Research Article
Administration of Melatonin in Diabetic Retinopathy Is Effective and Improves the Efficacy of Mesenchymal Stem Cell Treatment

Samraa H. Abdel-Kawi and Khalid S. Hashem

1Department of Medical Histology and Cell Biology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt 62511
2Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt 62511

Correspondence should be addressed to Khalid S. Hashem; khaled.mohamed2@vet.bsu.edu.eg

Received 23 October 2021; Accepted 26 March 2022; Published 11 April 2022

1. Introduction

Diabetes mellitus (DM) affects the body’s metabolism. Diabetes complications have recently become a prominent issue [1]. These complications include many disorders such as retinal, renal, neuronal, and cardiovascular disruption [2].

Diabetic retinopathy (DR) is defined as a vascular alteration in the retinal cells. This change has the potential to cause blindness and vision loss. The pathophysiological mechanisms of DR are not fully understood [3]. Long-term hyperglycemia increases the production of reactive oxygen species (ROS) in the retina, which is the most well-established mechanism that could explain DR. [4] Furthermore, inflammatory mediators such as TNF, interleukins (ILs), and cyclooxygenase-II (COX-II) play a crucial role in the etiology of DR. [5]

In response to diabetic-induced tissue injury, pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) are formed [6] and it is thought that VEGF is the primary cause in the vascular alterations and abnormalities in DR. [7] A prior study discovered a link between excessive VEGF production and lipid peroxidation in DR. [8] Apolipoprotein A1 (APOA1) and retinol-binding protein (RBP4) have recently been discovered to be more significantly expressed in retinal degenerative diseases and retinal detachment [9]; such overexpression

Stem cell transplantation is a promising therapeutic technique for the treatment of a variety of diseases; nevertheless, stem cell therapy may not always work as well as it could. The goal of this study was to test the hypothesis that employing a powerful antioxidant like melatonin improves stem cell transplantation success and potentiates stem cell function in the therapy of diabetic retinopathy. For this purpose, 50 adult male rats were divided into the following: control group: this group received 0.5 ml of 0.1 M of sodium citrate buﬀer (pH = 4.5) (intraperitoneal (I.P.)). The conﬁrmed diabetic rats were divided into 4 groups: diabetic group: conﬁrmed diabetic rats received no treatments with a regular follow of the blood glucose proﬁle for 8 weeks; melatonin group: conﬁrmed diabetic rats received melatonin (5 mg/kg/day); stem cell group: the conﬁrmed diabetic rats were given intravitreal injection of stem cells (2μl cell suspension of stem cells (3×10⁴ cells/μl)); and melatonin+stem cell group: conﬁrmed diabetic rats received melatonin (5 mg/kg/day), orally once daily for 8 weeks, and 2μl cell suspension of stem cells (3×10⁴ cells/μl) was carefully injected into the vitreous cavity. Our results showed that administration of melatonin and/or stem cell restored the retinal oxidative/antioxidant redox and reduced retinal inﬂammatory mediators. Coadministration of melatonin and stem cells enhanced the number of transplanted stem cells in the retinal tissue and signiﬁcantly reduced retinal BDEF, VEGF, APOA1, and RBP4 levels as compared to melatonin and/or stem alone. We may conclude that rats treated with melatonin and stem cells had their retinal oxidative/antioxidant redox values restored to normal and their histological abnormalities reduced. These ﬁndings support the hypothesis that interactions with the BDEF, VEGF, APOA1, and RBP4 signaling pathways are responsible for these effects.

Copyright © 2022 Samraa H. Abdel-Kawi and Khalid S. Hashem. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
indicates the massive damage of the barriers of the retina and so a dysfunction and damage of retina is developed. APOA1 is one of the most important components of high-density lipoprotein (HDL). Although, RBP4 is a hepatic adipocyte that acts as a transporter protein, many previous studies reported that RBP4 is incorporated in retinal dysfunction and degeneration [10, 11].

Many limitations and restrictions have been reported to the current therapies of DR, and most of these traditional therapies are not effective. So, it becomes a provoked need to find an effective approach for managing DR. [12] Moreover, controlling blood glucose and blood pressure is considered a potent protocol to control the massive progression of retinal damage in DR. [13] In the therapy of progressed damage retina in DR, photocoagulation by laser, intraocular injection of anti-VEGF, and steroid injection are all viable therapies. [14] As mentioned previously, many researchers seek alternative effective therapies for DR.

The transplantation of stem cell is considered a modern effective promising interference which can restore the damaged tissues and cells. This effect could be attributed to stem cells’ ability to proliferate and differentiate [15].

Stem cell transplantation in the retinal tissue is an effective and appealing technique for replacing or repairing damaged retinal pigment epithelium and photoreceptors [16]. Retinal stem cell transplantation is a viable alternative therapy for patients with DR who want to regain their vision [17, 18].

Transplantation of stem cells in the retina has many advantages, but there are many limitation and disadvantages of using stem cells such as low survival rate. This is considered one of the key reasons which restricts the efficiency of stem cell treatment [19, 20]. Inflammation and oxidative damage are considered the primary bulwark against the proper survival and implantation of stem cells. Many earlier studies suggested that the antioxidant content of the stem cells has the ability to inhibit the ROS and inflammatory response at the injured tissue. As a result, stem cell transplantation could protect against ROS-induced apoptosis [21]. As information regarding the effect of using an exogenous antioxidant on the antistress ability of stem cells against exogenous stresses, particularly on DR, is lacking, our aim was to investigate the effect of using a well-established antioxidant on the efficacy of stem cell transplantation in DR.

Melatonin’s unique molecular structure confers a powerful antioxidant action since it is both lipophilic and hydrophilic. The unique characteristics of melatonin enable it to easily traverse all body barriers and enter in numerous organelles, including mitochondria, where ROS are formed [22]. It also possesses direct free radical scavenging characteristics due to its ability to create a variety of antioxidant enzymes which regulate the oxidative/antioxidant redox such as glutathione peroxidase (GPx) and glutathione reductase (GR) which have an important role in regulating GSH metabolism, catalase (CAT), and superoxide dismutase (SOD) which have free radical scavenging activity [23].

Melatonin has the ability to reduce the inflammatory reaction via inhibiting many inflammatory mediators [24]. This anti-inflammatory action could be linked to its capacity to suppress NF-B activation, which expresses various inflammatory genes like IL-6, TNF, and IL-1 [8]. Furthermore, melatonin has an anti-inflammatory impact through inhibiting COX-II [25]. In light of this, our target is to evaluate the role of melatonin in increasing stem cell anti-ROS capability and therapeutic efficiency in DR, as well as the relationship between ROS, inflammatory, and vasculogenic mediators.

2. Materials and Methods

2.1. Chemicals: Streptozotocin (STZ). The streptozotocin vial (1 g) was provided by Sigma Company (Sigma-Aldrich, Egypt) in the form of powder.

2.2. Isolated and Cultivation of Bone Marrow-Derived Mesenchymal Stem Cells (BM-Derived MSCs). MSCs were isolated for a period of four weeks. Rats were sacrificed; then, the bilateral femora and tibias were extracted and put in Dulbecco’s modified Eagle medium (DMEM; Gibco/BRL) under sterile circumstances. MSCs were isolated and cultivated according to Jiang et al. [26].

