Original Article

# Histological and Biochemical Evaluation of the Therapeutic Influence of Bone Marrow Mesenchymal Stem Cells Versus Genistein on Induced Diabetic Retinopathy in Adult Male Albino Rats

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## ABSTRACT

**Background:** Diabetic retinopathy is a diabetes-related eye disease that can result in vision loss. Mesenchymal stem cells from bone marrow (BMSC) hold great promise for new medical therapies. Genistein is a naturally occurring compound found in soy products. It has been used to alleviate and regulate blood glucose levels.

**Aim:** The goal of this research was to assess and compare the potential efficacy of Genistein versus BMSC in the treatment of diabetic retinopathy.

**Materials and Methods:** 53 adult male albino rats were split into four groups: control group (I), diabetic group (II) (single STZ dose 60 mg / kg I.P), Diabetic + BMSCs group (III) (0.2 ml of PKH26 labelled MSCs suspension in BPS 3x106 cells/ml) intravitreal to diabetic rats and Diabetic + Genistein group (IV) (0.18 mg/kg orally). Animals were anesthetized and sacrificed at the end of the study (8 weeks). Both animal eyes were removed for histological, immunohistochemistry, and electron microscopic analyses. Biochemical and morphometric studies had been carried out.

**Results:** Diabetic group had a reduced total retinal thickness as well as destructed photoreceptor layer. The inner nuclear layer has thinned and exposing darkly stained nuclei. The ganglion cells showed pyknotic nuclei. Vacuolation was evident in the plexiform layers. The immunoexpression of caspase-3, COX-2, and vimentin increased significantly. Ultrastructural changes revealed pigmented epithelium degradation, disorganized and vacuolated photoreceptors associated with condensed nuclei, cytoplasmic vacuolization, and distorted mitochondria of bipolar cells. Biochemically, antioxidant enzymes including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were declined, while malondialdehyde (MDA) levels increased dramatically. Both BMMSCs and Genistein improved histological structure of diabetic retina.

**Conclusion:** Though both BMSCs and Genistein were found to be effective potential intervention therapy for diabetic retinopathy, Genistein could be a promising therapy that delays the development of early pathological processes in diabetics prior to vision loss.

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**Key Words:** BMSCs, diabetic retinopathy, genistein.

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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia, which is caused by insulin resistance, deficient insulin production or both. Both vascular and nonvascular complications can result from prolonged exposure to chronic hyperglycaemia[1]. The prevalence of diabetes mellitus has risen sharply in recent years, despite new drugs and advancements in clinical therapies.

One of the microvascular complications of diabetes mellitus is a diabetic retinopathy (DR). It is still the leading cause of vision loss and blindness among working-age people in developing countries. This complication affected more than 100 million patients worldwide in 2010, and is predicted to grow to > 190 million by 2030[2]. The number of people who are at risk of losing their vision due to diabetes is predicted to double in the next 30 years[3].

Rather than waiting for vision-threatening lesions to occur, it is important to establish better methods for identifying, preventing, and treating retinopathy in its early stages. Improvement in these areas necessitates a new look at the problem, which involves the role of the neural retina and insulin resistance[4]. DR is marked by progressive harm to retinal microvasculature with neuro-degeneration of the retina. Microvascular variations as well as changes in all of the retina's major cell types are involved[5]. For advanced DR, current therapies are recommended, but they have important adverse effects and have experienced only limited success. New therapies are therefore required for the early stages of DR and for prophylaxis against DR and vision loss[6].

Therapeutic techniques, including vitreo-retinal surgery, laser photocoagulation and corticosteroids, have achieved only limited effectiveness[7]. No therapy for regeneration of the damaged retinal vasculature caused by diabetes mellitus has been developed yet. Mammalian mature retinas were assumed to be incapable of regeneration; however, studies have revealed that a number of retinal stem cells near the pigmented ciliary margin can differentiate into a variety

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of retinal cells, including photoreceptors, bipolar cells, and Müller cells[8].

Cell therapy is a set of procedures that use live cells for medical purposes. The aim of this treatment is to repair or restore the biological function of the damaged tissue. The use of stem cells in cell therapy is being investigated in numerous medical fields[9]. In order to prevent neurovascular harm and to encourage regeneration of the damaged retina, cell-based therapies may be an achievable choice[10]. Bone marrow-derived stem cells (BMSCs) can cure optic nerve damage by differentiating into ganglion cells via intravitreal administration in mice[11]. In rats with ischemic retinal injury, intravitreal administration of BMSCs helped the retina survive[12].

Genistein (GEN) [5, 7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one] is a naturally occurring isoflavone derived from a wide range of plant-derived foods, particularly soybeans and soy-based foods[13]. It was first obtained in 1931 from 90% methanol extract of a soybean meal. The chemical name GEN is derived from dyer's green herb (Genista tinctoria). Because of its structural resemblance to human estrogen, it is also recognized as phytoestrogen[14]. The glucoside genistin is the primary source of GEN. The action of acid hydrolysis and bacterial enzymes in the stomach and intestine releases GEN from Genistin[15]. Genistein also present in fruit, vegetables, and nuts. Many studies have reported a wide range of its biological effects as antioxidant, antiangiogenic, anthelmintic, anticancer, antidiabetic, and antiobesity activities[16]. Because of its defensive influence on pancreatic β -cells, it has strong anti-diabetic properties. Clinical studies revealed that Genistein reduced the risk of diabetic complications in obese women[17].

The objective of this research was to assess whether Genistein or bone marrow mesenchymal stem cell (MSC) could help to treat experimentally induced diabetic retinopathy in adult male albino rats using histological, immunohistochemical, and morphometric tests.

## MATERIALS AND METHODS

### *Drugs and Chemicals*

Streptozotocin (Sigma Chemical Co., St. Louis, USA) was purchased as a white powder. For each rat, freshly prepared STZ (60 mg/kg, dissolved in 0.1 M cold Sodium citrate buffer, pH 4.5) was given as a single intraperitoneal (IP) injection[18].

Genistein (GEN) Synthetic, ~98% (HPLC), powder, 100 mg in poly bottle (Sigma-Aldrich, St. Louis, MO, USA). In this analysis, a dosage of 0.18mg/kg of Genistein dissolved in corn oil was used[16].

Bone marrow mesenchyme stem cells (BM-MSCs) (labelled PKH67 Green Fluorescent Cell Linker) were obtained from the stem cell research unit of Cairo University's Faculty of Medicine's Department of Biochemistry. In the Stem Cell Unit, Central Laboratory, Alexandria University, Faculty of Medicine, the BMMSCs were injected intravitreally at a dose of 0.2 ml of solution containing (3x106 cells / ml) BMMSCs for each rat[19]. A fluorescent microscope (Olympus BX50F4, No. 7M03285, Tokyo, Japan) was used to map and detect the cells labelled with PKH67 in retinal tissues (Figure 1).

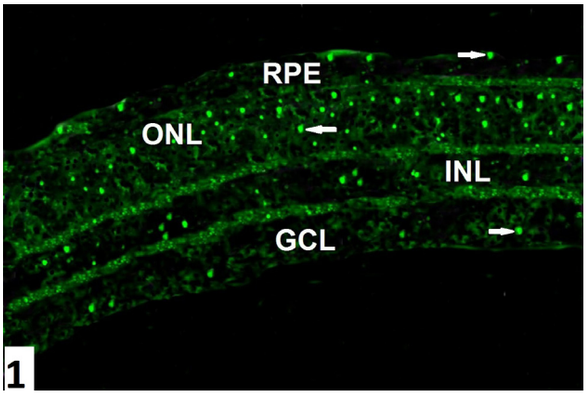



**Fig. 1:** A section in rat`s retina, three days after stem cell administration showing distribution of PKH67-labeled cells (arrows), appearing as bright green dots within RPE, ONL ,INL and GCL. (Fluorescent Microscope X 200)

### *Animals*

In this study, 53 adult male albino rats weighing 240-250 g (4-6 months) were included. Throughout the research, animals were held at a constant temperature (24±2 ° C). They were stored on a standard rodent pellet, and water was available ad libitum. The animal ethics committee approved the animal care and experimental procedures in compliance with the Guide for the Treatment and Use of Laboratory Animals from the University of Menoufia in Egypt.

### *Diabetes Triggering*

Overnight-fasted rats received a single intraperitoneal (IP) injection of STZ (60 mg/kg body weight) dissolved in 0.1 M sodium citrate buffer for TIDM induction. Animals were fasted for 72 hours following STZ injection (experiment day 0), and blood samples were collected from the tail vein for blood glucose monitoring. Rats were considered diabetic when their glucose levels reached 250 mg/dl[20].

### *Design of experiment*

Rats were given unlimited supply of food and water for 7 days prior to experimentation. Then they were randomly subdivided into four groups:

**Group I (control group; n= 20):** rats were randomly assigned to equal four subgroups, 5 rats each:

Subgroup IA: animals were not untreated (negative control).

Subgroup IB: each animal received single intravitreal injection of 0.2 ml phosphate buffer saline containing (3x106 cells / ml) BMMSCs[19].

Subgroup IC: through oral gavage, animals were administered 0.18 mg/kg of Genistein dissolved in corn oil[16].

Subgroup ID: intraperitoneal injection of 0.2 mL/kg body weight sodium citrate buffer into rats (STZ vehicle). All subgroups were treated for eight weeks.

**Group II (Diabetic group; n= 10):** single intraperitoneal injection of freshly prepared STZ (60 mg / kg, dissolved in 0.1 M sodium citrate buffer , pH 4.5) was administered to each rat[18]. After 72 h, a glucose analyzer was used to assay fasting blood glucose levels. Animals have been considered to be diabetic and included in our sample with blood glucose levels equal or higher than 250 mg/dl. In order to increase survival rates, diabetic rats were given subcutaneous insulin (2-4U) (Humulin-N; Eli Lilly & Co., Indianapolis, Indiana, USA) twice a week[21]. Retinal specimens were taken eight weeks after establishment of DM.

**Group III (Diabetic + Stem Cells group; n= 13):** induction of diabetes as group II was done, then each animal was injected with stem cell in the same dose and route of administration of subgroup IB. Animal retinae were taken eight weeks after stem cell administration.

After 3 days of the start of the experiment, three animals from this group were sacrificed and retina samples were obtained, processed to evaluate and prove the homing of fluorescent labeled BMSCs in the retina.

**Group IV (Diabetic + Genistein group; n=10):** diabetic animals were treated with genistein in the identical daily dosage and administration route as subgroup IC for eight weeks.

At the end of the experiment (8 weeks after induction of DM), animals were ether-anesthetized, sacrificed and tissues were fixed via animal perfusion. Both eyeballs were dissected from all groups. Specimens were prepared for histological, biochemical, immunohistochemical, and transmission electron microscopic examination.

### *I- Biochemical analysis of oxidant and antioxidant markers in retinal tissue*

One eyeball was taken from each rat and put on blotting paper to determine oxidant and antioxidant enzymes. 5 mm behind the limbus, an incision was made to separate the poles from the anterior and posterior. Retina was then separated from the choroid complex with a scalpel blade and forceps. Retina samples have been removed. They were homogenised in 1 ml PBS, pH 7.4, and centrifuged for 15 minutes at 4 ° C at 4500 rpm, then the supernatant was collected using a spectrophotometer for evaluation of malondialdehyde (MDA)[22],(SOD)[23],Catalase (CAT) and Glutathione (GSH)[24].

### *II- Histological and Imunohistochemical research*

Eye specimens from all rats were fixed in a 10% buffered formalin solution, dehydrated in ascending grades of ethanol, and embedded in paraffin. Serial parts with a thickness of 5-7 m were cut and stained with the preceding stains:

1. Staining of Haematoxylin and Eosin (H&E): to analyse the histological results in the various groups[25].
2. Immunohistochemical staining
    A. Caspase-3 (apoptosis marker): Anti-Caspase-3 (rabbit polyclonal antibody, Thermo Science, Fermont, CA 94539, USA at a dilution 1/50) was used.The primary antibody used for caspase-3 was ready-to-use rabbit polyclonal antibody (CAT-No. RB-3425-R2).
    B. Cyclooxygenase-2 (COX-2) (inflammatory marker): anti-mouse polyclonal antibody (SAB4200576; Sigma) (dilution 1: 200) was used. COX-2 expression indicates inflammation and increase production of cytotoxic prostaglandins.
    C. Vimentin (gliosis index): The primary monoclonal mouse antibody for Vimentin (Santa Cruz Biotechnology, Santa Cruz, California, USA, 1:300 wits PBS) was used to detect Müller cells. Peroxidase-labelled streptavidin-biotin technique was used[26]. Diaminobenzidine (DAB) (Dakopatts, Glostrup, Denmark) was applied to the slides for chromogenic purposes. Distilled water was used to clean the slides. Mayer's haematoxylin was used to counter-stain sections later. Normal lymphoid tissue was used as a positive control for caspase-3, kidney was used as a positive control for COX-2 while smooth muscle tissue used as a positive control for vimentin. Negative controls were omit primary antibody[27].

