the cornea revealed normal nonkeratinized stratified squamous epithelium, substantia propria and endothelium in group A (Figure 4a). In group B, single layer of epithelial cells with focal epithelial disruption, epithelial and endothelial surface cells with round nuclei, congested blood vessels and cellular disorganization were seen in the substantia (Figures 4 b, c). Reduced epithelial thickness was found in group C (Figure 4d). In group D vacuolation of some basal and suprabasal epithelial cells was observed (Figure 4e). Accidental vacuolated epithelial cells were detected in group E (Figure 4f). The endothelium appeared normal in the treated groups.

Sections in the cornea stained by Masson's trichrome showed intact anterior limiting membrane (ALM) (Bowman's membrane) and parallel compact collagen fibers in the substantia in group A (Figure 5a). Group B demonstrated disrupted ALM and extensive separation between multiple collagen fibres (Figure 5b). In group C, obvious separation between some collagen bundles was noted (Figure 5c). There was minimal separation between multiple collagen bundles in group D (Figure 5d), while only few separated collagen bundles were found in group E (Figure 5e). ALM was intact in all treated groups.

Prussian blue stained sections demonstrated negative staining in group A (Figure 6a), groups B and C. In group D, multiple Pb positive cells were observed near the epithelium and among the substantia (Figure 6b). Less numerous positive cells were detected near epithelium and among the substantia in group E (Figure 6c).

Cytokeratin3 immunostained sections showed obvious immunoexpression (IE) in all epithelial cells in group A (Figure 7a), while minimal in all epithelial cells in group B, except the surface layer which was extensive (Figure 7b). Obvious IE was found in some epithelial cells and at the surface in group C (Figure 7c), in multiple epithelial cells in group D (Figure 7d) while it was detected in all epithelial cells in group E (Figure 7e).

Caspase 3 immunostained sections demonstrated positive IE in few epithelial and endothelial cells in group A (Figure 8a), while group B revealed positive IE in multiple epithelial cells and multiple keratocytes (Figure 8b). Positive IE was evident in some cells of epithelium and some keratocytes in group C (Figure 8c), while in group D it was observed in few cells, few keratocytes and accidental vascular lining cells (Figure 8d). In group E, only few epithelial cells and accidental keratocytes showed positive IE (Figure 8e).

CD44 immunostained sections revealed negative IE in group A (Figure 9a), while group B showed few positive cells at the epithelium, in substantia and at the endothelium (Figure 9b). Less numerous positive cells were visualized at epithelium, in addition to substantia in group C (Figure9c). The positive cells appeared numerous at the epithelial surface and in the substantia in group D (Figure 9d), while less numerous positive cells were evident in group E (Figure9e).

Morphometric Results (Tables 2,3)

The mean thickness of corneal epithelium recorded a decrease in group B, group C versus to groups A, D and E. Similarly, a decrease was found in the mean area % of collagen fibers in group B.

Significant decrease in the mean area of CK3 and increase in caspase3 IE were documented in group B versus other groups, in group C versus groups A, D and E. A significant decrease was detected in mean area of CK3 in group D versus groups A and E.

Concerning the count of Pb positive cells, a decrease was noted in group E versus group D. The count of CD44 positive immunoexpression recorded a decrease in group B versus other groups and in group C versus group D. In addition to group E compared to group D.

PCR results

The measurements of PCR for TNFα were in picogram (pg)/mg. The mentioned measurements indicated an increase in group B versus all groups, in group C versus groups A, D and E, in group D versus groups A and E (Table 1) (Figure 2c).

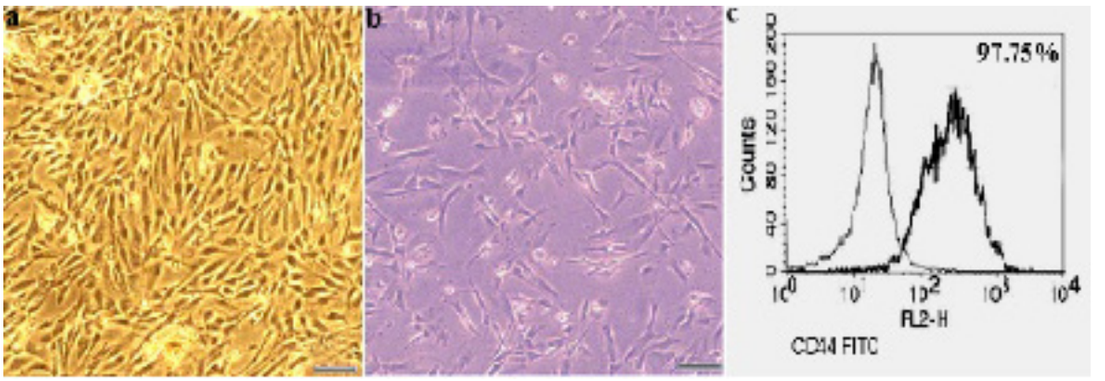

Fig. 1 showing: (a) CD44 +ve spindle cells. (b) CD34 -ve immunoreactivity, (Phase contrast microscopy x 100). (c) Immunophenotyping of AMSCs 97.75 % are +ve for CD44 (Flow Cytometry).