2.3. Animals. The experiment involved 50 mature male albino rats weighing 200-250 g on average. Before the experiments, the rats were given a two-week acclimatization period in the laboratory. They were housed in metal cages (3 rats in each cage) and kept in conventional laboratory conditions, including (25 ± 2°C) room temperature, 70 percent relative humidity, and a 12-hour dark-light cycle with free access to food and water. The National Institutes of Health (NIH) standard for the laboratory animal use was followed for all procedures involving drug administration and tissue and blood collection (NIH Publications No. 8023, revised 1978).

2.4. Induction of Diabetes. STZ (60 mg/kg BW, I.P.) was injected to create diabetes [7, 27, 28]. All animals were starved for twenty-four hours before induction of diabetes. Diabetic rats were defined as those that had blood glucose level of 200 mg/dl or higher after 48 hours of the injection and were monitored for 8 weeks [29].

2.5. Experimental Animals. After the end of the acclimation period, these rats were divided into five equal groups (n = 10): control group: animals were I.P. injected with 0.5 ml of 0.1 M sodium citrate buffer (pH = 4.5) (intraperitoneal (I.P.)). The confirmed diabetic rats were divided into 4 groups: diabetic group: confirmed diabetic rats received no treatments with a regular follow of the blood glucose profile for 8 weeks; melatonin group: confirmed diabetic rats were orally administered melatonin (5 mg/kg/day) [30], as a suspension in 1 mL of 0.1 M sodium citrate buffer (pH = 4.5) once daily till the end of the 8th week; stem cell group: 4 weeks later to the confirmation of diabetes [31], the confirmed diabetic rats were given intravitreal injection of stem cells. To begin, diabetic rats were sedated with 2 percent pentobarbital sodium intraperitoneally, and the limbs and head were well secured to allow access to the eyes. From the corneoscleral limbus of the eye, a 101 microsyringe
linked to a 30 G needle was inserted into the vitreous cavity, and 21 stem cell suspension (3104 cells/l) was gently injected into the vitreous cavity. All injections were effective, as evidenced by no bleeding after 30 seconds of observation [32], and rats were kept under careful observation for about 4 weeks later [33]. Melatonin+stem cell group: confirmed diabetic rats were given melatonin (5 mg/kg body weight once daily for 8 weeks) [30], as an oral suspension. For preparing the suspension, 1 mL of 0.1 M sodium citrate buffer (pH = 4.5) was used. Then, 2 μl suspension of stem cells (3 × 10⁴ cells/μl) was carefully injected into the vitreous cavity [32] and still under observation 28 days later. At the end of the experiment, blood samples were collected from the medial canthus of the eye, and then, rats were sacrificed by decapitation [33] (Figure 1).

2.6. Vitreous Sample Collection. Vitreous samples were suctioned directly into a 5 ml syringe using a three-port 25-gauge transconjunctival suture-less vitrectomy device (TSV25G; Alcon Constellation; Alcon Laboratories, Fort Worth, TX) according to Ding et al. [34].

2.7. Retina Collection for Biochemical Investigations. The retinas of the left eyes were dissected as soon as rats were killed and kept refrigerated at -70°C until biochemical studies could be performed. For the biochemical experiments, retina samples were homogenized using a tissue homogenizer in a cold phosphate-buffered saline (diluted as 1:5; pH 7.2) (Ortaolresa, Spain). After centrifuging homogenates at 10,000 g for 30 minutes, the supernatants were stored at -80°C for biochemical tests.

2.8. Fluorescent Microscopic Examination. To elucidate fluorescent-labeled mesenchymal stem cells, sections of stem cells and melatonin+stem cells were examined under fluorescent microscopy (Figure 2). The bromodeoxyuridine (BrDU-) positive cells in the sections were identified by staining the section with rat anti-BrDU (1:100, Neomarkers) and goat anti-rat Ig GFITC (1:100, Kpl) [35] (Figure 2).

2.9. Measurement of Blood Glucose, Glycated Hemoglobin A1C (Hba1C), and C-Peptide Linkage. A glucose assay kit (Sigma-Aldrich, Egypt, Cat. No. GAGO20) was used to determine the blood glucose level. For detecting HbA1C, glycatedhemoglobin absorbance and total hemoglobin fraction were measured at 415 nm compared to the prepared standard hemoglobin [36]. A C-peptide ELISA assay kit (Sigma-Aldrich, Egypt, Product no. EZRMCP2) was used for detecting C-peptide linkage.

2.10. Measurement of Retinal Oxidant/Antioxidant Redox. All the used kits for measuring the retinal oxidant/antioxidant status were purchased from Sigma-Aldrich, Egypt. A GSH (reduced glutathione) test kit (Cat. no. 099M4064V) [37], glutathione reductase (GR) test kit (Cat. no. GRSA) [38], catalase (CAT) assay kit (Cat. no. CAT 100) [39], superoxide dismutase activity (SOD) assay kit (Cat. no. BCCC1068) [40], total antioxidant capacity (TAC) (Cat. no. 059M4154V) [41], and malondialdehyde (MDA) assay kit (Cat. no. 6A20K07390) [42] were used to evaluate the retinal oxidant/antioxidant redox.

2.11. Measurement of Retinal Inflammatory Markers. All the used kits for measuring the retinal inflammatory markers were purchased from Sigma-Aldrich, Egypt. A tumor necrosis factor-α (TNF-α) ELISA kit (Cat. no. RAB0479), interleukin 1-β (IL-1β) ELISA kit (Cat. no. RAB0278), interleukin 6 (IL-6) ELISA kit (Cat. no. RAB0311), and cyclooxygenase II (COX-II) ELISA kits (Cat. no. RAB1034) were used.

2.12. Measurement of BEDF, VEGF, APOA1, and RBP4 by Using the Western Blot Technique. The concentrations of cerebral BEDF, VEGF, APOA1, and RBP4 were determined by immunoblotting with the appropriate antibody, and the proteins were subsequently separated by gel electrophoresis according to their molecular weight and the intensity of the bands was compared to that of β-actin using the image analysis programme ChemiDoc MP imager (Markham Ontario L3R 8T4, Canada) [43, 44].

2.13. Retina Preparation for Histological Study. Each rat’s tissue (retina) was evaluated graphically after the eye was removed. The tissue was taken for histological examination, washed in normal saline, and immediately immersed in 10% buffered formalin. According to conventional methods, they were gradually dehydrated, paraffin embedded, sectioned into 5 µm slices, and stained with hematoxylin and eosin for histologic evaluation [45].

2.14. Immunohistochemical Study for Detection of COX-II and VEGF. To remove paraffin, the sections were submerged in dimethyl benzene for 30 minutes and then rinsed in aqueous ethanol for 5 minutes each time. Endogenous peroxidases were then blocked for 15 minutes with 0.3 percent hydrogen peroxidase before being rinsed with ultrapure water. After that, the sections were treated by microwaving them for 10 minutes at 700 W in 0.01 M citric acid buffer (pH 6.0). An anti-VEGF polyclonal antibody (RB-222-R7; Lab Vision Corporation Laboratories, Fremont, California, USA) and anti-COX-II polyclonal antibody (Cat. No. 06-735; Sigma-Aldrich, Egypt) were incubated overnight at 4°C on the sections after thorough washing. After that, the pieces were immersed in DAB and multispectral image analysis was used to quantify them [46].