### *III-Transmission Electron Microscopic Analysis (TEM)*

In phosphate buffer, the eyes were fixed for 1 hour with 1% glutaraldehyde and 4% paraformaldehyde. Every eye ball's anterior segment was removed, and the posterior segments were sliced, dehydrated, and embedded in epoxy resin after being post-fixed in 1 percent osmium tetroxide. Ultrathin retinal sections (70-90 nm) were obtained and stained on copper grids with uranyl acetate and lead citrate. The ultrastructure of the tissues was examined using a Jeol electron microscope[28]. TEM processing and analysis is carried out by the Electron Microscopy Unit of Alexandria University's Faculty of Science.

### *IV-Morphometric and Statistical analysis*

All quantitative data was collected using the "Leica Qwin 500 C" image analyser automated data processing system Ltd. (Cambridge, England). The thickness of the

retina (measured from the ILM to the OLM), outer nuclear, and inner nuclear layers were measured in H&E stained sections using the distance parameters in the interactive measurement menu, with 10 random non-overlapping fields evaluated using 10 objective lenses. With a total magnification of X400, the area percentage (%) of Caspase-3, Vimentin, and Cox-2 immunoreactivity was determined within 10 fields of each rat using a Leica DML B2/11888111 microscope supplied with a Leica DFC450 camera. The measured variance was estimated using the software version K1.45 of Image J in anatomy department, faculty of Medicine, Menoufia University. Significant differences between groups were assessed using the Student t-test and variance test analysis. Histomorphometric measurement data and biochemical results were expressed as mean SD, and significant discrepancies between groups were assessed using the Student t-test and variance test study. The P value of 0.01 was deemed statistically significant[29].

## RESULTS

The animals were in good general condition and displayed normal conduct, behaviour, and appetite. A significant decrease in the animal body weight with excessive urination was found in the diabetic group when opposed to the control group (Table 1).

### *Biochemical results*

In the control group, there was no significant difference between any of the subgroups (IA, IB, IC and ID). When compared to the control group and diabetic animals treated with genestein, diabetic animals showed a significant reduction in GSH, whereas diabetic animals treated with BMSC showed no difference from the control group. MDA level showed a significant increase in the diabetic group (II) and diabetic + genistein group relative to the control group. The antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activity in diabetics has also decreased significantly ($P < 0.001$). Treatment with either BMSCs or genistein increased SOD and CAT levels significantly ($P<0.001$) in diabetic rats. All parameters have been set out in (Table 1, Histogram 1).

### *Histological Results:*

### *Light microscopic examination*

Using fluorescent microscope, examination of retinal sections, 3 days after stem cell injection to evaluate and prove the homing of fluorescent labelled BMSCs in the retina, showed distribution of PKH67-labeled cells as bright green dots in pigmented epithelium, outer nuclear layer, inner nuclear layer and ganglion cell layer (Figure 1).

Haematoxylin and eosin retinal-stained sections of all control subgroups (group I) exhibited normal histological architecture, demonstrating well organized retinal layers consisted of photoreceptor layer (PRL), outer nuclear layer (ONL), containing deeply stained nuclei of rods and cones, outer plexiform layer (OPL) appeared as a narrow pale area and inner nuclear layer (INL) having large and pale nuclei. Inner plexiform layer (IPL) appeared as pale zone between INL and ganglion cell layer (GCL) that had large vesicular nuclei (arrows). Nerve fiber layer (NFL) was also visible (Figure 2A).

The retinae of diabetic animals (group II) showed a significant reduction in total retinal thickness, ONL and INL (Table 1, Histogram 2). There was loss of some photoreceptor processes, a focal widening of the intercellular spaces of inner and outer nuclear layers. Vacuoles between the nerve fibres in IPL and OPL and vacuolated GCL with presence of some ghost of nuclei were also seen (Figure 2B).

Group III (diabetic + stem cells group) displayed almost normal histological architecture. The animals showed average retinal thickness as well as normal outer and inner nuclear layers (Table 1, Histogram 2). Most of ganglion cells were seen having large euchromatic nuclei. However, few ganglionic cells were still having small and darkly stained nuclei (Figure 2C).

Group IV (diabetic + Genistein group) revealed regularly arranged retinal layers with retained structure. Small empty spaces between nuclei of (ONL) and (INL) were present (Figure 2D). Normal retinal thickness, outer and inner nuclear layers were observed (Table 1, Histogram 2).

Toluidine blue-stained semi-thin sections of control retina showed retinal pigmented epithelium, photoreceptor layer, ONL having closely-packed dark nuclei arranged in rows. OPL appeared as loose reticular layer containing blood capillaries. The nuclei of the INL were pale and large. Pale stained inner plexiform layer was seen. GCL had a single row of ganglion cells with lightly stained cytoplasm and large vesicular nuclei. Intact inner limiting membrane was observed (Figure 3A). Semi-thin sections of diabetic group showed widely separated nuclei of ONL and INL. Presence of ghost cells and shrunken pyknotic nuclei in INL were recorded. Shrunken pyknotic nuclei of ganglion cells and congested blood capillary extending into the inner plexiform layer were present (Figure 3B).

Semi-thin sections of group III (diabetic + stem cells group) showed an improvement of the retinal architecture, although there were minimal intercellular spaces between the nuclei of INL and ONL. Some GC had pale nuclei while, others having shrunken darkly stained nuclei (Figure 3C). Group IV (diabetic + Genistein group) also exhibited remarkable histological improvement in retinal layers, however, minimal spacing between nuclei of INL and OLN was still seen (Figure 3D).

Immunohistochemically, anti-caspase-3 immune-marker expression of control group (I) revealed negative immune expression in all retinal layers (Figure 4A). Group II (diabetic group) showed a strong significant cytoplasmic reaction of caspase-3 in ONL, INL, IPL and GCL (Figure 4B) compared to control group. There was

negative reaction of caspase-3 in retinae of rats of group III (Figure 4C) and group IV (Figure 4D, Histogram 3).

Regarding to Cox-2 immunohistochemical staining, control group showed negative immunoreaction for Cox-2 in all retinal layers (Figure 5A). Group II displayed an obvious significant intense cytoplasmic positive immunoreaction of Cox-2 in nerve fibre layer, ganglion cell layer and outer plexiform layer compared to control (Figure 5B). However, these layers displayed negative Cox-2 immunoreaction in almost retinal layers of group III (Figure 5C) and group IV (Figure 5D, Histogram 3).

Anti-Vimentin immune expression of control group (group I) exhibited minimal positive vimentin immunoreactivity of Muller cell end feet and its radial processes, which appear as long filaments extending in NFL, GCL, IPL, INL, OPL and ONL (Figure 6A). Group II showed significant increase positive cytoplasmic vimentin immunostaining observed throughout most retinal layers (Figure 6B).There was mild positive immune expression of vimentin of retinae of animals of group III (Figure 6C) while group IV showed moderate positive vimentin reaction (Figure 6D, Histogram 3).

### *Electron microscope examination*

Ultrastructurally, retinal specimens of the control group showed retinal pigment epithelial cell with large euchromatic oval nucleus, mitochondria, melanin granules, long apical microvilli and phagocytosed photoreceptors outer segments (Figure 7). The photoreceptors outer segments contained regular flattened membranous lamellae (Figure 8). The outer nuclear layer had tightly backed cells with no intercellular spaces. The cells have rounded nuclei with centrally highly condensed heterochromatin and surrounded by a thin rim of cytoplasm containing mitochondria (Figure 9). The inner nuclear layer contained bodies of bipolar cells, tightly packed to each other and having euchromatic rounded nuclei and their cytoplasm filled with mitochondria and rough endoplasmic reticulum (Figure 10). IPL having intact nerve axons was seen (Figure 11). Ganglion cell had large rounded euchromatic nucleus with uniformly staining nucleoplasm, mitochondria, rough endoplasmic reticulum and scattered ribosomes (Figure 12).Ultra-structurally retinal sections of diabetic group showed retinal pigmented cell having severely destructed broken apical microvilli, small shrunken oval nucleus and mitochondria with destructed cristae. Large phagosomes and vacuolization were present in its cytoplasm (Figure 13). Disorganized and vacuolated photoreceptor outer segments were also seen. Some photoreceptors had irregular membranous lamellae, others were degenerated and lost, leaving wide areas (Figure 14). Severe vacuolization of cells of ONL and wide spaces filled with debris between the nuclei were observed (Figure 15). Bipolar cells had condensed nuclei, many cytoplasmic vacuolization and mitochondria with distorted cristae. Rarefied cytoplasm was also seen (Figure 16). IPL had extensively destructed nerve axons (Figures 17,18). Ganglion cells contained indented irregular nucleus with areas of electron-dense heterochromatin and destructed nuclear membrane. Its cytoplasm exhibited severe vacuolization (Figure 18).

Electron microscopic examination of diabetic + stem cells group showed nearly normal retinal pigment epithelium with intact euchromatic nucleus, apical microvilli, melanin granules and phagocytosed photoreceptors segments. However, some cytoplasmic vacuoles were still present (Figure 19). Almost normal outer segment photoreceptors with typical lamellar appearance were identified (Figure 20). Cells of ONL exhibited almost normal nuclei with heterochromatin surrounded by thin rim of cytoplasm. Little intercellular spaces between cells and synaptic region of OPL containing mitochondria were observed (Figure 21). Bipolar cells were tightly packed together, having euchromatic nuclei. Slight vacuolization of the cytoplasm was still present (Figure 22). Nearly normal IPL had intact nerve axons (Figure 23). There was ganglion cell with large euchromatic nucleus and normal nuclear envelope. The cytoplasm contains rough endoplasmic reticulum and swollen mitochondria (Figure 24)

Regarding to electron microscopic examination of the retina of diabetic + Genistein group, retinal pigment cell was similar to normal, having an euochromatic nucleus and many long apical microvilli enclosing the photoreceptor outer segments. Large phagosome and few broken microvilli were noticed (Figure 25). Standard lamellar appearance of outer segments slightly space-separated was observed (Figure 26). The ONL showed tightly packed photoreceptors cells containing rounded nuclei with its characteristic dense chromatin (Figure 27). Bipolar cells had large euchromatic nuclei and mitochondria (Figure 28). The IPL was occupied by normal nerve axons (Figure 29). Almost normal ganglion cell having large euchromatic nucleus and normal nuclear envelope was detected. Its cytoplasm contained rough endoplasmic reticulum and mitochondria (Figure 30).