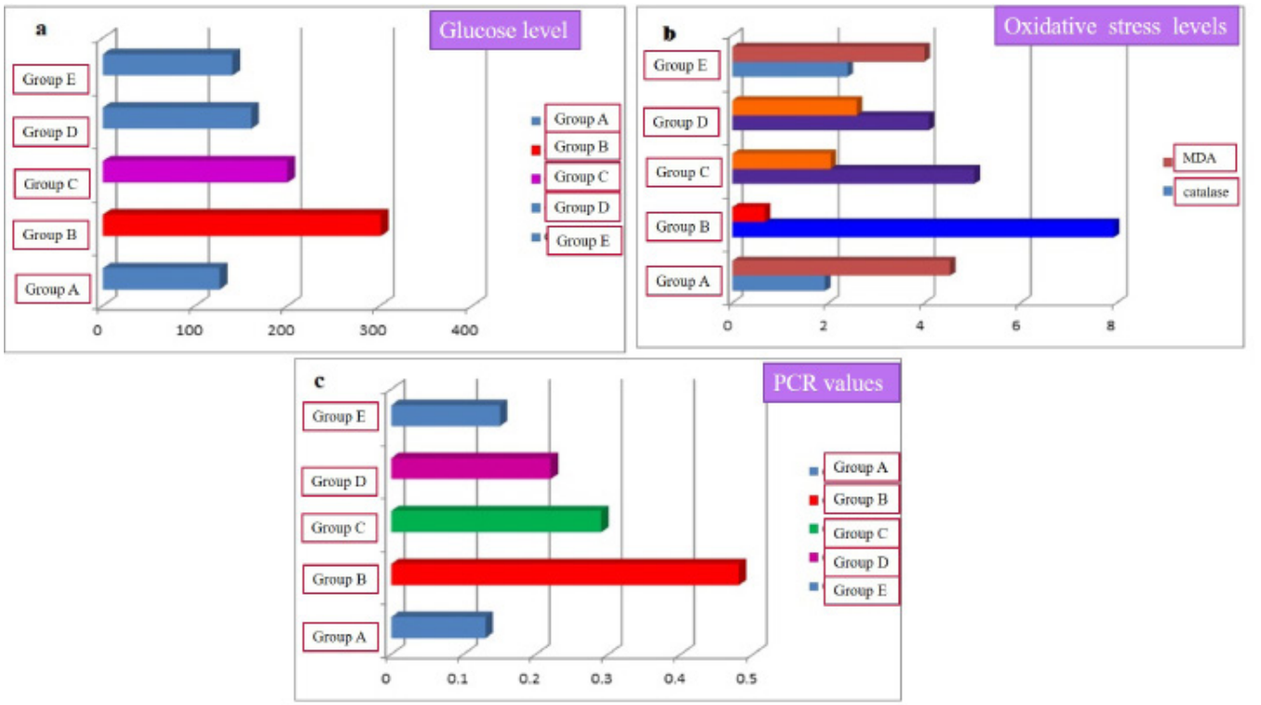

Fig. 2 showing histograms of: (a) blood glucose level (b) Oxidative stress levels: MDA and catalase parameters (c) PCR values