2.15. Morphometric Study. The morphometric measurements were done by using a Leica Qwin 500 LTD image analyzer (Leica, Cambridge, UK). All measurements were taken in five sections from VEGF and anti-cox-2 immunostained sections from each animal. The mean area percentage of COX-II and VEGF immunoreactivity was measured in 10 random nonoverlapping fields per section using a binary mode with ×40 objective lens.

2.16. Statistical Analysis. SPSS software, version 16, was used for statistical analysis. All data were expressed as mean value ± standard error (SE). For comparison of rat groups, a one-way analysis of variance (ANOVA) test will be utilized, followed by the Tukey–Kramer post-ANOVA test.
$P$ value less than 0.05 ($P < 0.05$) was considered to indicate a significant difference.

3. Results

3.1. Changes in the Glucose Profile of Different Treated Groups. Our results in Table 1 showed a significant increase in blood glucose level and HbA1C (74.4%, 271.6%), respectively, as compared to the control group. Melatonin administration significantly reduced blood glucose level and HbA1C (29.6%, 37.1%), respectively, in comparison to the diabetic group. Stem cell administration showed a nonsignificant decrease in blood glucose level and HbA1C as compared to the diabetic group. Coadministration of melatonin and stem cells in the melatonin+stem cell group significantly decreased the blood glucose level and HbA1C (30.7%, 42.3%) as compared to the diabetic group. Moreover, C-peptide linkage showed a significant reduction in the diabetic group (92.7%) as compared to the control group. Melatonin administration significantly increased C-peptide linkage (600%) as compared to the diabetic group. Stem cell administration showed a nonsignificant difference in C-peptide linkage as compared to the diabetic group. Coadministration of melatonin and stem cells in the melatonin+stem cell group showed a significant increase in C-peptide linkage (733%) as compared to the diabetic group.

3.2. Changes in Retinal Oxidative/Antioxidant Status. Our results in Table 2 showed a significant decrease in GSH concentration, GR, CAT, and SOD activities, and TAC content...
with a significant increase in MDA level as compared to the control group ($P < 0.05$). Melatonin administration significantly increased GSH concentration, GR, CAT, and SOD activities, and TAC content and significantly reduced MDA level as compared to the diabetic group at $P < 0.05$. Also, stem cell administration significantly increased GSH concentration, GR, CAT, and SOD activities, and TAC content and significantly reduced MDA level as compared to the diabetic group at $P < 0.05$. Moreover, there were no significant differences of oxidant/antioxidant status between melatonin, stem cell, and melatonin+stem cell, and control groups at $P < 0.05$.

### 3.3. Changes of Retinal Inflammatory Markers in Different Treated Groups

Our results in Table 3 showed a significant increase in TNF-$\alpha$, IL-1$\beta$, IL-10, and COX-II levels as compared to the control group at $P < 0.05$. Melatonin administration significantly reduced TNF-$\alpha$, IL-1$\beta$, IL-10, and COX-II levels as compared to the diabetic group at $P < 0.05$. Also, stem cell administration significantly decreased TNF-$\alpha$, IL-1$\beta$, IL-10, and COX-II levels as compared to the diabetic group at $P < 0.05$. Coadministration of melatonin and stem cells in the melatonin+stem cell group significantly decreased TNF-$\alpha$, IL-1$\beta$, IL-10, and COX-II levels as compared to the diabetic group at $P < 0.05$. Moreover, there were no significant differences of oxidant/antioxidant status between melatonin, stem cell, and melatonin+stem cell, and control groups at $P < 0.05$.

### Table 1: Changes in the blood glucose profile in different treated groups.

|               | Blood sugar | HbA1C   | C-peptide linkage |
|---------------|-------------|---------|-------------------|
| Control       | 69 ± 1.2    | 4.31 ± 0.56 | 4.16 ± 0.02      |
| Diabetic      | 270 ± 2.1$^a$ | 15.98 ± 0.93$^a$ | 0.3 ± 0.001$^a$ |
| Melatonin     | 190 ± 1.1$^{a,b}$ | 10 ± 0.86$^{a,b}$ | 2.1 ± 0.01$^{a,b}$ |
| Stem cells    | 268 ± 2.3$^a$ | 14.2 ± 1.05$^a$ | 0.45 ± 0.001$^a$ |
| Melatonin + stem cells | 187 ± 1.2$^{a,b}$ | 9.04 ± 0.23$^{a,b}$ | 2.5 ± 0.01$^{a,b}$ |

Values were expressed as means ± SE. $^a$ indicates a significant difference as compared to the control group at $P$ value $< 0.05$, and $^b$ indicates a significant difference as compared to the diabetic group at $P$ value $< 0.05$. 

---

**Figure 2:** Photomicrograph of sections of retinas shows a positive immunofluorescent: (a, c) stem cell group (50 $\mu$m and 100 $\mu$m, respectively) and (b, d) melatonin+stem cell group (50 $\mu$m and 100 $\mu$m, respectively).
Table 2: Changes in retinal oxidative/antioxidant status.

|               | GSH  | GR   | CAT  | SOD  | TAC  | MDA  |
|---------------|------|------|------|------|------|------|
| Control       | 50 ± 1.09 | 70 ± 0.76 | 90 ± 0.43 | 66 ± 0.76 | 210 ± 1.87 | 27.54 ± 1.09 |
| Diabetic      | 10.12 ± 0.98<sup>a</sup> | 20 ± 0.23<sup>a</sup> | 8.43 ± 0.06<sup>a</sup> | 15 ± 0.73<sup>a</sup> | 51.32 ± 1.33<sup>a</sup> | 85 ± 2.09<sup>a</sup> |
| Melatonin     | 45 ± 1.11<sup>b</sup> | 66.2 ± 2.01<sup>b</sup> | 87.22 ± 2<sup>b</sup> | 63.12 ± 1.3<sup>b</sup> | 200 ± 1.02<sup>b</sup> | 26.32 ± 1.08<sup>b</sup> |
| Stem cells    | 44.22 ± 1.11<sup>b</sup> | 63 ± 1.71<sup>b</sup> | 84 ± 1.04<sup>b</sup> | 61.2 ± 2.09<sup>b</sup> | 197 ± 2.09<sup>b</sup> | 30.12 ± 1.03 |
| Melatonin+stem cells | 48.2 ± 0.76<sup>b</sup> | 67.32 ± 1.09<sup>b</sup> | 88.43 ± 1.06<sup>b</sup> | 64.41 ± 0.72<sup>b</sup> | 208 ± 1.54<sup>b</sup> | 25.1 ± 1.6<sup>b</sup> |

Values were expressed as means ± SE. <sup>a</sup> indicates a significant difference as compared to the control group at P value < 0.05, and <sup>b</sup> indicates a significant difference as compared to the diabetic group at P value < 0.05.

|               | TNF-α | IL-1β | IL-6 | COX-II |
|---------------|-------|-------|------|--------|
| Control       | 25 ± 0.19 | 20 ± 0.76 | 32 ± 0.13 | 30 ± 0.96 |
| Diabetic      | 90.13 ± 0.98<sup>a</sup> | 110 ± 0.53<sup>a</sup> | 160.8 ± 0.96<sup>a</sup> | 200 ± 0.49<sup>a</sup> |
| Melatonin     | 45 ± 1.12<sup>ab</sup> | 66.2 ± 2.01<sup>ab</sup> | 89 ± 1.1<sup>ab</sup> | 51.14 ± 0.53<sup>ab</sup> |
| Stem cells    | 46.24 ± 1.1<sup>ab</sup> | 85 ± 1.71<sup>ab</sup> | 90 ± 1.4<sup>ab</sup> | 67.2 ± 0.99<sup>ab</sup> |
| Melatonin+stem cells | 27.02 ± 0.6<sup>b</sup> | 24.36 ± 0.12<sup>b</sup> | 35.44 ± 0.16<sup>b</sup> | 32.11 ± 0.12<sup>b</sup> |

Values were expressed as means ± SE. <sup>a</sup> indicates a significant difference as compared to the control group at P value < 0.05, and <sup>b</sup> indicates a significant difference as compared to the diabetic group at P value < 0.05.

compared to the diabetic group at P < 0.05. Moreover, there were no significant differences in TNF-α, IL-1β, IL-10, and COX-II levels in the stem+melatonin group and control group at P < 0.05.