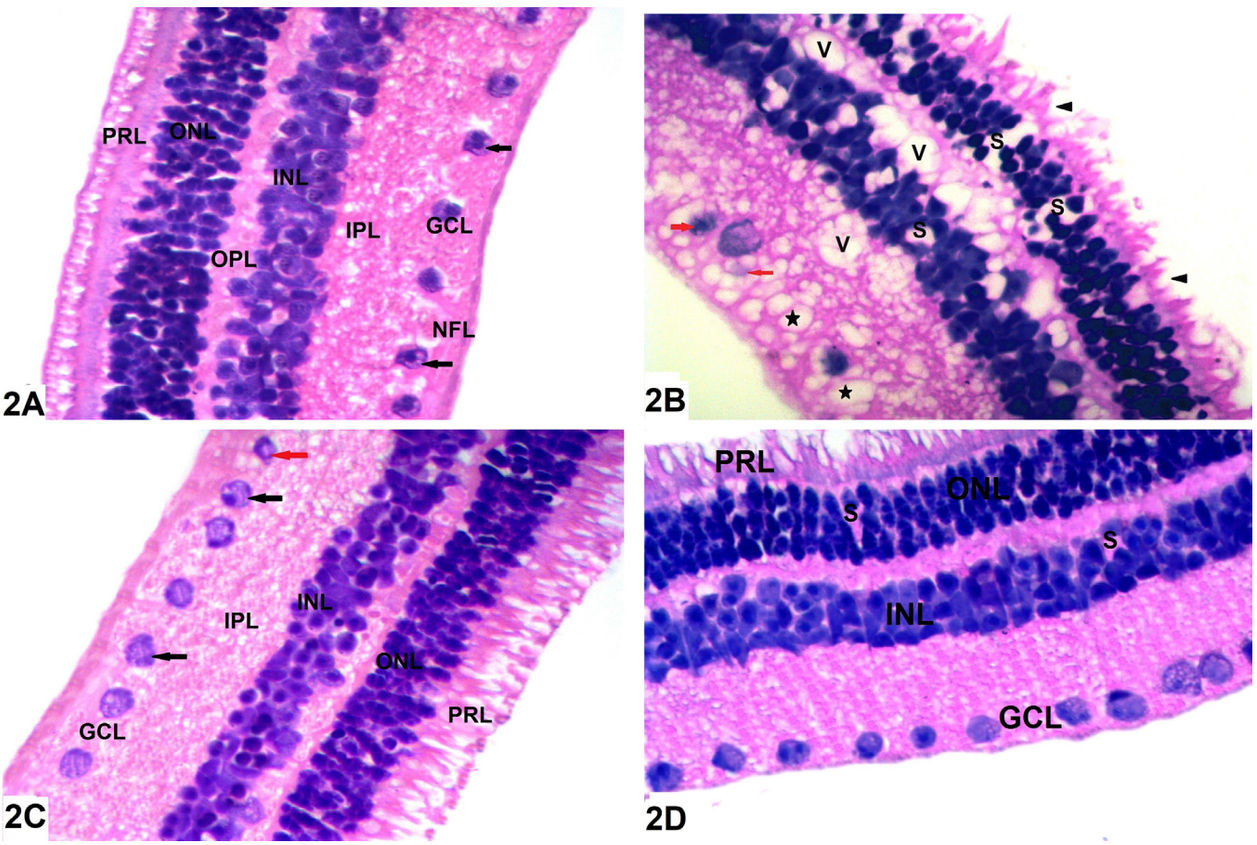



**Fig. 2:** A section in the retina (A) Control group (I) revealed photoreceptor layer (PRL), outer nuclear layer (ONL),containing deeply stained nuclei of rods and cones, outer plexiform layer (OPL) appeared as a narrow pale area and inner nuclear layer (INL) having large and pale nuclei. Inner plexiform layer (IPL) appeared as pale zone between INL and ganglion cell layer (GCL) that have large vesicular nuclei (arrows). Nerve fibre layer (NFL) is also visible.
(B) Diabetic retina (group II) demonstrating loss of some photoreceptor processes (arrow head), focal widening of intercellular spaces (S) of the inner nuclear layer (INL) and outer nuclear layer (ONL), Vacuoles between nerve fibres are also found in IPL and OPL (V). Note vacuolated GCL (star) with presence of some ghost of nuclei (red arrows). (C) Group III (diabetic + stem cells) showing nearly normal histological architecture. Most of ganglion cells are seen with large euchromatic nuclei (black arrows). Few ganglionic cells are still having small and darkly stained nuclei (red arrow). (D) Group IV (diabetic + genistein) demonstrating normal arranged retinal layers with preserved structure. Small empty spaces (S) between the nuclei of outer nuclear layer (ONL) and inner nuclear layer (INL) are seen. (H&E X 400)

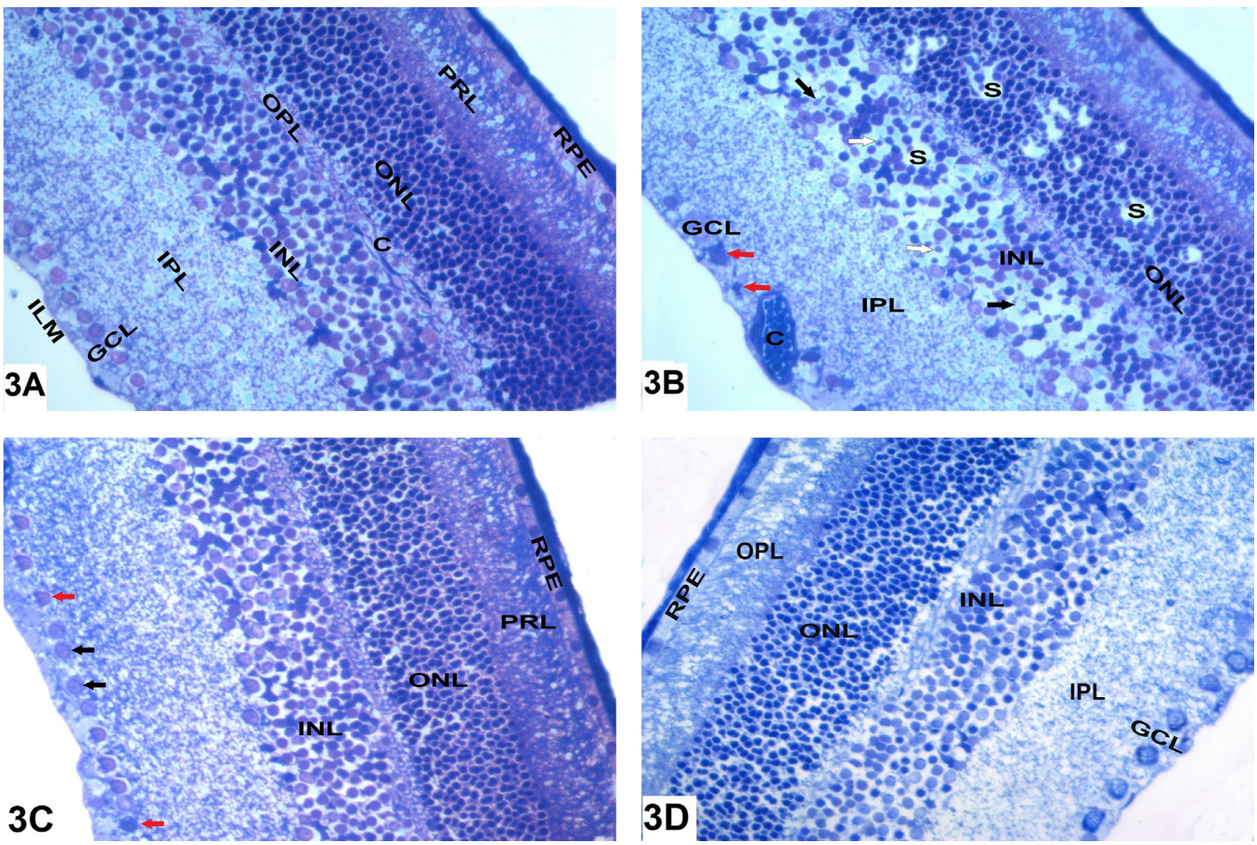



**Fig. 3:** A semi-thin section of the retina (A) Group I (control) showing retinal pigmented epithelial cells (RPE), photoreceptor layer (PRL), and ONL with tightly packed dark nuclei of rods and cones cells. The OPL is evident as a loose reticular layer with blood capillaries (C). The INL nuclei are pale and large. The inner plexiform layer (IPL) was visible as a pale area. A single row of ganglion cells (GC) with lightly stained cytoplasm and large vesicular nuclei is evident. Note the intact inner limiting membrane (ILM).
(B) Diabetic animal (group II) showing widely separated nuclei (S) of INL and ONL. Presence of ghost cells (black arrows) and shrunken pyknotic nuclei (white arrows) in the INL. Shrunken pyknotic nuclei of ganglion cells (red arrow) in GCL and congested blood capillary (C) extending into the inner plexiform layer (IPL) are seen. (C) Group III (diabetic +stem cells) showing retinal pigment epithelial cells (RPE), photoreceptor layer (PRL) and ONL with dark nuclei. The INL revealed large and pale nuclei. Minimal intercellular spaces in INL and ONL are seen. Some GC having pale nuclei (black arrows) while, others having shrunken darkly stained nuclei (red arrow). (D) Group IV (diabetic + genistein) showing normal appearing of retinal layers. However, minimal spacing between nuclei of ONL and INL is still seen. (Toluidine blue X 200)

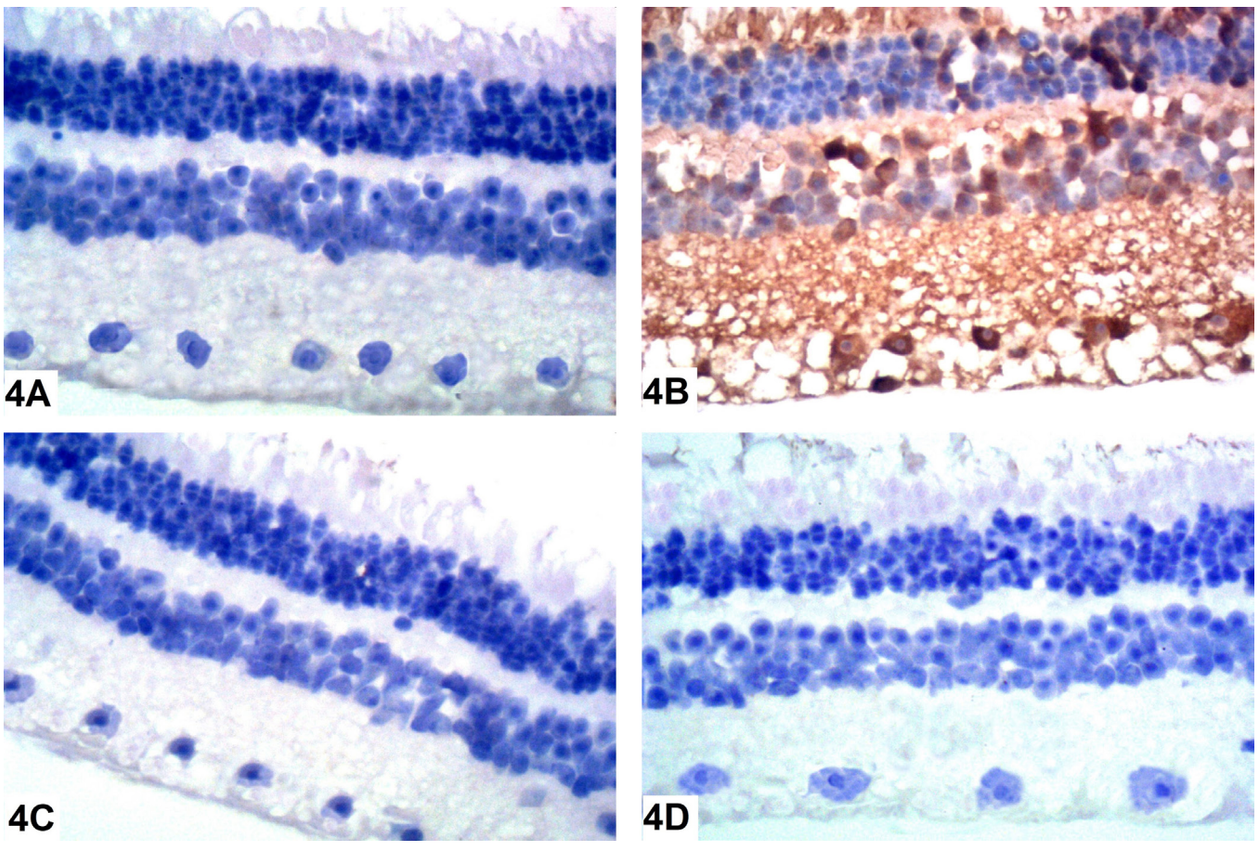



**Fig. 4:** A section of retina (A) A control rat (group I) showing negative immune expression of Caspase-3 in all retinal layers. (B) Diabetic rat (group II) showing strong cytoplasmic reaction of caspase-3 in ONL, INL, IPL and GCL. (C) Diabetic +stem cells treated rat (group III) showing negative reaction of caspase-3. (D) Diabetic + Genistein treated rat (group IV) showing negative reaction of caspase-3. (Immunostaining for Caspase-3 X 400)