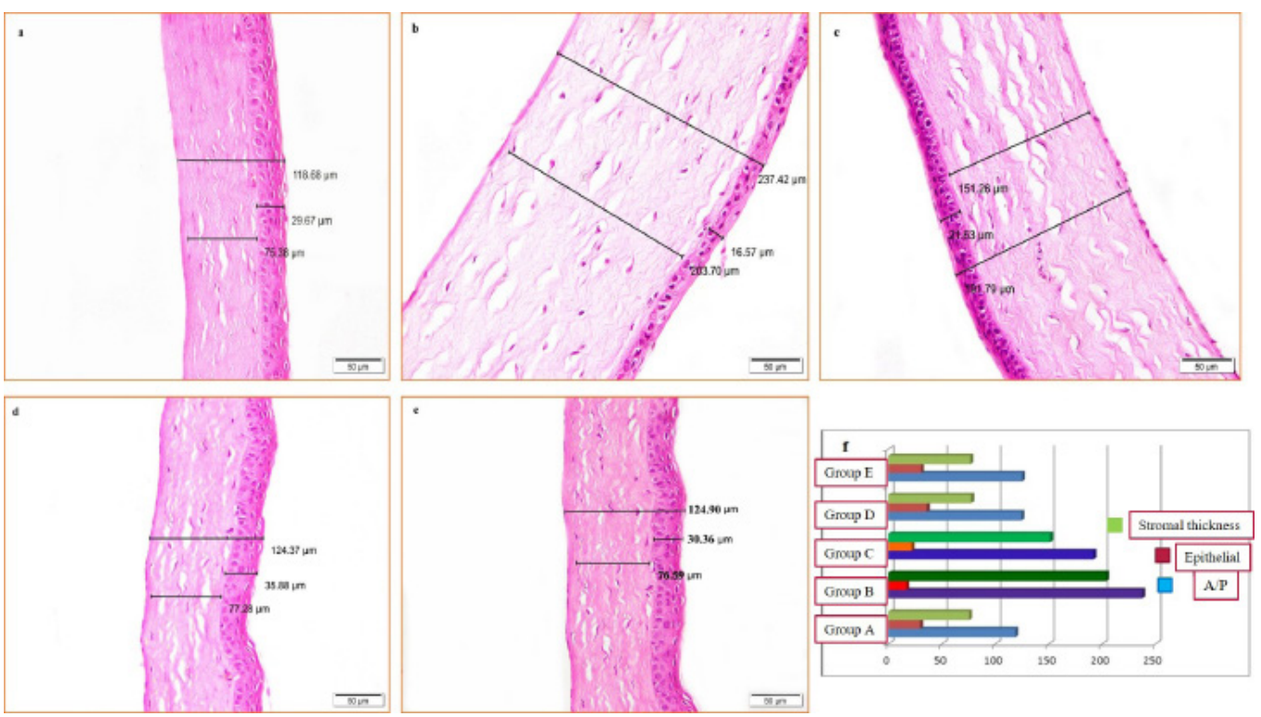

Fig. 3 showing corneal dimensions: (a) in group A. (b) in group B (c) in group C (d) in group D (e) in group E (H&E, x200). (f) histogram of means (A-P, epithelial and stromal thicknesses).

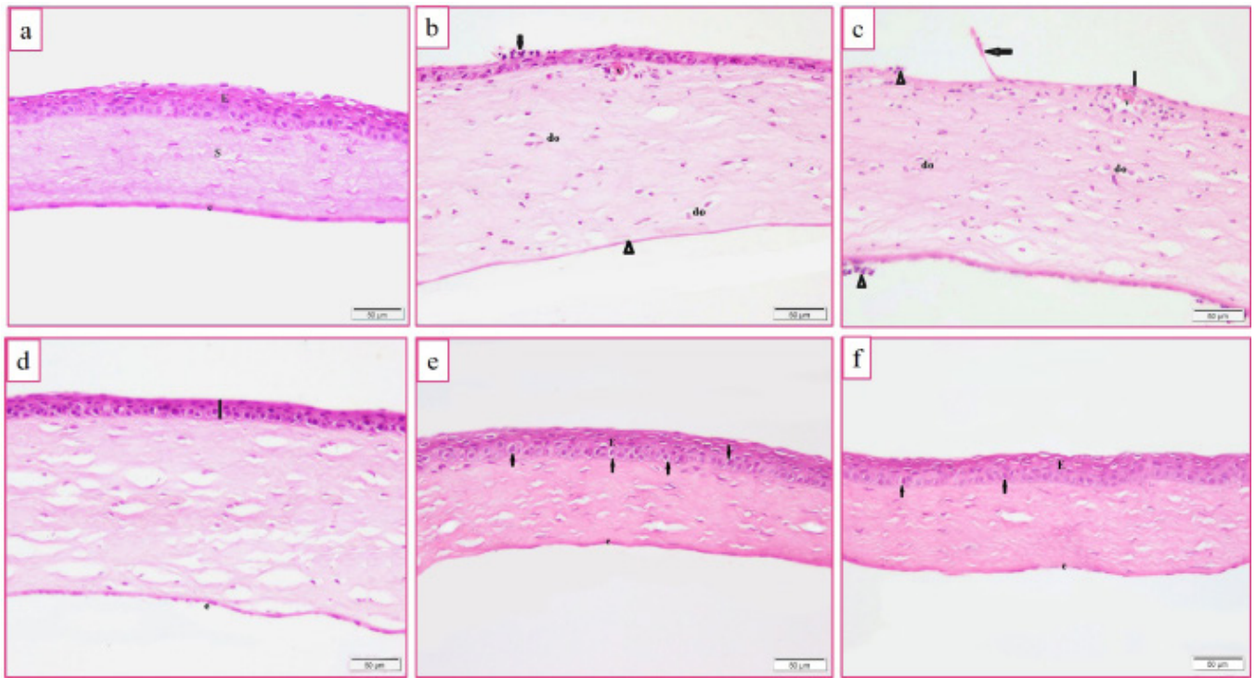

Fig. 4 showing: (a) epithelium (E), substantia propria (S) and endothelium. (b) epithelial surface cells with round nuclei (arrowhead), congested blood vessel (v) and cellular disorganization (do) in the substantia. (c) single layer of epithelial cells (line) with focal epithelial disruption (arrow), epithelial and endothelial surface cells with round nuclei (arrowheads), congested blood vessel (v) and cellular disorganization (do). (d) reduced epithelial thickness (line), apparently normal endothelium (e). (e) vacuolation (arrows) of some cells in the epithelium (E), normal endothelium (e). (f) few vacuolated (arrows) cells in the epithelium (E) and normal endothelium (e). (a: group A, b: group B, c: group B, d: group C, e: groupD, f: group E, (H&E, x200).