3.4. Changes of BEDF, VEGF, APOA1, and PRP4 of Different Treated Groups. Our results in Figure 3 showed a significant increase in BEDF, VEGF, APOA1, and PRP4 protein concentrations as compared to the control group at P < 0.05. Melatonin administration significantly reduced BEDF, VEGF, APOA1, and PRP4 protein concentrations as compared to the diabetic group at P < 0.05. Also, stem cell administration significantly decreased BEDF, VEGF, APOA1, and PRP4 protein concentrations as compared to the diabetic group at P < 0.05. Coadministration of melatonin and stem cells in the melatonin+stem cell group significantly decreased BEDF, VEGF, APOA1, and PRP4 protein concentrations as compared to the diabetic group at P < 0.05. Moreover, there were no significant differences in BEDF, VEGF, APOA1, and PRP4 protein concentrations in the stem+melatonin group and control group at P < 0.05.

3.5. Histopathological Study of Retinal Tissues. As shown in Figure 4(a), the control group shows the normal architecture of well-organized layers of the retina consisting of the photoreceptor layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, and ganglion cell layer. The diabetic group shows disorganization of retinal layers with near-total disappearance of nuclei of the outer nuclear layer, disruption of the outer plexiform layer, and appearance of empty spaces within the inner nuclear layer. Neovascularization in the ganglion cell layer with dilated congested blood capillaries is also noticed (Figure 4(b)). It shows apparent marked reduction of retinal thickness in comparison with the control group and marked disorganization of retinal layers with the appearance of spaces within the outer nuclear layer and many spaces in the inner nuclear layer. Multiple cavities within the photoreceptor layer are also detected. Retinal folding with disruption of the outer plexiform layer is seen, and dilated congested blood vessels and a reduction in the counts of ganglion cells in the ganglion cell layer can be detected (Figure 4(c)). It shows neovascularization in the ganglion cell layer (Figure 4(d)). It shows an increased number of supporting cells with dark nuclei within the ganglion cell layer (Figure 4(e)). Melatonin and stem cells groups, respectively, show well-organized retinal layers but with the appearance of many small empty spaces in the photoreceptor layer, outer nuclear layer, and inner nuclear layer. Neovascularization is still found in the ganglion cell layer (Figures 4(f) and 4(g)). In the melatonin+stem cell group, the total retinal thickness is preserved, the inner nuclear layer appears with higher cell density, the inner plexiform layer preserves its reticular appearance with no widening of the spaces between its fibers, and the ganglion cell layer consisted of one row of ganglion cells (Figure 4(h)).

3.6. Changes of COX-II Immunohistochemical Reaction in Retinal Tissues in Different Groups. As shown in Figure 5(a), the control group showed no immunoreactivity. In Figure 5(b), the diabetic group showed a strong positive COX-II immunoreactivity (brown color). In Figures 5(c) and 5(d), melatonin and stem cell groups, respectively, displayed a positive immunoreactivity (brown color). In
Figure 3: Continued.
Figure 3: Continued.
Figure 3: The changes of BDEF, VEGF, ABOA1, and PRP4 protein expression in different groups. The effect of melatonin and/or stem cell on diabetic rats on the expression levels of BDEF (a), VEGF (b), ABOA1 (c), and PRP4 (d) and (e) images of western blotting bands of the measured protein expression in different groups. Data are presented as mean ± SE (n = 10). a indicates a significant difference compared to the control group, and b indicates a significant change compared to the diabetic group at P<0.05 using ANOVA followed by Tukey-Kramer as the post-ANOVA test.

3.7. Changes of VEGF Immunohistochemical Reactions in Retinal Tissues of Different Groups. As shown in Figure 6(a), the control group showed no immunoreactivity. In Figure 6(b), the diabetic group showed a strong positive VEGF immunoreactivity (brown color) in the ganglion cell layer (GCL). In Figures 6(c) and 6(d), the melatonin and stem cell groups, respectively, displayed a positive immunoreactivity (brown color) in GCL. In Figure 6(e), the melatonin+stem cell group showed a negative immunoreactivity. In Figure 6(f), the optical density of VEGF was represented as means ± SE (n = 10). a indicates a significant difference as compared to the diabetic group at P<0.05. b indicates a significant difference as compared to the diabetic group at P<0.05. The magnification is ×200.

4. Discussion

Diabetic retinopathy (DR) is provoked microvascular sequelae of diabetes mellitus (DM) which may cause a vision loss [47]. The rapid progression of DM and its complication worldwide give DR great importance [48, 49]. Oxidative and inflammatory stressors are considered crucial limiting factors in the formation of DR. [50]

The overproduction of ROS impairs the retinal vessels, which leads to the development of DR. Diabetes-induced oxidative damage in the retina could be attributed to the activation of nuclear factor-κB (NF-κB) [51] and reduction nuclear factor erythroid 2-related factor 2 (Nrf2) expression [52]. Based on our obtained results, the disturbance of retinal oxidative/antioxidant redox is manifested by reduction of retinal GSH concentration, GR, CAT, and SOD activities, and TAC with a concurrent increase in MDA concentration. Even if blood glucose levels return to normal, oxidative damage produced by diabetes might linger for a long time [53]. Additionally, hyperglycemia triggers many inflammatory mediators in the retinal vasculature such as IL-1β, TNF-α, IL-6, and COX-II [54]. Moreover, inflammation, oxidative stress, and autophagy all play a role in the etiology of diabetic retinopathy [23].

PEDF is a glycoprotein that was discovered in foetal human retinal pigment epithelial cell culture for the first time [55]. It has strong antioxidant properties and is abundantly expressed in the retina. PEDF expression was shown to be higher in the diabetic group compared to the control group, which is consistent with previous findings [56]. Other researchers, on the other hand, found that hyperglycemia caused a considerable drop in PEDF expression [57]. The overexpression of PEDF expression could be a protective response of the retina to the diabetic effect [58, 59].

VEGF overexpression has been observed in the retinas and vitreous humors of diabetic animals and people in a number of earlier studies [60]. VEGF is produced in retinal tissue in response to a variety of stimuli [61]. In early DR, VEGF increases the retinal vascular permeability [62]. The fundamental cause of mediating VEGF and the development of DR is thought to be oxidative stress [63].

Lipids have an important role in the onset of DR. APOA1 is one of the fundamental proteins which enters in the formation of HDL. It removes deleterious oxidized lipids from the retinal tissue [64].