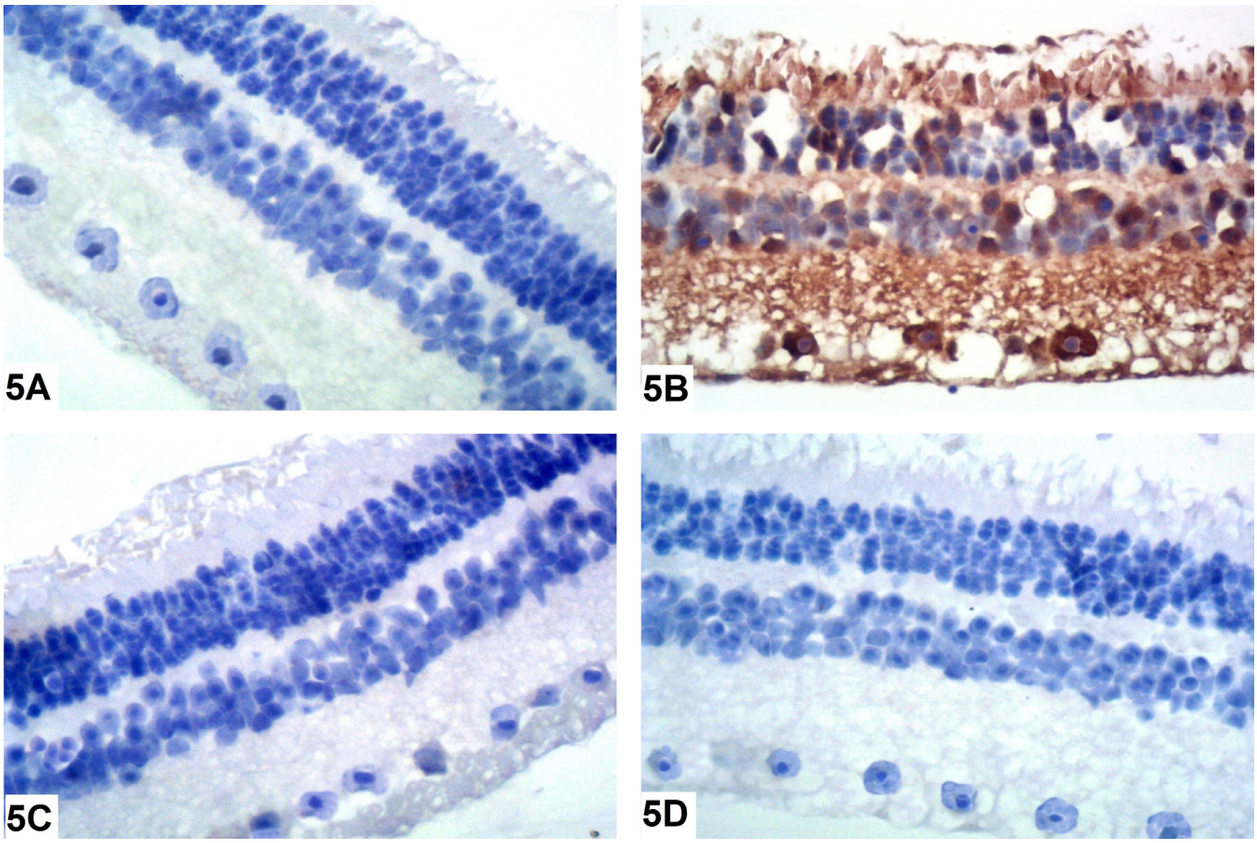



**Fig. 5:** A section in the retina of (A) Group I (control group) showing negative immunoreaction for Cox-2 in almost all retinal layers. (B) Group II (diabetic group) showing obvious intense cytoplasmic positive immunoreactions of Cox-2 in nerve fibre layer, ganglion cell layer and outer plexiform layer. (C) Diabetic +stem cell treated rat (group III) showing negative reaction of Cox-2 in almost retinal layers. (D) Diabetic + Genistein treated rat (group IV) showing negative reaction of Cox-2 in retinal layers. (Immunostaining for Cox-2 X 400)

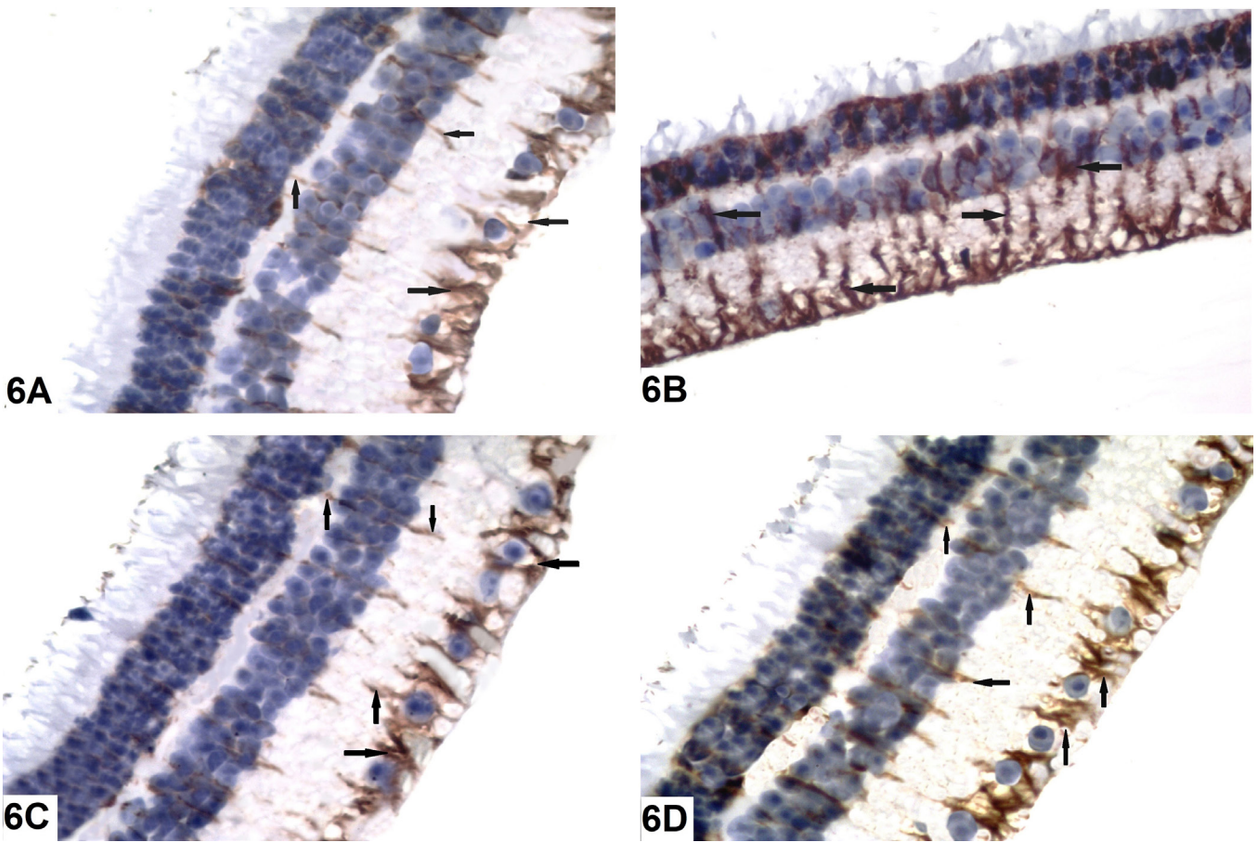



**Fig. 6:** A section in the retina of (A) Group I (control group) showing minimal positive vimentin immunoreactivity (arrows) of Muller cell end feet and its radial processes, extending in NFL, GCL, IPL, INL, OPL and ONL. (B) Group II (diabetic group) showing marked positive cytoplasmic vimentin immunostaining (arrows) in most retinal layers.
(C) Diabetic +stem cell treated rat (group III) showing mild positive immune expression of Vimentin (arrows) in retinal layers. (D) Diabetic + Genistein treated rat (group IV) showing moderate positive vimentin reaction (arrows) in nearly all layers of the retina. (Immunostaining for Vimentin X 400)

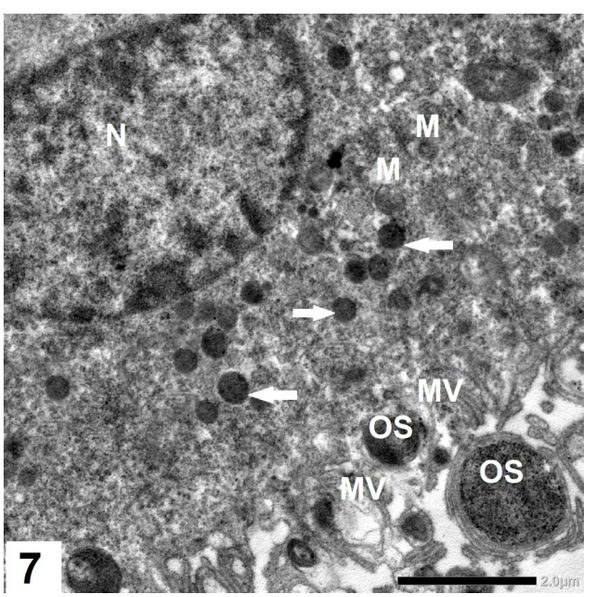



**Fig. 7:** An electron micrograph of the retina of group I (control group) showing retinal pigment epithelial cell (RPE) having large euchromatic oval nucleus(N), mitochondria (M), melanin granules (arrows), long apical microvilli (MV) and phagocytosed photoreceptors outer segments (OS). (TEMX4000)

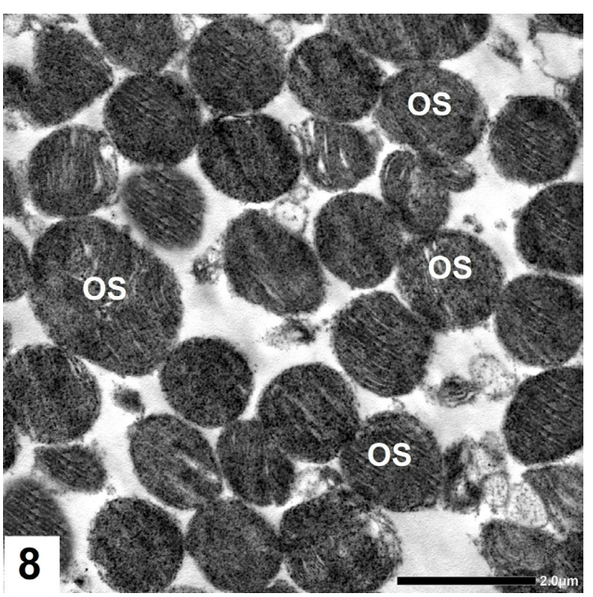



**Fig. 8:** An electron micrograph of the retina of group I (control group) showing photoreceptors outer segments (OS) with regular flattened membranous lamellae. (TEMX4000)

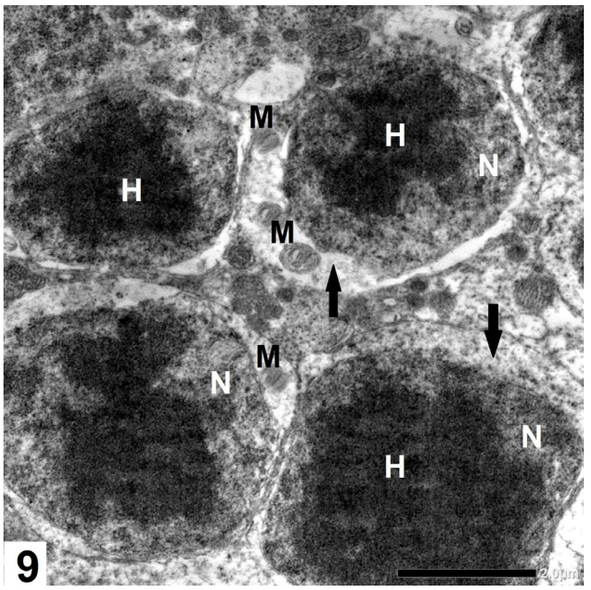



**Fig. 9:** An electron micrograph of the retina of group I (control group) from the outer nuclear layer showing tightly backed cells with no intercellular spaces. The cells have rounded nuclei (N) with centrally highly condensed heterochromatin (H) and surrounded by a thin rim of cytoplasm (arrow) containing mitochondria (M). (TEM X4000)