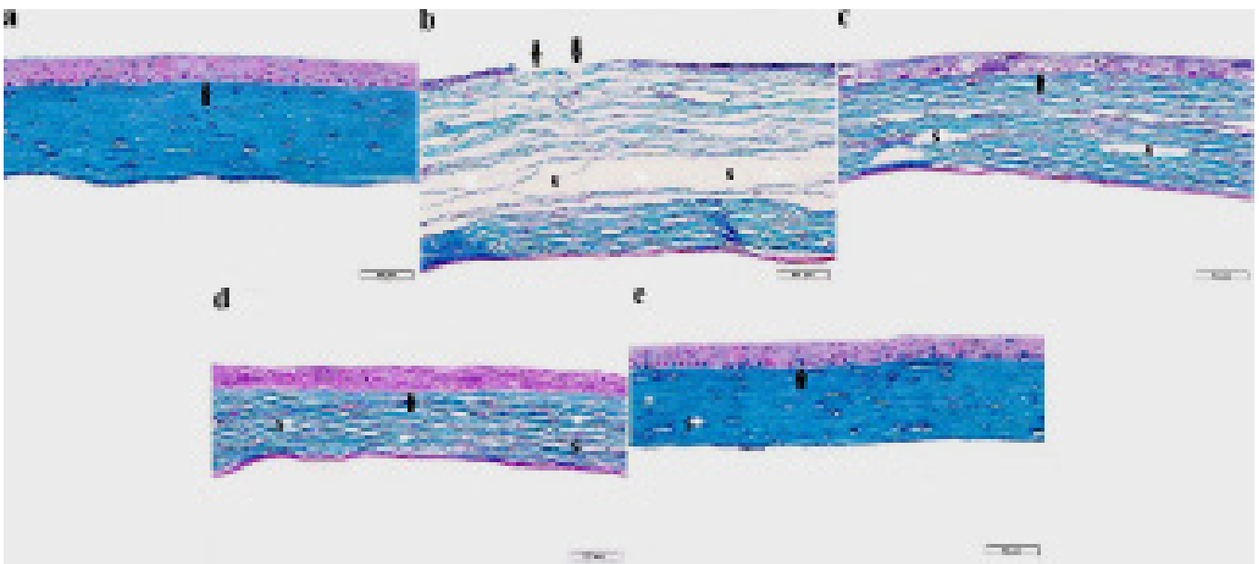

Fig. 5 showing: (a) intact Bowman's membrane (arrow), parallel compact collagen bundles in the substantia. (b) disrupted Bowman's membrane (arrows), extensive separation between multiple collagen fibres. (c) intact Bowman's membrane (arrow), obvious separation between some collagen bundles. (d) intact Bowman's membrane (arrow), minimal separation between multiple collagen bundles. (e) intact Bowman's membrane (arrow), few separated collagen bundles (a: group A, B: group B, c: group C, d: group D, e: group E, Masson's trichrome, x200).

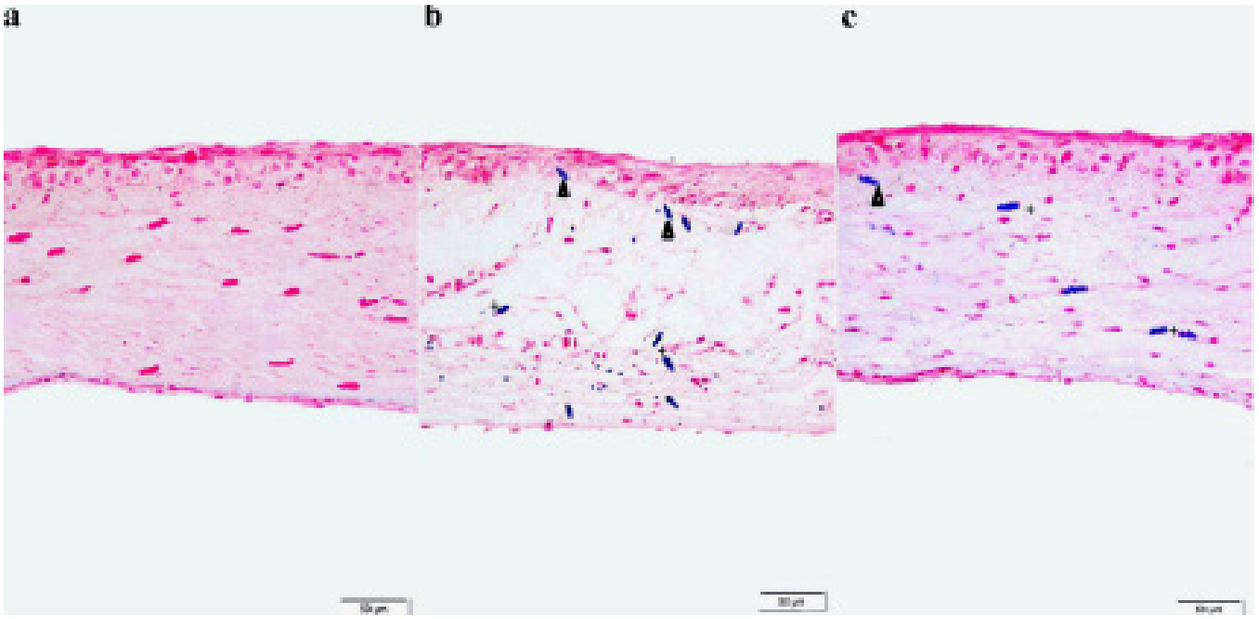

Fig. 6 showing: (a) Prussian blue -ve staining. (b) multiple Prussian blue +ve spindle cells at and near the epithelium (arrowhead) and among the substantia (+). (c) some +ve spindle cells near the epithelium (arrowhead) and among the substantia (+) (a: group A, B: group D, c: group E, Prussian blue, x200).