In agreement with our findings, Simó et al. recorded that vitreous humor content of APO1 significantly increased in DR. [65] The overexpression of APOA1 could be attributed to its potent scavenging power for oxidative reactants [66] and anti-inflammatory effect [67].

RBP4 is considered one of the most important cardiovascular protective agents, and it is closely related to insulin resistance [68]. Previous studies documented the role of RBP4 in early vascular dysfunction [69, 70].

Many proinflammatory mediators such as TNF-α, IL-1β, IL-6, and COX-II were stimulated by RBP4 [71]. The overexpression of RBP4 in the diabetic group induces endothelial cell inflammation, which, followed by an impairment of retinal vascularity, leads to too much increase in the retinal vascular permeability [34] and progressive retinal degeneration [72].

The results of our histology analysis showed empty spaces between the outer and inner nuclear layers. An apparent loss in retinal thickness and disorganized retinal layers are detected in DR. It was also shown that the outer and
Figure 4: Continued.
As mentioned previously, treatment with a well-known potent anti-inflammatory and an antioxidant agent such as melatonin has a great effect on DR. In diabetic rats, a significant reduction of melatonin formation has been recorded. This reduction could be attributed to the low expression of melatonin production-regulating enzymes such as aryl alkyl amine N-acetyl transferase (AANAT) [76]. Consistent with our results, the concentration of melatonin was lower in patients with DR than non-DR individuals [77]. Melatonin has also been shown to reduce oxidative stress, which helps to alleviate histopathological changes in the retina [78].

Melatonin activates the PI3K/Akt-Nrf2 signaling pathway, which boosts cellular antioxidant defences and lowers VEGF production [8]. Melatonin’s capacity to reduce ROS and malondialdehyde (MDA) as well as control apoptosis and inflammation in diabetic retinopathy rats by regulating the MAPK pathway could explain the lower BEDF in the melatonin group compared to the diabetic group [79]. The protective effect of melatonin on the retinal tissue was previously studied by Djordjevic et al. [80]. The significant reduction of APOA1 in the melatonin group might be due to the consumption of APOA1 in HDL synthesis [81]. RBP4 expression was reduced in the melatonin group compared to the diabetic group. As previously discussed, RBP4 is released in response to retinal vascular degeneration. In accordance with our results, a significant reduction of retinal vascular damage, cytokines, and other inflammatory mediators was recorded with melatonin administration [7].

The use of stem cells to heal injured neural tissues becomes a viable option. Following the transplantation, there was a gradual integration of the transplanted stem cells into the retinal milieu, as well as proliferation and differentiation of the transplanted stem cells into target cells [82]. However, these therapies’ efficacy falls short of expectations [83]. There are many challenges and limitations of the use of stem cell transplantation in retinal degeneration and DR. Following stem cell transplantations, many previous studies have reported retinal detachment, visual loss [84], and inflammation [85]. Moreover, the low survival rate of transplanted stem cells represents one of the main obstacles in stem cell therapy. The low survival rate could be attributed to the oxidative stress and inflammatory environment at the injured site [86]. So it is a logical approach to counter these effects by using a potent antioxidant and anti-inflammatory substance. Many previous
cotreatments in stem cell therapy have been studied, such as N-acetylcysteine (NAC), which dramatically enhanced the survival of muscle-derived stem cells (MDSCs) and cardiac function in an acute myocardial infarction model [87].

In diabetic retinopathies, there are not enough data on antioxidant cotreatment with stem cells. So, in this work, we looked into the therapeutic effects of melatonin in combination with MSCs on DR.

MSCs reduced hyperglycemia-induced histological alterations in the retinas of STZ-induced diabetic rats, according to our findings. Furthermore, we noticed that MSCs’ therapeutic activity is due to their ability to decrease oxidative stress and suppress VEGF release. Because of their anti-inflammatory and antiangiogenic properties, MSCs have therapeutic potential in DR.

In the present study, histopathological examination and morphometric analysis revealed that MSCs were able to improve histopathological alterations of DR by preserving retinal thickness and organization of its different layers. The protection of the retina against oxidative stress and downregulation of VEGF expression may be responsible for the improvement shown with MSCs.

Figure 2 shows the capacity of melatonin to improve stem cell survival and implantation in retinal tissue. This

![Figure 5: Photomicrograph of COX-II immunohistochemically stained retinal sections (magnification, ×200). (a) Control group showed no immunoreactivity. (b) Diabetic group showed a strong positive COX-II immunoreactivity (brown color). (c, d) Melatonin and stem cell groups, respectively, displayed a positive immunoreactivity (brown color). (e) The melatonin+stem cell group showed a negative immunoreactivity. (f) The optical density of COX-II was represented as means ± SE (n = 10). a indicates a significant difference as compared to the control group at P < 0.05. b indicates a significant difference as compared to the diabetic group at P < 0.05.](image-url)
finding is supported by a previous study which indicated that melatonin medication improved the efficacy of adipose-derived mesenchymal stem cell (ADSC) therapy in rats with acute interstitial cystitis [88].

5. Conclusion

In conclusion, the present study demonstrates that administration of melatonin alleviates oxidative and inflammatory changes in DR. Administration of melatonin and stem cell in DR restores retinal oxidative/antioxidant redox and reduces retinal inflammatory mediators. Melatonin is a promising supportive therapy with stem cell transplantation as it has the capability to regulate retinal BEDF, VEGF, APOA1, and RBP4 gene expression.

Data Availability

Data are available when requested.

Ethical Approval

The Experimental Animal Ethics Committee of Beni-Suef University’s Faculty of Veterinary Medicine was the guide
of this study, and all experimental procedures followed the National Institutes of Health’s (NIH) standard for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

K.H. and S.A. were responsible for the conceptualization, methodology, software, and investigation and wrote the original draft. K.H. was responsible for the formal analysis and reviewed and edited the paper. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors acknowledge the staff members of histological and biochemical laboratories, Beni-Suef University, for their kind support and help during all the steps of this work.