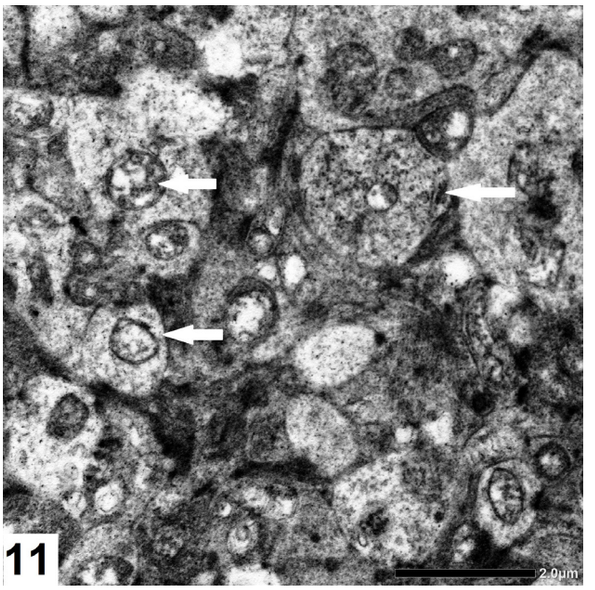



**Fig. 11:** An electron micrograph of the retina of group I (control group) showing IPL having intact nerve axons (arrows). (TEM X4000)

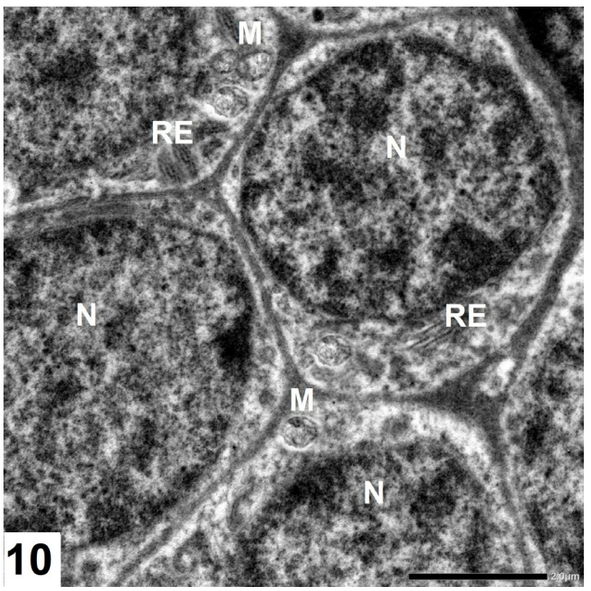



**Fig. 10:** An electron micrograph of the retina of group I (control group) from the inner nuclear layer showing the cell bodies of bipolar cells, tightly packed to each other and having euchromatic rounded nuclei (N) and their cytoplasm filled with mitochondria (M) and rough endoplasmic reticulum (RE). (TEM X4000

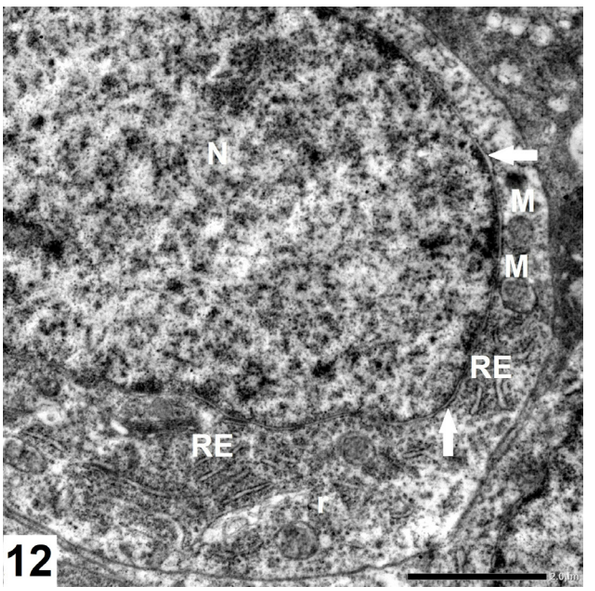



**Fig. 12:** An electron micrograph of the retina of group I (control group) showing ganglion cell having large rounded euchromatic nucleus (N) with uniformly staining nucleoplasm. Mitochondria (M), Rough endoplasmic reticulum (RE)and scattered ribosomes (r) are observed. Intact nuclear envelope (white arrow) is present. (EM X4000)

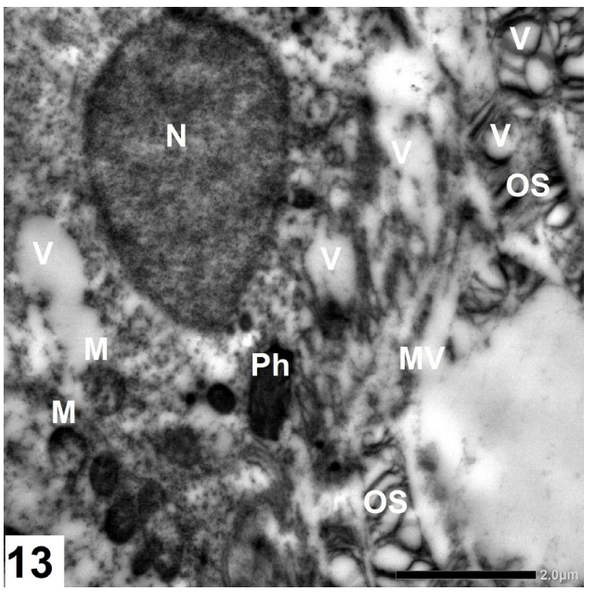



**Fig. 13:** An electron micrograph of the retina of group II (diabetic group) showing retinal pigmented cell (RPE) with severely destructed broken apical microvilli (MV), small shrunken oval nucleus (N) and mitochondria with destructed cristae (M) .There are large phagosomes (ph) and vacuolization (V) in the cytoplasm. The photoreceptor outer segments (OS) are disorganized and vacuolated (V). (TEM X4000)

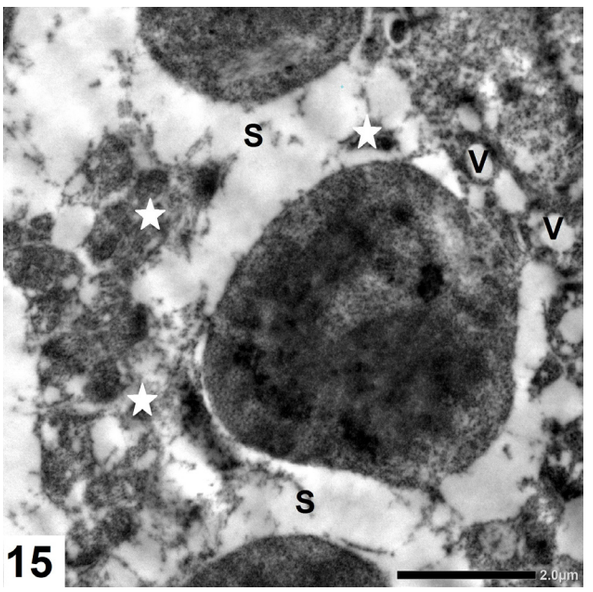



**Fig. 15:** An electron micrograph of a diabetic retina (group II) showing wide spaces between photoreceptor nuclei (S) filled with debris (star) and multiple cytoplasmic vacuolization (V). (TEM X4000)

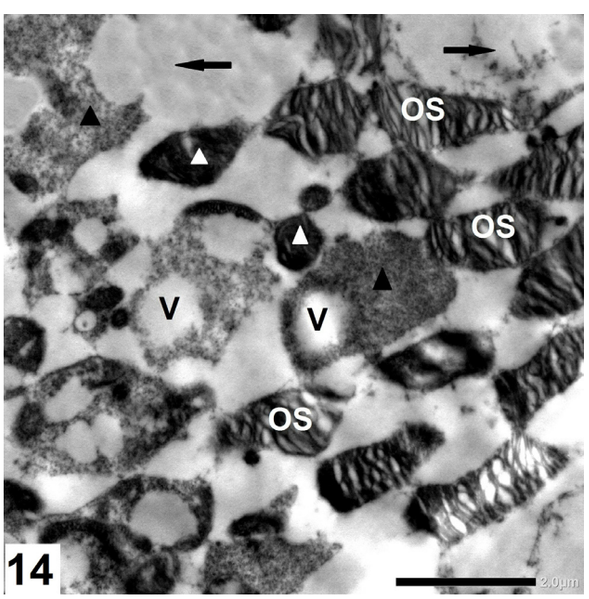



**Fig. 14:** An electron micrograph of the retina of group II (diabetic group) showing disorganized photoreceptor outer segments with irregular membranous lamellae (OS) and vacuolization (V). There are degenerated segments (arrowheads) and wide areas of lost outer segments (arrows). (TEM X4000)

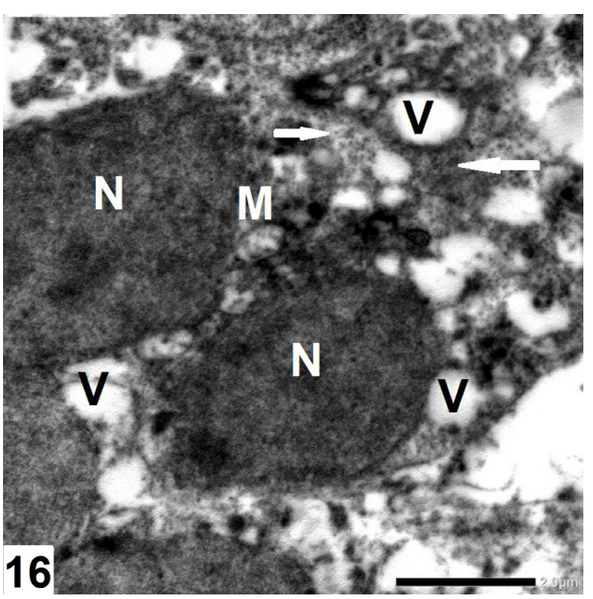



**Fig. 16:** An electron micrograph of a diabetic retina(group II) showing bipolar cells having condensed nuclei (N), many cytoplasmic vacuolization (V) and mitochondria with distorted cristae (M).Rarified cytoplasm can be seen (arrow). (TEM X4000)

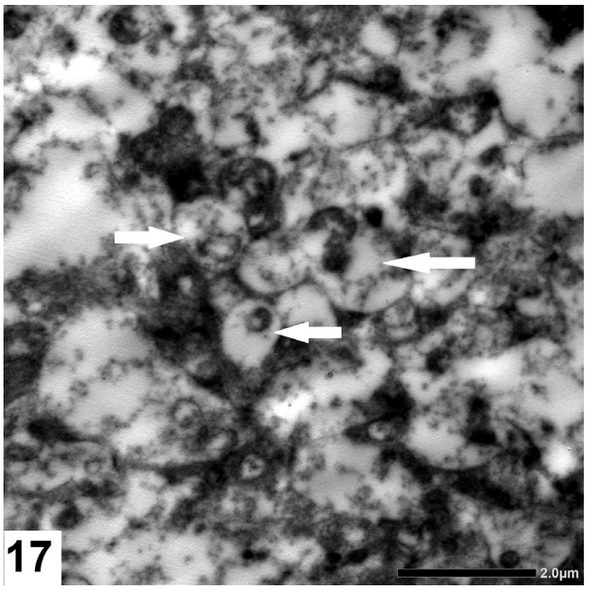



**Fig. 17:** An electron micrograph of the retina (diabetic group) showing IPL having extensively destructed nerve axons (arrows). (TEM X4000)