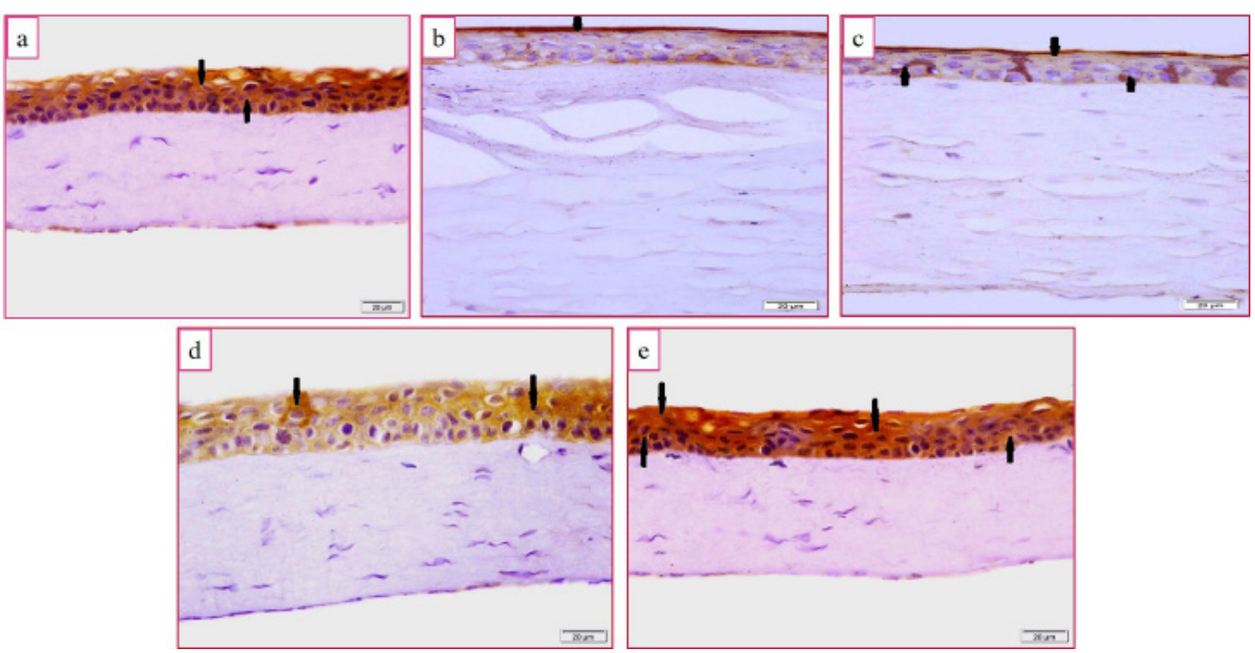

Fig. 7 showing: (a) obvious immunoexpression (arrow) in all epithelial cells. (b) minimal IE in all epithelial cells, except the surface layer (arrow) shows extensive IE. (c) obvious IE (arrows) in some epithelial cells and at the surface. (d) obvious IE (arrows) in multiple epithelial cells. (e) obvious IE (arrows) in all epithelial cells (a: group A, B: group B, c: group C, d: group D, e: group E, (CK3 immunostaining, x400).