References

[1] B.-Y. Peng, N. K. Dubey, V. K. Mishra et al., “Addressing stem cell therapeutic approaches in pathobiology of diabetes and its complications,” Journal of Diabetes Research, vol. 2018, 16 pages, 2018.
[2] N. E. Babiker, A. Gassoum, N. E. Abdelrahem et al., “The progress of stem cells in the treatment of diabetes mellitus type 1,” Progress in Stem Cell, vol. 4, no. 1, pp. 175–188, 2017.
[3] A. K. W. Lai and A. C. Lo, “Animal models of diabetic retinopathy: summary and comparison,” Journal of Diabetes Research, vol. 2013, 29 pages, 2013.
[4] A. M. A. El-Asrar, “Role of inflammation in the pathogenesis of diabetic retinopathy,” Middle East African Journal of Ophthalmology, vol. 19, no. 1, pp. 70–74, 2012.
[5] Y. Woolf, S. M. Man, R. Aggio-Bruce, R. Natoli, and N. Fernando, “IL-1 family members mediate cell death, inflammation and angiogenesis in retinal degenerative diseases,” Frontiers in Immunology, vol. 10, p. 1618, 2019.
[6] T. Curtis, T. Gardiner, and A. Stitt, “Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis,” Eye, vol. 23, no. 7, pp. 1496–1508, 2009.
[7] G. Özdemir, Y. Ergün, S. Bakarıș, M. Kilinc, H. Durdu, and E. Ganiyusufoglu, “Melatonin prevents retinal oxidative stress and vascular changes in diabetic rats,” Eye, vol. 28, no. 8, pp. 1020–1027, 2014.
[8] T. Jiang, Q. Chang, J. Cai, J. Fan, X. Zhang, and G. Xu, “Protective effects of melatonin on retinal inflammation and oxidative stress in experimental diabetic retinopathy,” Biochemistry Research International, vol. 2016, 13 pages, 2016.
[9] Z. Wu, N. Ding, M. Yu et al., “Identification of potential biomarkers for rhegmatogenous retinal detachment associated with choroidal detachment by vitreous t-TRAP-based proteomic profiling,” International Journal of Molecular Sciences, vol. 17, no. 12, p. 2052, 2016.
[10] C. L. Cioffi, N. Dobri, E. E. Freeman et al., “Design, synthesis, and evaluation of nonretinoïd retinol binding protein 4 antagonists for the potential treatment of atrophic age-related macular degeneration and Stargardt disease,” Journal of Medicinal Chemistry, vol. 57, no. 18, pp. 7731–7757, 2014.
[11] C. L. Cioffi, B. Racz, E. E. Freeman et al., “Bicyclic octahydrocyclopenta pyrrolo antagonists of retinol binding protein 4: potential treatment of atrophic age-related macular degeneration and Stargardt disease,” Journal of Medicinal Chemistry, vol. 58, no. 15, pp. 5863–5888, 2015.
[12] Y. Wang, X. Meng, and H. Yan, “Niaspan inhibits diabetic retinopathy-induced vascular inflammation by downregulating the tumor necrosis factor-α pathway,” Molecular Medicine Reports, vol. 15, no. 3, pp. 1263–1271, 2017.
[13] C. Hernández, A. Simó-Servat, P. Bogdanov, and R. Simó, “Diabetic retinopathy: new therapeutic perspectives based on pathogenic mechanisms,” Journal of Endocrinological Investigation, vol. 40, no. 9, pp. 925–935, 2017.
[14] A. Fiori, V. Terlizzi, H. Kremer et al., “Mesenchymal stromal/stem cells as potential therapy in diabetic retinopathy,” Immunobiology, vol. 223, no. 12, pp. 729–743, 2018.
[15] M. L. Graham, J. L. Janecek, J. A. Kittredge, B. J. Hering, and H. J. Schuurman, “The streptozotocin-induced diabetic mouse model: differences between animals from different sources,” Comparative Medicine, vol. 61, no. 4, pp. 356–360, 2011.
[16] W. Zakrzewski, M. Dobrzyński, M. Szymonowicz, and Z. Rybak, “Stem cells: past, present, and future,” Stem Cell Research & Therapy, vol. 10, no. 1, pp. 1–22, 2019.
[17] K. Ben M’Barek and C. Monville, “Cell therapy for retinal dystrophies: from cell suspension formulation to complex retinal tissue bioengineering,” Stem Cells International, vol. 2019, Article ID 4568979, 14 pages, 2019.
[18] K. B. M’Barek, W. Habeler, and C. Monville, “Stem cell-based RPE therapy for retinal diseases: engineering 3D tissues amenable for regenerative medicine,” Retinal Degenerative Diseases, vol. 1074, pp. 625–632, 2018.
[19] K.-H. Ryu, S. Y. Kim, Y. R. Kim et al., “Tonsil-derived mesenchymal stem cells alleviate concanavalin A-induced acute liver injury,” Experimental Cell Research, vol. 326, no. 1, pp. 143–154, 2014.
[20] M. Yuasa, K. Ishiwata, T. Sugio et al., “Herpes simplex virus type 2 fulminant hepatitis after umbilical cord blood transplantation for acute myeloid leukemia,” [Rinsho ketsueki] The Japanese Journal of clinical hematology, vol. 55, no. 6, pp. 682–686, 2014.
[21] T. He, T. E. Peterson, E. L. Holmuhamedov et al., “Human endothelial progenitor cells tolerate oxidative stress due to intrinsically high expression of manganese superoxide dismutase,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 24, no. 11, pp. 2021–2027, 2004.
[22] C. Venegas, J. A. Garcia, G. Escames et al., “Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations,” Journal of Pineal Research, vol. 52, no. 2, pp. 217–227, 2012.
[23] E. Dehdashtian, S. Mehrzadi, B. Yousefi et al., “Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress,” Life Sciences, vol. 193, pp. 20–33, 2018.
[24] R. Jahanban-Esfahan, S. Mehrzadi, R. J. Reiter et al., “Melatonin in regulation of inflammatory pathways in rheumatoid arthritis and osteoarthritis: involvement of circadian clock genes,” British Journal of Pharmacology, vol. 175, no. 16, pp. 3230–3238, 2018.
[25] P. Trivedi, G. B. Jena, K. B. Tikoo, and V. Kumar, “Melatonin modulated autophagy and Nrf2 signaling pathways in mice with colitis-associated colon carcinogenesis,” Molecular Carcinogenesis, vol. 55, no. 3, pp. 255–267, 2016.

[26] T.-S. Jiang, L. Cai, W. Y. Ji et al., “Reconstruction of the corneal epithelium with induced marrow mesenchymal stem cells in rats,” Molecular Vision, vol. 16, pp. 1304–1316, 2010.

[27] A. Gajdosik, A. Gajdosikova, M. Stefek, J. Navarova, and R. Hozova, “Streptozotocin-induced experimental diabetes in male Wistar rats,” General Physiology and Biophysics, vol. 18, pp. 54–62, 1999.

[28] A. Akbarzadeh, D. Norouzian, M. R. Mehrabi et al., “Induction of diabetes by streptozotocin in rats,” Indian Journal of Clinical Biochemistry, vol. 22, no. 2, pp. 60–64, 2007.

[29] M. Cesaretti, M. Ginoza, A. B. Ribeiro, and O. Kohlmann Jr, “Arq Brasileiros de Endocrinologia e Metabologia,” vol. 54, no. 9, pp. 842–851, 2010.

[30] V. Brazão, F. H. Santello, R. P. Colato et al., “Melatonin downregulates steroid hormones, thymocyte apoptosis and inflammatory cytokines in middle-aged T. cruzi infected rats,” Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, vol. 1866, no. 11, article 165914, 2020.

[31] H. Kajiyama, T. S. Hamazaki, M. Tokuhara et al., “Crude enzyme preparation from C. luteus for the direct determination of reducing agent more stable and efficient,” vol. 54, no. 9, pp. 842–851, 2010.

[32] X. Feng, P. Chen, X. Zhao, J. Wang, and H. Wang, “Transplanted embryonic retinal stem cells have the potential to repair the injured retina in mice,” BMC Ophthalmology, vol. 21, no. 1, pp. 1–11, 2021.

[33] P. K. Ngoc, P. V. Phuc, T. H. Nhung, D. T. Thuy, and N. T. M. Nguyet, “Improving the efficacy of type 1 diabetes therapy by transplantation of immunosolated insulin-producing cells,” Human Cell, vol. 24, no. 2, pp. 86–95, 2011.