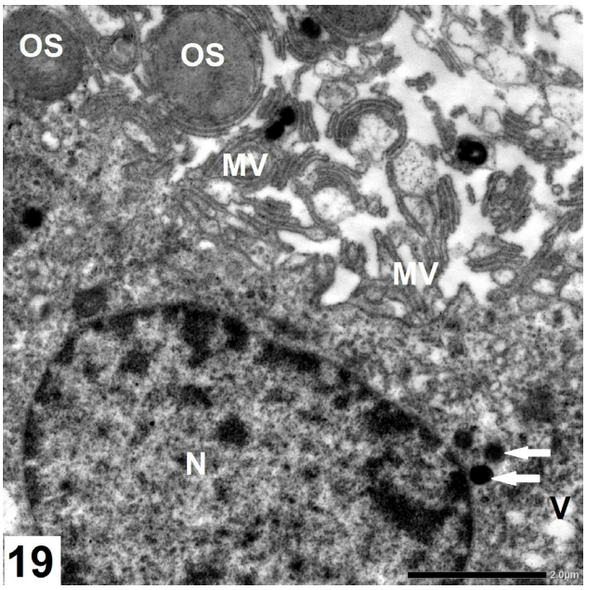



**Fig. 19:** An electron micrograph of the retina (Diabetic + Stem Cells) showing retinal pigmented cell with preserved euchromatic nucleus (N), apical microvilli (MV), melanin granules (arrows) and phagocytosed photoreceptors outer segments (OS). However, some cytoplasmic vacuoles (V) are still present. (TEMX4000)

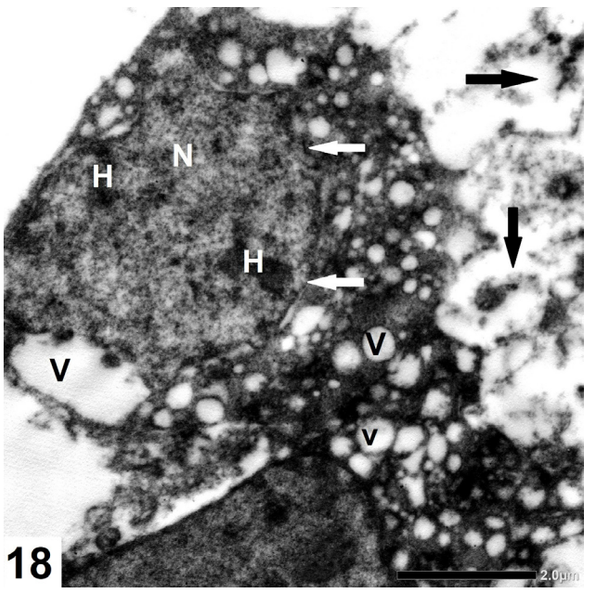



**Fig. 18:** An electron micrograph of the retina (diabetic group) showing ganglion cell with indented irregular nucleus (N), exhibiting areas of electron-dense heterochromatin (H) and destructed nuclear membrane (white arrows). The cytoplasm is heavily vacuolated (V). Note destructed nerve fibres (black arrows) in IPL. (TEM X4000)

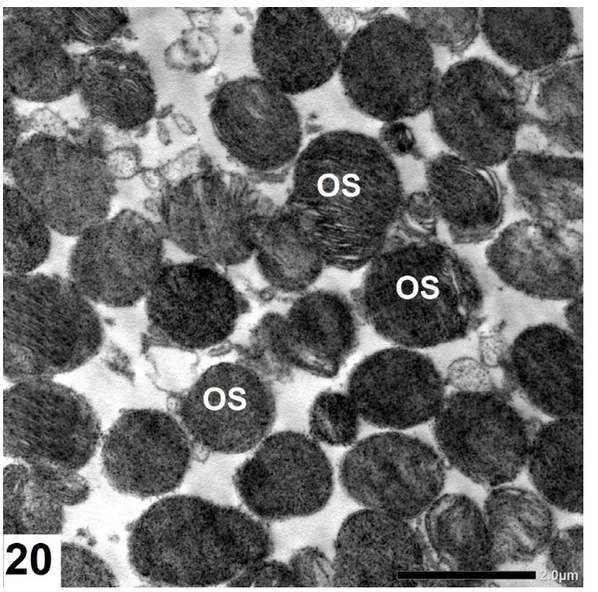



**Fig. 20:** An electron micrograph of the retina of group III (Diabetic + Stem Cells) showing nearly normal photoreceptors outer segments (OS) with regular lamellar appearance. (TEMX4000)

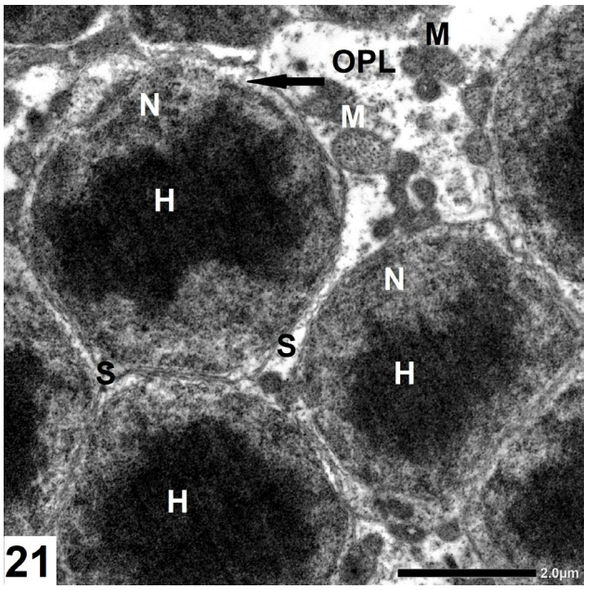



**Fig. 21:** An electron micrograph of the retina of group III (Diabetic + Stem Cells)from the outer nuclear layer showing nearly normal nuclei (N) with heterochromatin (H) surrounded by thin rim of cytoplasm (arrow) with little intercellular spaces (S).Synaptic region of outer plexiform layer (OPL containing mitochondria (M)) can be seen. (TEMX4000)

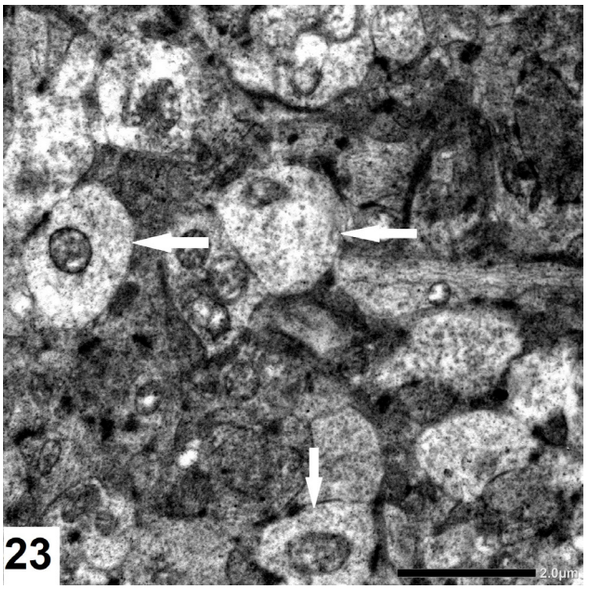



**Fig. 23:** An electron micrograph of the retina of group III (diabetic +Stem cells group) showing nearly normal IPL having intact nerve axons (arrows). (TEM X4000)

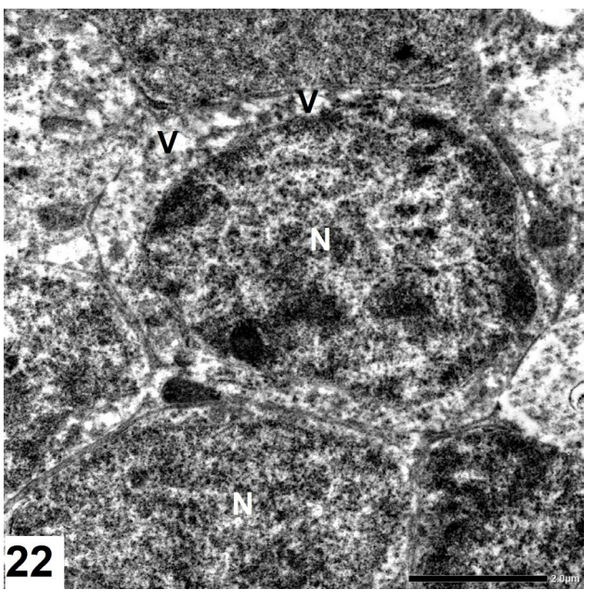



**Fig. 22:** An electron micrograph of the retina of group III (Diabetic + Stem Cells)from the inner nuclear layer showing bipolar cells, tightly packed together and having euchromatic nuclei (N). Slight vacuolization (V) of the cytoplasm is still present. (TEMX4000)

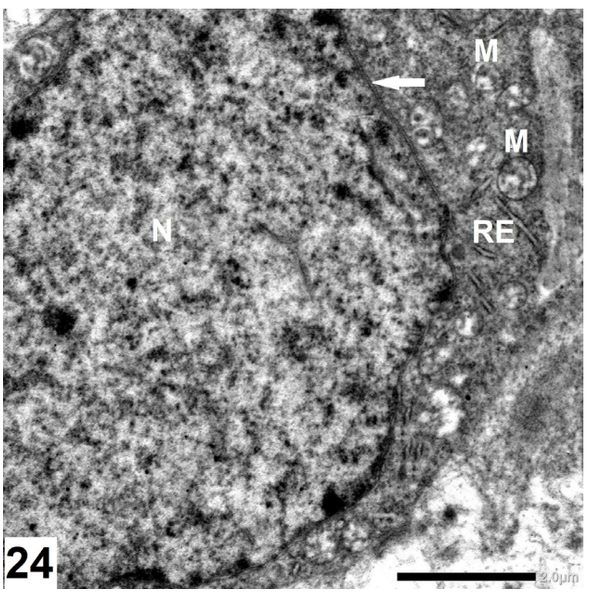



**Fig. 24:** An electron micrograph of the retina of group III (Diabetic + Stem Cells) showing ganglion cell with large euchromatic nucleus (N) and intact nuclear envelope (arrow). The cytoplasm contains rough endoplasmic reticulum (RE) and swollen mitochondria (M). (TEM X4000)

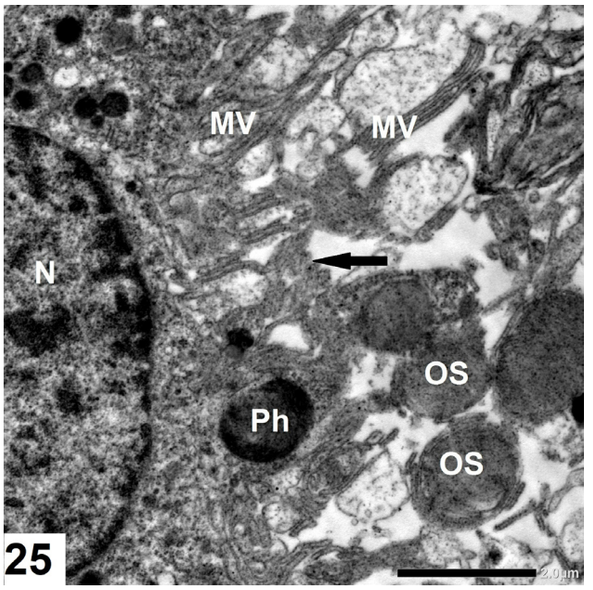



**Fig. 25:** An electron micrograph of the retina of group IV (Diabetic +Genistein) showing nearly normal retinal pigment epithelium (RPE) having euchromatic nucleus (N)and many long apical microvilli (MV) enclose photoreceptor outer segments (OS). Notice large phagosome (ph) and few broken microvilli (arrow). (TEM X4000)

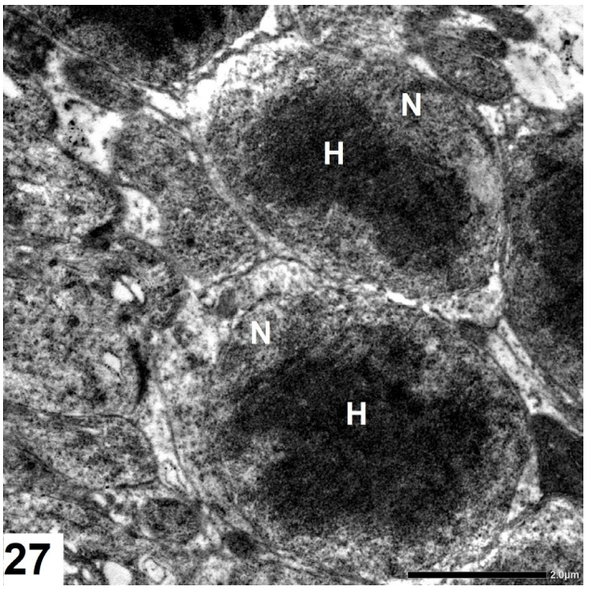



**Fig. 27:** An electron micrograph of the retina of groupIV (Diabetic +Genistein)showing tightly packed photoreceptors cells containing rounded nuclei (N) with its characteristic dense chromatin (H). (TEM X4000)