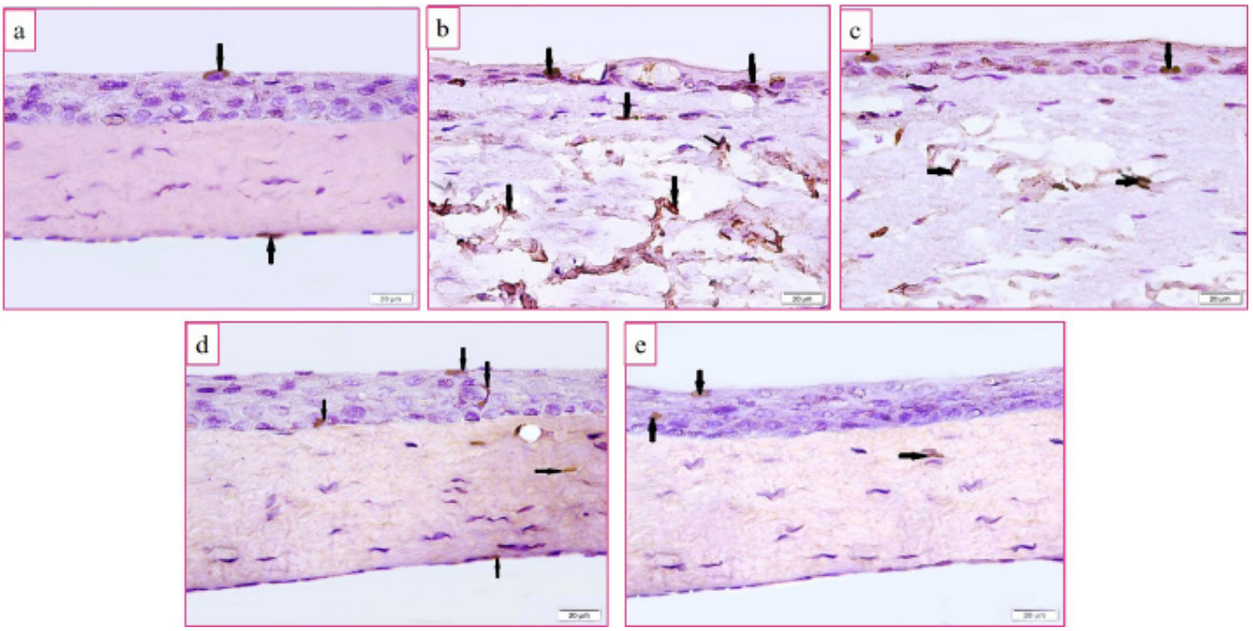

Fig. 8 showing: (a) +ve IE (lines) in an epithelial and an endothelial cell. (b) +ve IE (arrows) in multiple epithelial cells and multiple keratocytes. (c) +ve IE (arrows) in some epithelial cells and some keratocytes. (d) +ve IE (arrows) in few epithelial cells, few keratocytes and an endothelial cell. (e) +ve IE (arrows) in few epithelial cells and a keratocyte (a: group A, B: group B, c: group C, d: group D, e: group E, (Caspase 3 immunostaining, x400).

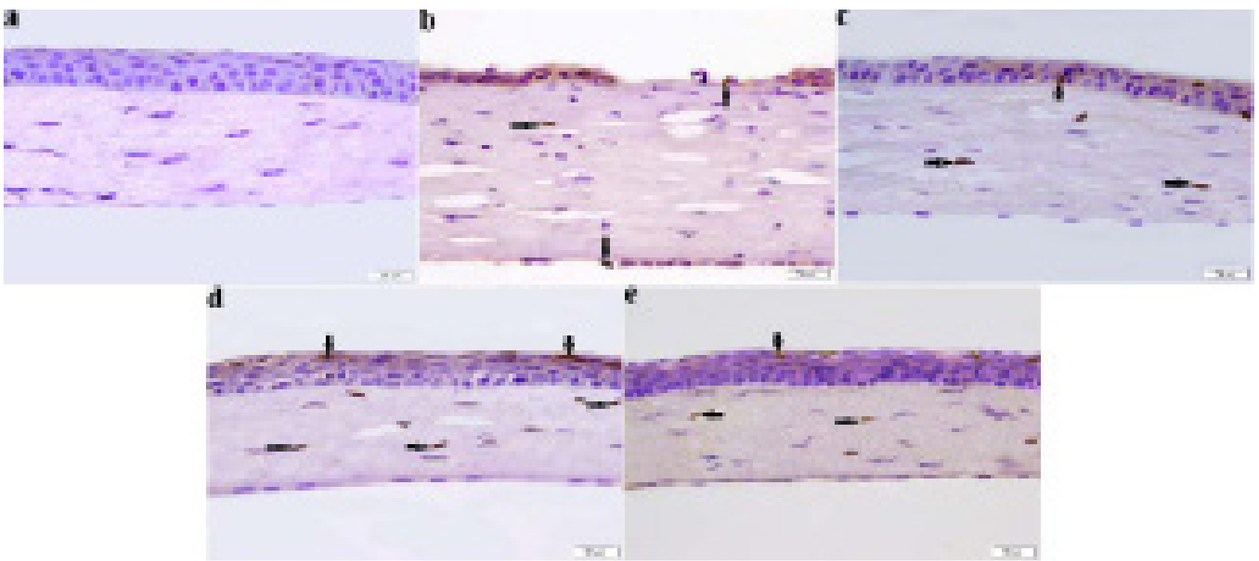

Fig. 9 showing: (a) -ve IE. (b) few +ve spindle cells (arrows) at the epithelium, in the substantia and at the endothelium. (c) some +ve spindle cells (arrows) at the epithelium and in the substantia. (d) numerous +ve spindle cells (arrows) at the surface of the epithelium and in the substantia. (e) multiple +ve spindle cells (arrows) at the surface of the epithelium and in the substantia (a: group A, B: group B, c: group C, d: group D, e: group E, (CD44 immunostaining, x400).

Table 1: Mean ± standard deviation of serum glucose, MDA and Catalase values

Groups	Serum glucose level mg/dl	MDA value (nM/mg)	Catalase value U/mg	PCR value pg/mg
Group A	126.20±4.99	1.92±0.22	4.52±1.03	0.13±0.02
Group B	300.80±12.21*	7.93±0.50*	0.67±0.11*	0.48±0.07*
Group C	199.80±20.40^	5.03±0.61^	2.04±0.09^	0.29±0.05#
Group D	160.30±15.25	4.08±0.08^	2.58±0.31^	0.22±0.04^
Group E	140.20±5.31	2.39±0.26	3.99±0.23	0.15±0.03

Sig $P \leq 0.05$ *versus all groups ^ versus groups A & E #versus groups A, D & E

Table 2: Mean ± standard deviation of A-P, epithelial and stromal thicknesses (µm)