[34] N. Ding, S. Luo, J. Yu, Y. Zhou, and Z. Wu, “Vitreous levels of apolipoprotein A1 and retinol binding protein 4 in human rhegmatogenous retinal detachment associated with choroidal detachment,” Molecular Vision, vol. 24, pp. 252–260, 2018.

[35] M. T. Abdel Aziz, M. A. Wassef, L. A. Rashed, S. Mhfouz, and N. Omar, “Mesenchymal Stem Cells Therapy in Acute Renal Failure: Possible Role of Hepatocyte Growth Factor,” Journal of Stem Cell Research & Therapy, vol. 1, no. 3, pp. 2–7, 2011.

[36] W. Chenene, S. Suleman, T. Yemane, and G. Abebe, “Assessment of glycemic control using glycated hemoglobin among diabetic patients in Jimma University specialized hospital, Ethiopia.” BMC Research Notes, vol. 9, no. 1, pp. 1–10, 2016.

[37] E. Beutler, “Improved method for the determination of blood glutathione,” The Journal of Laboratory and Clinical Medicine, vol. 61, pp. 882–888, 1963.

[38] J. C. Han and G. Y. Han, “A procedure for quantitative determination of Tris(2-carboxyethyl)phosphine, an odorless reducing agent more stable and effective than dithiothreitol,” Analytical Biochemistry, vol. 220, no. 1, pp. 5–10, 1994.

[39] S. P. Sichak and A. L. Dounce, “Analysis of the peroxidatic mode of action of catalase,” Archives of Biochemistry and Biophysics, vol. 249, no. 2, pp. 286–295, 1986.

[40] M. Nishikimi, N. A. Rao, and K. Yagi, “The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen,” Biochemical and Biophysical Research Communications, vol. 46, no. 2, pp. 849–854, 1972.

[41] D. Koracevic, G. Koracevic, V. Djordjevic, S. Andrejevic, and V. Cosic, “Method for the measurement of antioxidant activity in human fluids,” Journal of Clinical Pathology, vol. 54, no. 5, pp. 356–361, 2001.

[42] J. A. Buege and S. D. Aust, “Microsomal lipid peroxidation,” Methods in Enzymology, vol. 52, pp. 302–310, 1978.

[43] H. M. Marks, The Progress of Experiment: Science and Therapeutic Reform in the United States 1900-1990, Cambridge University Press, 2000.

[44] J. Wang, K. Eeden, R. Manzer et al., “Differentiated human alveolar epithelial cells and reversibility of their phenotype in vitro,” American Journal of Respiratory Cell and Molecular Biology, vol. 36, no. 6, pp. 661–668, 2007.

[45] H. Dawood Yassa, A. Alsayed Mohammed, A. Saleh Moawad, A. Yahiya Sedeek, and G. Hassan Abdelfatah, “Role of stem cell therapy in diabetic cardiomyopathy in rats: histological and immunohistochemical study,” Egypt Journal of Medical Research, vol. 2, no. 1, pp. 36–55, 2021.

[46] J. D. Bancroft and M. Gamble, Theory and practice of histological techniques, Elsevier health sciences, 2008.

[47] R. Williams, M. Airey, H. Baxter, J. Forrester, T. Kennedy-Martin, and A. Girach, “Epidemiology of diabetic retinopathy and macular oedema: a systematic review,” Eye, vol. 18, no. 10, pp. 963–983, 2004.

[48] N. Maniadakis and E. Konstantakopoulou, “Cost effectiveness of treatments for diabetic retinopathy: a systematic literature review,” PharmacoEconomics, vol. 37, no. 8, pp. 995–1010, 2019.

[49] K. W. Lee, S. M. Ching, V. Ramachandran et al., “Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis,” BMC Pregnancy and Childbirth, vol. 18, no. 1, pp. 1–20, 2018.

[50] J. Tang and T. S. Kern, “Inflammation in diabetic retinopathy,” Progress in Retinal and Eye Research, vol. 30, no. 5, pp. 343–358, 2011.

[51] A. Sui, X. Chen, A. M. Demetriades et al., “Inhibiting NF-κB signaling activation reduces retinal neovascularization by promoting a polarization shift in macrophages,” Investigative Ophthalmology & Visual Science, vol. 61, no. 6, pp. 4–4, 2020.

[52] W. P. Miller, S. Sunilkumar, J. F. Giordano, A. L. Toro, A. J. Barber, and M. D. Dennis, “The stress response protein REDD1 promotes diabetes-induced oxidative stress in the retina by Keap1-independent Nrf2 degradation,” Journal of Biological Chemistry, vol. 295, no. 21, pp. 7350–7361, 2020.

[53] R. G. Miller and T. J. Orchard, “Understanding metabolic memory: a tale of two studies,” Diabetes, vol. 69, no. 3, pp. 291–299, 2020.

[54] A. R. Santiago, R. Boia, I. D. Aires, A. F. Ambrósio, and R. Fernandes, “Sweet stress: coping with vascular dysfunction in diabetic retinopathy,” Frontiers in Physiology, vol. 9, p. 820, 2018.

[55] B. Boehm, G. Lang, O. Volpert et al., “Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retinopathy,” Diabetologia, vol. 46, no. 3, pp. 394–400, 2003.

[56] M. Matsuoka, N. Ogata, K. Minamino, A. Higuchi, and M. Matsumura, “High levels of pigment epithelium-derived...
factor in the retina of a rat model of type 2 diabetes,” *Experimental Eye Research*, vol. 82, no. 1, pp. 172–178, 2006.

[57] B. Wang, P. Atherton, R. Patel, G. Manning, and R. Donnelly, “Antiangiogenic effects and transcriptional regulation of pigment epithelium-derived factor in diabetic retinopathy,” *Microvascular Research*, vol. 80, no. 1, pp. 31–36, 2010.

[58] A. N. Witmer, “Vascular endothelial growth factors and angiogenesis in eye disease,” *Progress in Retinal and Eye Research*, vol. 22, no. 1, pp. 1–29, 2003.

[59] J. Penn, A. Madan, R. B. Caldwell, M. Bartoli, R. W. Caldwell, and M. E. Hartnett, “Vascular endothelial growth factor in eye disease,” *Progress in Retinal and Eye Research*, vol. 27, no. 4, pp. 331–371, 2008.

[60] J. M. Sundstrom, C. Hernández, S. R. Weber et al., “Proteomic analysis of early diabetic retinopathy reveals mediators of neuregenerative brain diseases,” * Investigative Ophthalmology & Visual Science*, vol. 59, no. 6, pp. 2264–2274, 2018.

[61] J. Patel, G. M. Saleh, P. G. Hykin, Z. J. Gregor, and I. A. Cree, “Concentration of haemodynamic and inflammatory related cytokines in diabetic retinopathy,” *Eye*, vol. 22, no. 2, pp. 223–228, 2008.

[62] Y. Wu, Y. Zuo, R. Chakrabarti, B. Feng, S. Chen, and M. H. Davidson, “Proteomic analysis of diabetic retinopathy: new concepts in pathophysiology and treatment,” *Cell & Bioscience*, vol. 4, no. 1, pp. 1–14, 2014.

[63] D. do Carmo Bueno, R. A. Peliciari-Garcia, F. G. do Amaral et al., “Early-stage retinal melatonin synthesis impairment in streptozocin-induced diabetic Wistar rats,” *Investigative Ophthalmology & Visual Science*, vol. 52, no. 10, pp. 7416–7422, 2011.