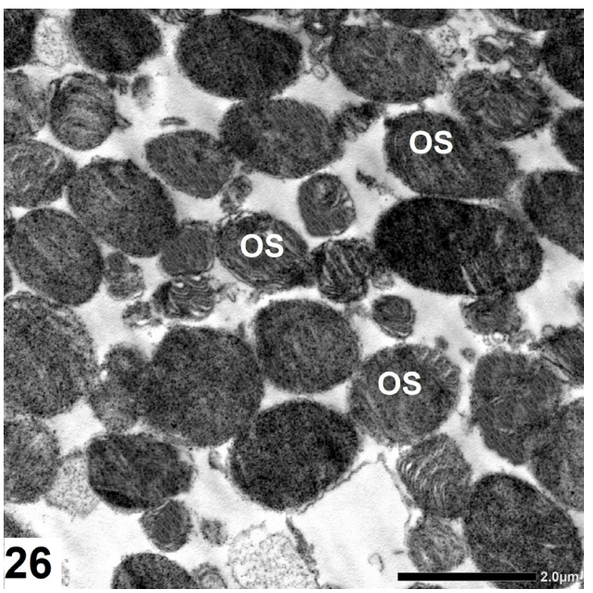



**Fig. 26:** An electron micrograph of the retina of groupIV (Diabetic +Genistein) showing multiple photoreceptors outer segments (OS), mostly having normal lamellar appearance. (TEM X4000)

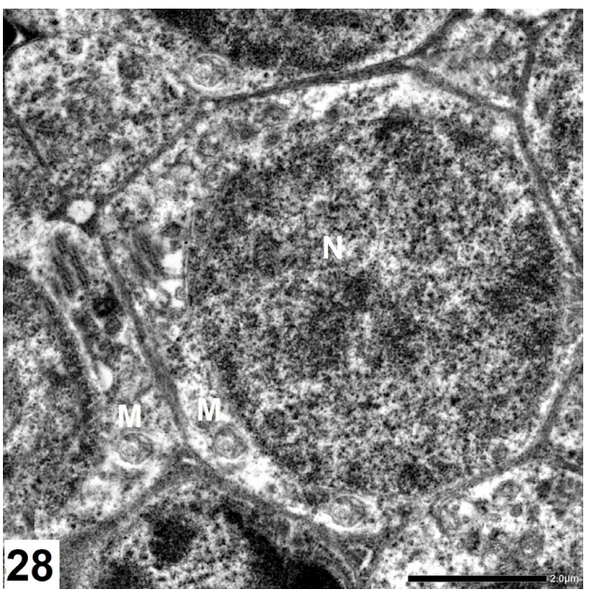



**Fig. 28:** An electron micrograph of the retina of groupIV (Diabetic +Genistein) showing bipolar cells having large euchromatic nuclei (N). The cytoplasm filled with mitochondria (M). (TEM X4000)

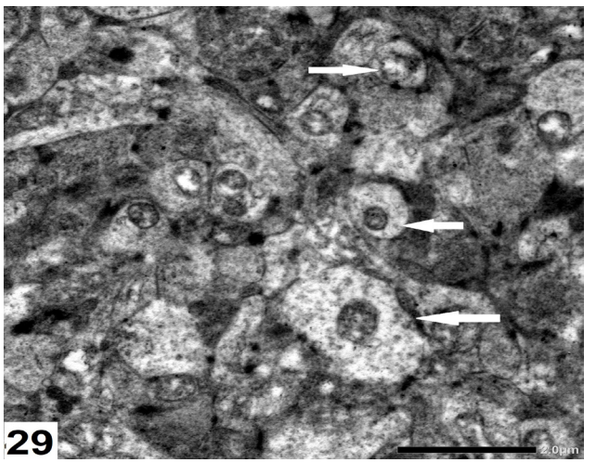



**Fig. 29:** An electron micrograph of the retina of group IV (diabetic +Genistein group) showing normal nerve axons of IPL (arrows). (TEM X4000)

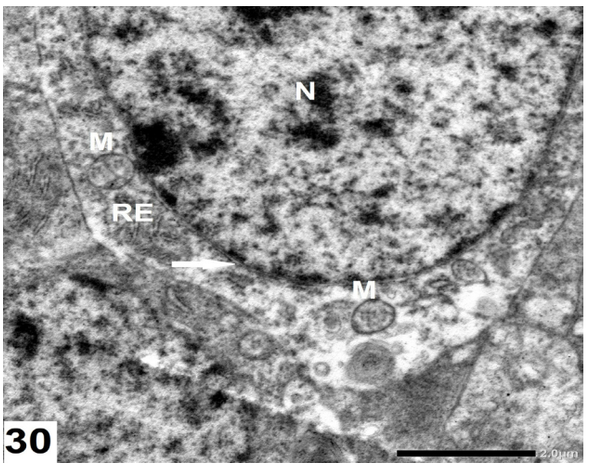
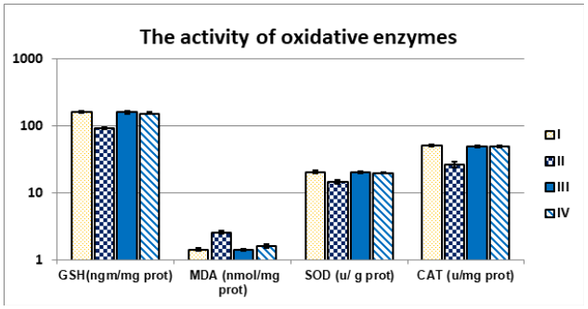



**Fig. 30:** An electron micrograph of the retina of group IV (Diabetic +Genistein) showing ganglion cell with large an euchromatic nucleus (N) and normal nuclear envelope (arrow). The cytoplasm contains rough endoplasmic reticulum (RE) and mitochondria (M). (TEM X4000)

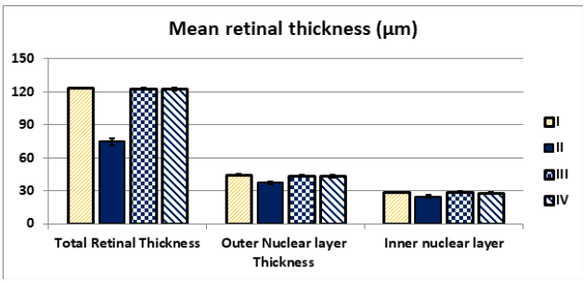
**Table 1:** Effect of different treatments on mean 0f body weight, levels of MDA, SOD and CAT, retinal thickness, outer and inner nuclear layers, and immune reactivity of Caspase-3, COX-2 and Vimentin of all groups of rats

| | $\bar{X}$±SD | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | *P-Value* |
| Body weight in grams | 200.8±8.1 | 190.9±6 | 203.2±2.75 | 199.7±3.1 | P1= 0.024<br>P2= 0.33<br>P3= 0.7 |
| GSH (µmol/gm) | 163.5±4.86 | 92.9±3.39 | 160.5±5.97 | 155.6±5.7 | P1= 0.0001<br>P2= 0.19<br>P3= 0.0001<br>P4= 0.0001 |
| MDA (nmol/mg protein) | 1.46±0.06 | 2.63±0.14 | 1.43±0.06 | 1.63±0.11 | P1= 0.0001<br>P2= 0.2<br>P3= 0.0001<br>P4= 0.001 |
| SOD (u/mg protein) | 20.78±0.84 | 14.8±0.95 | 20.42±0.61 | 20.12±0.34 | P1= 0.0001<br>P2= 0.1<br>P3= 0.08<br>P4=0.0001 |
| CAT (u/mg protein) | 51.10±2.1 | 26.80±2.82 | 49.88±2.1 | 49.83±1.82 | P1= 0.0001<br>P2= 0.2<br>P3= 0.08 |
| Total Retinal Thickness (µm) | 123.27±0.43 | 74.6±2.9 | 122.95±1.58 | 122.27±1.22 | P1= 0.0001<br>P2= 0.53<br>P3= 0.06 |
| Outer Nuclear layer Thickness (µm) | 44.09±0.86 | 37.11±1.1 | 43.79±1.17 | 43.09±1.27 | P1= 0.0001<br>P2= 0.34<br>P3= 0.084 |
| Inner Nuclear layer Thickness (µm) | 28.19±0.26 | 24.81±1.24 | 28.66±0.62 | 27.89±O.88 | P1= 0.0001<br>P2= 0.06<br>P3= 0.28 |
| Caspase-3 immune reactivity | 3.28±0.6 | 63.6±4.97 | 3.74±0.42 | 3.77±0.65 | P1= 0.0001<br>P2= 0.1<br>P3= 0.07 |
| COX-2 immune reactivity | 2.67±0.61 | 51.50±4.97 | 3.04±0.42 | 3.03±0.65 | P1= 0.0001<br>P2= 0.09<br>P3= 0.07 |
| Vimentin immune reactivity | 7.54±0.57 | 31.10±2.85 | 7.95±0.76 | 8.03±1.05 | P1= 0.0001<br>P2= 0.1<br>P3= 0.16 |

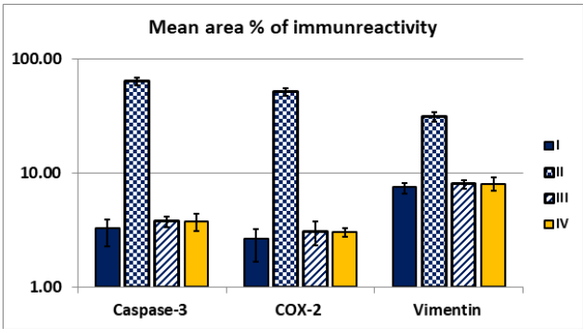
$\bar{X}$= the mean value. SD= the standard deviation.
Significant = (*P value*≤ 0.001).
P1=Group 2 compared to control
P2=Group 3 compared to control
P3=Group 4 compared to control
P4=Group 4 compared to group 3




**Histogram 1:** The activity of the antioxidant enzymes




**Histogram 2:** Mean of total retinal thickness, outer nuclear layer and inner nuclear layer (µm)




**Histogram 3:** Mean area % of immunoreactivity

## DISCUSSION

Diabetic retinopathy is a complication of diabetes that results in injury to the retina due to disruption of its vasculature. In developed countries, it is a leading cause of vision loss[30]. Genistein has a strong anti-diabetic efficacy due to its protecting role on pancreatic β-cells[17]. Stem cell therapy can mitigate the effects of such retinal degenerative processes[12].

The goal of current study was to compare and contrast the therapeutic benefits of BMSC versus genistein on diabetic retinopathy. Diabetic animals have shown a significant decrease in body weight as previously stated that a reduction in body weight could be due to breakdown of tissue proteins in diabetic rats[31]. MDA levels increased significantly, while GSH, CAT, and SOD levels decreased significantly. Similar to the data recorded by other studies[32,33] found that reducing enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH) cause oxidative stress in diabetic retina. The retina has the highest levels of oxygen uptake and glucose oxidation, as well as a high concentration of unsaturated fatty acids. As a result, the retina is more susceptible to oxidative stress[34].

Reduced retinal thickness and multiple photoreceptor damage were discovered on histological examination in this work. With cell loss, ganglion cells were found to have pyknotic nuclei. The intercellular spaces between the inner and outer nuclear layers have expanded, revealing pyknotic nuclei in the INL, as well as empty IPLs and OPLs. This was in line with previous research that showed streptozotocin-treated rats had pyknotic nuclei in their neural cells[35] and were associated with a decrease in the total number of neurons, ganglion cells, and nerve fibres in the retina[36]. Retinal thickness was substantially decreased in diabetic patients with DR due to reduced blood flow, resulting in retinal degeneration and tissue loss[37]. Lim and his colleagues reported a marked apoptotic loss in the ganglion cell and a reduction in the inner plexiform layer in diabetic retinopathy, which may be linked with neuro-degeneration due to prolonged glucose metabolism disorders and microvascular disruption[38]. In mice, DM induced thickness declines in the INL, ganglion cell layer (GCL), and outer nuclear layer (ONL)[39].