Groups	A-P thickness	Epithelial thickness	Stromal thickness
Group A	118.68±14.32	29.67±2.75	75.38±9.08
Group B	237.42±15.99*	16.57±2. 33*	203.70±20.01*
Group C	191.79±16.58^	21.63±4.02^	151.26±13.89^
Group D	124.37±9.98	35.88±5.05	77.28±10.11
Group E	124.90±18.54	30.36±3.09	76.59±9.55

Sig $P \leq 0.05$ *versus all groups ^ versus groups A, D and E

Table 3: Mean ± standard deviation of corneal area % of collagen fibres (Cf), area of CK3 IE, area of caspase3 IE (Cp), count of Pb +ve and of CD44 +ve cells

Groups	Cf	CK 3	Cp	Pb	CD44
Group A	24.27±2.95	257.87±10.34	6.11±0.90	-	-
Group B	12.90±1.34^	52.46±7.08^	36.38±5.92^	-	3.30±0.21^
Group C	23.47±1.22	99.32±15.23*	16.27±3.05*	-	6.60±1.34*
Group D	25.01±4.05	159.75±12.59#	7.81±2.00	9.90±1.01	15.60±2.11
Group E	23.99±4.00	259.37±15.09	6.46±0.98	4.90±0.30$	9.30±1.18∞

Sig $P \leq 0.05$ ^ versus other groups * versus groups A, D, E #versus A, E $ ∞ versus D

DISCUSSION

In the current work, streptozotocin has been injected into rodents to create a model of diabetic keratopathy. Afterwards, the effect of therapy of AMSCs individually or combined with GTE was compared in treatment of induced type1 diabetes (T1D) keratopathy in adult male rats.

As regards serological findings, an increase in blood glucose values was recorded in group B before sacrifice and in group C versus groups A and E. Stepien *et al*[29] postulated that GTE exerts hypoglycemic effect in different doses. However, the present work confirmed the potentiation of this effect by the regenerative plasticity of SCs.

Concerning biochemical parameters of oxidative stress, significant increase in MDA and a decrease in catalase were recorded in group B, in groups C and D versus groups A and E. The mean values of PCR for TNFα indicated an increase in group B, in group C versus groups A & E and in group D compared to group A. In accordance, oxidative stress derived inflammation was found in diabetes, and it was hypothesized that oxidative stress is the key regulator in development and aggravation of diabetic complications[30]. GTE was recently documented to inhibit TNF-α effect[31]. In support. AMSCs isolated from rat were proved to have anti-inflammatory, antioxidative, and cytoprotective properties[32].

Regarding, corneal dimensions, significant reduction of epithelial thickness and significant increase in stromal thickness were found in the diabetic group versus all groups, and in GTE group versus control and AMSCs treated groups. The previous findings indicated impaired epithelial regeneration and the incidence of stromal edema in diabetic and to less extent in GTE treated group. In accordance, Hashem[33] referred increased stromal thickness to stromal edema that appears as wide spacing of collagen bundles. In addition, Segars *et al*[34] related the regain of normal corneal epithelial and stromal thicknesses to stem cells migration, regeneration and repair.

In the diabetic group (Group B), lymphocytes at the epithelial and endothelial surfaces, extensive separation of collagen fibers, appearance of vessels and cellular disorganization in the substantia propria were evident. The previously mentioned changes indicated inflammatory process developing in the cornea. In accordance, Mirabelli *et al*[35] stated that edema and erythema are suggestive of inflammation and inflammatory corneal neovascularization is a pathological process that might lead to blindness.

In addition, group B showed epithelial disruption and disrupted ALM denoting degenerative changes. Positive caspase3 IE was found in multiple epithelial cells and multiple keratocytes, proving apoptosis. In support, Shih *et al*[36] reported recurrent corneal erosions and consequent degeneration with defective tissue regeneration in diabetes.

Concerning CK3 immunostaining, minimal IE was noted in all epithelial cells of group B, except the surface layer, in which the IE was extensive. This was supported morphometrically, indicating disruption of cytoskeletal elements and keratinization at the surface, which interferes with corneal transparency.\Concomitantly, Kitazawa *et al*,[37] pointed to that CK3 is essential in maintaining the epithelial morphology and differentiation as it is the cytoskeleton. In addition, surface keratinization interferes with integrity of vision.

Group B recruited few CD44 positive cells at the epithelium, in substantia and at the endothelium. This can be referred to the demonstration of endogenous SCs only in diabetic corneas, which were not capable of regression of morphological changes. Li *et al*,[38] proved that postnatal mesenchymal stem/progenitor cells are retrieved from bone marrow or adipose tissue. They were described as fibroblastic cells, plastic-adherent and exhibit a surface marker profile positive for CD73, CD44, CD90 and CD105. They were defined by the International Society for Cellular Therapy as MSCs. Zickri *et al*[39] added that diabetes has non-desirable effects on plasticity and transdifferentiation of corneal and limbal resident cells.

In group C (GTE Group), intact ALM and less separation between fewer collagen bundles were observed. Obvious CK3 IE was found in some epithelial cells and at the surface, confirming an increase in CK3 IE, and caspase 3 positive IE was detected in less epithelial cells and less keratocytes. These findings denoted amelioration of inflammatory and degenerative changes versus diabetic group. Concomitantly, Camellia sinensis extract, similar to GTE was found to exert high antinflammatory and antioxidant radical scavenging properties, referred to the high phenolic and flavonoid content[40].