[64] S. Simão, D. B Bitoque, S. M Calado, and G. A Silva, “Oxidative stress modulates the expression of VEGF isoforms in the diabetic retina,” *New Frontiers in Ophthalmology*, vol. 2, no. 2, pp. 77–83, 2016.

[65] M. H. Davidson, “Apolipoprotein measurements: is more widespread use clinically indicated,” *Clinical Cardiology: An International Indexed and Peer-Reviewed Journal for Advances in the Treatment of Cardiovascular Disease*, vol. 32, no. 9, pp. 482–486, 2009.

[66] R. Simó, M. Higuera, M. García-Ramírez, F. Canals, J. García-Arumí, and C. Hernández, “Elevation of apolipoprotein A-I and apolipoprotein H levels in the vitreous fluid and overexpression in the retina of diabetic patients,” *Archives of Ophthalmology*, vol. 126, no. 8, pp. 1076–1081, 2008.

[67] F. Robbesyn, N. Augé, C. Vindis et al., “High-density lipoproteins prevent the oxidized low-density lipoprotein–induced endothelial growth factor receptor activation and subsequent matrix metalloproteinase-2 upregulation,” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 6, pp. 1206–1212, 2005.

[68] R. Simó, M. García-Ramírez, M. Higuera, and C. Hernández, “Apolipoprotein A1 is overexpressed in the retina of diabetic patients,” *American Journal of Ophthalmology*, vol. 147, no. 2, pp. 319–325, 2009.

[69] V. Lambadiari, N. P. E. Kadoglou, V. Stasinou et al., “Serum levels of retinol-binding protein-4 are associated with the presence and severity of coronary artery disease,” *Cardiovascular Diabetology*, vol. 13, no. 1, pp. 1–8, 2014.

[70] S. E. Park, D. H. Kim, J. H. Lee et al., “Retinol-binding protein-4 is associated with endothelial dysfunction in adults with newly diagnosed type 2 diabetes mellitus,” *Atherosclerosis*, vol. 204, no. 1, pp. 23–25, 2009.

[71] A. Solini, F. Stea, E. Santini et al., “Adipocytokine levels mark endothelial function in normotensive individuals,” *Cardiovascular Diabetology*, vol. 11, no. 1, pp. 103–107, 2012.

[72] K. M. Farjo, R. A. Farjo, S. Halsey, G. Moiseyev, and J. X. Ma, “Retinol-binding protein 4 induces inflammation in human endothelial cells by an NADPH oxidase-and nuclear factor kappa B-dependent and retinol-independent mechanism,” *Molecular and Cellular Biology*, vol. 32, no. 24, pp. 5103–5115, 2012.

[73] M. Du, L. Otalora, A. A. Martin et al., “Transgenic mice over-expressing serum retinol-binding protein develop progressive retinal degeneration through a retinoid-independent mechanism,” *Molecular and Cellular Biology*, vol. 35, no. 16, pp. 2771–2789, 2015.

[74] M. Saberi and S. Gholami, “An investigation on the effects of the Aloe Vera extract on the thickness of the retina in male diabetic rats,” *Iranian Journal of Veterinary Research*, vol. 13, no. 41, pp. 296–302, 2012.

[75] R. E. Marc, B. W. Jones, C. B. Watt, F. Vazquez-Chona, D. K. Vaughan, and D. T. Organick, “Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration,” *Molecular Vision*, vol. 14, pp. 782–806, 2008.

[76] X. Zhang, H. Zeng, S. Bao, N. Wang, and M. C. Gillies, “Diabetic macular edema: new concepts in pathophysiology and treatment,” *Progress in Retinal and Eye Research*, vol. 22, no. 1, pp. 1–14, 2014.

[77] D. do Carmo Bueno, R. A. Peliciari-Garcia, F. G. do Amaral et al., “Early-stage retinal melatonin synthesis impairment in streptozocin-induced diabetic Wistar rats,” *Investigative Ophthalmology & Visual Science*, vol. 52, no. 10, pp. 7416–7422, 2011.

[78] T. Hikichi, N. Tateda, and T. Miura, “Alteration of melatonin secretion in patients with type 2 diabetes and proliferative diabetic retinopathy,” *Clinical Ophthalmology (Auckland, NZ)*, vol. 5, p. 655, 2011.

[79] T. Göürpınar, N. Ekerbiçer, N. Uysal, T. Barut, F. Tarakçı, and M. I. Tuglu, “The effects of the melatonin treatment on the oxidative stress and apoptosis in diabetic eye and brain,” *Scientific World Journal*, vol. 2012, 5 pages, 2012.

[80] Y. Ma, Q. Zhao, Y. Shao, M. Z. Cao, M. Zhao, and D. Wang, “Melatonin inhibits the inflammation and apoptosis in rats with diabetic retinopathy via MAPK pathway,” *European Review for Medical and Pharmacological Sciences*, vol. 23, 3 Suppl, pp. 1–8, 2019.

[81] B. Djordjevic, T. Cvetkovic, T. J. Stoimenov et al., “Oral supplementation with melatonin reduces oxidative damage and concentrations of inducible nitric oxide synthase, VEGF and matrix metalloproteinase 9 in the retina of rats with streptozocin/nicotinamide induced pre-diabetes,” *European Journal of Pharmacology*, vol. 833, pp. 290–297, 2018.

[82] L. Ba, S. Cao, N. Ji, C. Ma, R. Wang, and D. Luo, “Exogenous melatonin treatment in the postharvest storage of pitaya fruits delays senescence and regulates reactive oxygen species metabolism,” *Food Science and Technology*, vol. 42, 2021.

[83] M. S. Singh, S. S. Park, T. A. Albini et al., “Retinal stem cell transplantation: balancing safety and potential,” *Progress in Retinal and Eye Research*, vol. 75, article 100779, 2020.

[84] S. N. Bhatia, G. H. Underhill, K. S. Zaret, and J. J. Fox, “Cell and tissue engineering for liver disease,” *Science Translational Medicine*, vol. 6, no. 245, p. 245sr2, 2014.

[85] A. J. Rong, B. L. Lam, Z. A. Ansari, and T. A. Albini, “Vision loss secondary to autologous adipose stem cell injections: a rising problem,” *JAMA Ophthalmology*, vol. 136, no. 1, pp. 97–99, 2018.
[85] H. Surendran, S. Nandakumar, V. B. Reddy K et al., “Transplantation of retinal pigment epithelium and photoreceptors generated concomitantly via small molecule-mediated differentiation rescues visual function in rodent models of retinal degeneration,” Stem Cell Research & Therapy, vol. 12, no. 1, pp. 1–17, 2021.

[86] W. Chang, B. W. Song, J. Y. Moon et al., “Anti-death strategies against oxidative stress in grafted mesenchymal stem cells,” Histology and Histopathology, vol. 28, no. 12, pp. 1529–1536, 2013.

[87] L. Drowley, M. Okada, S. Beckman et al., “Cellular antioxidant levels influence muscle stem cell therapy,” Molecular Therapy, vol. 18, no. 10, pp. 1865–1873, 2010.

[88] Y. T. Chen, H. J. Chiang, C. H. Chen et al., “Melatonin treatment further improves adipose-derived mesenchymal stem cell therapy for acute interstitial cystitis in rat,” Journal of Pineal Research, vol. 57, no. 3, pp. 248–261, 2014.