Immunohistochemical results revealed strong cytoplasmic reaction of anti caspase-3 in ONL, INL, IPL and GCL in diabetic group. This was consistent with the previous published data that confirmed the apoptotic death of photoreceptor[40], neural cells[35] and ganglion cells[38]in diabetic retina. Activated inflammatory microglial cells released a range of cytokines that led to microvascular complications and apoptosis during the inflammatory response[35]. COX-2 is not normally expressed throughout many tissues. It is raised by pathological triggers. COX-2 expression has been related to inflammation, ischemia, and can be constitutively produced by astrocytes and is generally considered as an "immediate early response gene" following damage to the CNS in *vivo*[41]. Prostaglandins (PGE2), which are formed by the cyclooxygenase reaction, impair retinal blood flow and weaken the blood–retinal/ blood–aqueous barrier[42]. Schoenberger *et al*,[42] reported that diabetes raises COX-2 levels in the retina. They also found that COX-2 or its products were also linked to retinal cell death. The role of cyclooxygenase-2 (COX-2) and PGE2 in the pathogenesis of diabetic retinopathy has been confirmed by recent research findings[43]. Individuals' vitreous fluid and animal models of diabetic retinopathy have been shown to have vastly greater PGE2 levels, a result of Cox-2. Prostaglandin inhibitors can interrupt or slow the progression of retinopathy[44]. Long-term exposure to elevated glucose concentrations and other diabetes risk factors, according to Roy *et al*.[45], enhanced COX-2 expression and activation which in term increased production of cytotoxic PGE2and inflammatory cytokines.

Our findings showed that diabetic rats had higher levels of vimentin expression, which is similar to previous

studies that found STZ to cause early gliosis in rats[10,46]. In diabetic Müller cell culture, high glucose levels and oxidative stress induced GFAP expression[47]. Muller cell expression increased in the DR in response to retinal inflammation[48] and toxic ROS release[49]. Müller cells produced and secreted cytokines that induced retinal neuronal and vascular cell dysfunction[48].

Electron microscopic results showed a degenerated retinal pigmented cell with broken apical microvilli containing pyknotic nucleus, degenerated mitochondria, phagosomes and vacuolation. Loss and disorganized vacuolated photoreceptor outer segments with irregular lamellae. Loss of some of ONL cells was observed. Bipolar cells had mitochondria with distorted cristae, cytoplasmic vacuolation and pyknotic nucleus. The IPL had extensively destroyed nerve axons. Ganglion cells contained heterochromatic indented nucleus with electron-dense areas, vacuolation and destruction of the nuclear membrane. Our findings were consistent with those of others who found that RPE cells were lost and degenerated. Many RPE cells have been found to contain disturbed organelle morphology, such as pyknotic nuclei, dilated and reduced endoplasmic reticulum, degenerated mitochondria and altered melanosomal distribution[50]. Six weeks diabetic rats had a 31% lower photoreceptor cell density. The photoreceptor's outer segments were found to be distorted and fewer in number[45] which may be linked to hyperglycaemia[51]. Rats treated with streptozotocin had degeneration and malfunction of the inner retinal neurons with pyknoticnuclei and cell loss[52]. Loss of GCs due to apoptosis coupled with morphological alterations can explain some of the functional disorders of diabetes[53]. Degenerated mitochondria were present in GCs and some were shrunk and had condensed cytoplasm, decreased organelles with undefined nuclear membrane and cytoplasmic vacuoles[52]. Morphological changes in IPL due to neurodegenerative alterations displayed as neuronal apoptosis[54]. We can deduce that hyperglycaemia associated with metabolic disorders caused degenerative retinal changes in DR by inducing microvascular complications, neovascularization, and a breakdown of the blood-retinal barrier[2]. Mitochondrial dysfunction resulted in toxic reactive oxygen species (ROS) being released, which caused retinal cell injury[55].

BM-MSCs are multipotent mesenchymal stromal cells known as self-renewing cells present in all postnatal tissues and organs[56]. In our results, the diabetic animal treated with BM-MSCs showed significant improvements in all oxidative markers relative to the diabetic group and almost normal histological architecture. Negative reactivity was observed for caspase-3 and cox-2.Mesenchymal stem cells (MSCs) induced an antioxidant capacity through the expression of sulfoxide reductase A, which absorbed reactive oxygen species[57].

MSCs may be suitable for therapeutic applications in terms of tissue replacement. It was thought that MSCs could differentiate into a variety of cells, even retinal neurons[8]. BMSCs were found to contribute to the protection of the ischaemic and diabetic retina in rats and had an anti-apoptotic effect[58-60]. MSCs have demonstrated neuroprotective effects in animal models of retinal degeneration and ischemia-damaged retinas[61]. Injection of adult stem cells into the rat eyes improved retinal structure and function[62]. Intravitreal MSC administration reduced oxidative stress and had a cytoprotective effect in diabetic mice's retinas via stimulating several factors such as nerve growth factor, basic fibroblast growth factor, and glial cell line-derived neurotrophic factor[63]. MSCs decreased inflammatory mediators such as COX-2 and TNF-α by suppressing activated microglia[64]. BMSCs greatly decreased vimentin expression. Previous research has found that BMSCs suppress vimentin expression in diabetic retinas[19,65]. When MSCs were injected into the retina, ROS-induced neurovascular damage was minimized, resulting in reduced death, vascular leakage, apoptosis, and inflammation[64,66].

Electron microscopic finding confirmed the light microscopic results, the retinal pigment epithelium and outer segment photoreceptors were nearly normal, the ONL had nearly normal nuclei, and the OPL was average. The euchromatic nuclei of bipolar cells were closely packed together. IPL and ganglion cell layer are nearly regular. Other researchers[65] supported the previous results, finding that MSC administration helps most retinal layers recover their normal structure. MSCs, according to Gaddam *et al*.[67], may be a highly effective treatment for diabetic retinopathy. The therapeutic benefit of MSCs could be due to their ability to differentiate into various types of retinal cells, including Ganglion cells[8], improved integrity of the blood–retinal barrier[68], and the secretion of anti-inflammatory and neuroprotective mediators[49]. MSCs have the ability to differentiate into pericytes and incorporate into the retinal blood vessels[69].

The retinal layers of the diabetic animal treated with genistein exhibited remarkable histological change. The total retinal thickness and ONL and INL thickness had no differences compared to control. There was mild positive immunoreaction for caspase-3 and mild positive Cox-2 immunostaining in NFL and OPL of retina. The previous results were in agreement with others[70] who discovered that genistein inhibited tyrosine phosphorylation and thus shielded the eyes from ischemic degenerative changes. Genistein protects diabetic retinopathy by inhibiting tyrosine kinase and inhibits TNF-α, ERK and P38 phosphorylation by microglial activation[71]. In diabetic retinopathy, genistein enhances histological alterations, resulting in healing characteristics that mimic those of normal retina[72]. Treatment with genistein reduced inflammation and oxidative stress in diabetic mice, as well as neuronal apoptosis and retinal degeneration. It helps reduce the retinal disorganization characterized by excessive intercellular spaces in the INL and ONL, as well as preventing Müller cell inflammation and swelling[71]. Also retinal epithelial pigment cells are preserved from

glucose toxicity by genistein. Genistein may be a potential therapy for complications related to diabetes, such as diabetic retinopathy[73].

Administration of genistein to diabetic animals decreased the expression of COX-2 in the retinal tissue. These were in accordance with Chae *et al*.,[74] who suggested that dietary isoflavonoids as genistein contributed to the prevention or inhibition of breast cancer through suppressing Cox-2 production.Vimentin expression was decreased after a genistein injection. Our results are consistent with those of others[75,76]. Genistein inhibits TNF-α exit by acting as a tyrosine kinase inhibitor, resulting in suppression of ERK and P38 phosphorylation in microglial cells[77,72].

Our data strongly indicated that conservative treatment with BMSCs or genistein was successful in reducing tissue damage caused by diabetic retinopathy in adult male albino rats in an experimentally induced diabetic model. Both BMSCs and genistein have been shown to be successful in the treatment of diabetic retinopathy. Genistein, on the other hand, is a naturally occurring compound that is less expensive, easier to use, less invasive, and accessible to a wide range of people. Furthermore, it can hold promise as a treatment for diabetic retinopathy. Further research is required to investigate the combined treatment of BMSCs and genistein in diabetic retinopathy patients.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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**الملخص العربى**

# التقييم الهستولوجى والبيوكيميائي للتأثير العلاجي للخلايا الجذعية الوسيطة للنخاع العظمي مقابل الجينيستين على اعتلال الشبكية السكري المستحث في ذكور الجرذان البيضاء

**هالة محمد الحرون ونادية سعيد بدوى**

**قسم الأنسجة ـ كلية الطب ـ جامعة المنوفية**

**المقدمة:** اعتلال الشبكية السكري هو مرض يصيب العين مرتبط بالسكري ويمكن أن يؤدي إلى فقدان البصر. تعد الخلايا الجذعية الوسيطة من نخاع العظام واعدة بعلاجات طبية جديدة. الجينيستين هو مركب طبيعي موجود في منتجات الصويا. تم استخدامه للتخفيف من مستويات السكر في الدم.

**الهدف:** كان الهدف من هذا البحث هو تقييم الفعالية المحتملة للجنستين والخلايا الجذعية الوسيطة من نخاع العظام في علاج اعتلال الشبكية السكري.

**المواد والطرق:** الفئران مقسمة إلى أربع مجموعات: المجموعة الضابطة ، مجموعة مرضى السكري (جرعة واحدة من STZ مجم ٦٠/ كجم حقنا بالغشاء البريتونى، مجموعة مرضى السكر بالخلايا الجذعية الوسيطة ٠,٢ مل من(٣ × ١٠ ٦ خليه/مللى من الخلايا الجذعية الوسيطة المسمى PKH26المسمى) داخل الجسم الزجاجي للفئران المصابة بداء السكري و مجموعة مرضى السكر + الجينيستين (٠,١٨ مجم / كجم عن طريق الفم). تم تخدير الحيوانات والتضحية بها في نهاية الدراسة (٨ أسابيع). تمت إزالة كلتا عينى الحيوانات لإجراء التحليلات النسيجية والكيمياء المناعية والمجهر الإلكتروني. تم إجراء الدراسات البيوكيميائية والشكلية مورفومترية.

**النتائج:** الفئران المصابة بمرض السكر انخفضت سماكة الشبكية الكلية ودمرت طبقات المستقبلات الضوئية. تميزت الطبقة النووية الداخلية بنوى رقيقة ذات بقع داكنة. أظهرت الخلايا العقدية نوى متجمعة. كانت هناك أيضًا مناطق متدهورة من طبقات الضفيرة. علاوة على ذلك ، زاد التعبير المناعي لـ caspase-3 و COX-2 وvimentin بشكل ملحوظ. لوحظت تغييرات في البنية التحتية في شكل تحلل الظهارة المصطبغة ، ومستقبلات ضوئية غير منظمة ومفرغة مرتبطة بالنوى المكثفة ، والفجوة السيتوبلازمية ، والميتوكوندريا المشوهة للخلايا ثنائية القطب. من الناحية الكيميائية الحيوية ، كان هناك انخفاض في إنزيمات مضادات الأكسدة ، الجلوتاثيون (GSH) ، ديسموتاز الفائق (SOD) ، والكتلاز (CAT) ، بالإضافة إلى زيادة كبيرة في MDA). )

يحسن كل من الخلايا الجذعية الوسيطة والجنيستين التركيب النسيجي للشبكية الناجم عن مرض السكري.

**الخلاصة:** على الرغم من أن كلا من BMSCs و Genistein علاجًا تدخليًا فعال لاعتلال الشبكية السكري ، يمكن أن يكون Genistein علاجًا واعدًا يؤخر تطور العمليات المرضية المبكرة لمرضى السكري قبل فقدان البصر.