In group C more CD44 positive spindle stem cells were detected compared to group B. Similarly, Zickri and Embaby[41] stated that resident SCs stimulation was recorded under the effect of green tea therapy, being considered an antioxidant with amelioration of morphological and morphometric findings associated with organ damage.

In group D (AMSCs group), vacuolation of some epithelial cells, intact ALM and minimal separation between multiple collagen bundles were detected. Obvious CK3 IE was evident in multiple epithelial cells, with decrease in area% versus groups A & E. Positive caspase3 IE was seen in few cells, few keratocytes and accidental vascular lining cells. It can be commented that AMSCs induced more obvious therapeutic effect compared to GTE treated group. In agreement, MSCs of different origins proved a regressing effect on systemic inflammatory responses and consequent tissue or organ damage[42].

In *vitro* characterization of cultured AMSCs was proved to be positive for CD44marker. Multiple Pb positive spindle cells and numerous CD44 positive cells appeared among the various layers of the cornea. The multiplicity of labeled undifferentiated cells can be referred to the migration of exogenous in addition to endogenous SCs. Ziaei *et al*[43] pointed to the potential therapeutic benefit of SCs in corneal epithelial, stromal and endothelial disorders.

In group E (AMSCs and GTE Group), accidental vacuolated epithelial cells and only few separated collagen bundles were found. Obvious CK3 IE was detected in all epithelial cells, only few epithelial cells and accidental keratocytes showed positive caspase3 IE. The previous results denoted the most remarkable ameliorating effect of combined GTE and AMSCs therapy, suggesting a potentiating and activating effect of the exogenous MSCs on the endogenous cells. In addition, Cazzola *et al*[44] proved the capability of GTE active ingredients to enhance cell plasticity.

Less Pb positive and CD44 positive cells were found near the epithelium and among substantia, with a decrease in the count versus AMSCs group. This finding may be explained by more pronounced differentiation and trans-differentiation of AMSCs when combined with GTE, being an antioxidant and an anti-inflammatory agent, it may provide a better environment for the SCs. This was confirmed by Edrees *et al*[45], who used vitamin E as an antioxidant combined with bone marrow MSCs.

CONCLUSION

In conclusion, T1D induced corneal inflammatory and degenerative morphological changes. Both GTE and AMSCS proved amelioration of morphological and quantitative parameters, the amelioration was more remarkable in response to AMSCs therapy. Combined GTE and AMSCs therapy guaranteed the most remarkable effect.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير المحتمل لمستخلص الشاي الأخضر والعلاج بالخلايا الجذعية الدهنية على إعتلال القرنية في النوع الأول من السكري المحدث تجريبي في نموذج الفأر

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الخلفية والأهداف: التغيرات المورفولوجية في قرنية مرضى السكري متكررة. مستخلص الشاي الأخضر هو منتج طبيعي ثبت أن له تأثيرات مضادة للأكسدة ومضادة للالتهابات. تُستخدم الخلايا الجذعية الوسيطة الدهنية (AMSCs) بشكل شائع. الهدف من الدراسه: يهدف العمل الحالي إلى دراسة ومقارنة تأثير العلاج AMSCs بشكل فردي أو بالاشتراك مع مستخلص الشاي الأخضر في اعتلال القرنية الذي يعقد مرض السكري من النوع الأول (T١D) في ذكور الجرذان البالغة.

الطرق والنتائج: تم تقسيم ٣٨ فأرًا ذكرًا بشكل عشوائي إلى: مجموعة الدراسة في المختبر: فئران للعزل وتجهيز AMSCs. مجموعة السيطرة (المجموعة أ): تتكون من ٨ حيوانات.لحدوث مرض السكري: تم تحفيز ٢٨ فأراً لتكون مصابة بالسكري عن طريق حقنة واحدة داخل الصفاق (IP) قدرها ٥٠ ملغم / كغم من الستربتوزوتوسين (STZ)، ثم بعد ٤ أسابيع من تحريض مرض السكري تم تقسيمهم إلى المجموعات التالية. مجموعة مرضى السكري (المجموعة ب): سبعة حيوانات تركت دون علاج. مجموعة مستخلص الشاي الأخضر (المجموعة ت): سبع حيوانات تم إعطاؤها مستخلص الشاي الأخضر ٥٠ ملغم / كغم يومياً عن طريق الفم. مجموعة AMSCs (المجموعة ج): سبعة حيوانات تم إعطاؤها حقنة في الوريد (IV) مكونة من ١ × ١٠٦ خلايا AMSCs ومجموعة AMSCs (المجموعة ع): سبعة حيوانات تم إعطاؤها مزيجًا من حقن AMSCs و مستخلص الشاي الأخضر.

النتائج: تم التضحية بالحيوانات من جميع المجموعات بعد ٨ أسابيع من بداية التجربة. تم إجراء أبعاد القرنية والدراسات المصلية، والكيميائية الحيوية والمورفولوجية والكمية. كشفت المجموعة (ب) عن تغيرات التهابية وتنكسية في الطبقات المختلفة التي تراجعت بسبب العلاجات المختلفة.