

Effects of Curcumin on Early Retinal Neuro-Degenerative Changes in Diabetic Albino Rats

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Abstract: Diabetic retinopathy (DR) is the leading cause of blindness in working age adults and it remains a major cause of morbidity in diabetic patients. The gradual loss of neurons in diabetic retina suggests that the progression of the disease is ultimately irreversible, since these cells cannot be replaced. A single large dose of Streptozotocin can produce diabetes in rodents or alternatively, multiple small doses can be used, curcumin administration prevents the decrease in the antioxidant capacity that is induced by diabetes and It has been considered to have beneficial effects on the antioxidant defense system. **Aim:** To assess the effect of curcumin in early retinal neurodegenerative changes in diabetic albino rats. **Methods:** Male Sprague-Dawley albino rats were divided into 3 groups (16 rats in each group). (1)Control group, (2) Diabetic group, (3) Diabetic and curcumin treated group . Each group was re-divided into 2 subgroups according to the duration of diabetes (4th and 8th weeks).Diabetes were induced in rats by a single intraperitoneal injection of streptozotocin (65 mg/kg/b.wt) and after three days, fasting blood glucose level was measured .These rats with blood glucose level greater than 250mg/dl were confirmed as diabetic rats. Third group were received only powdered diet supplemented with curcumin 0.5 g/kg . This type of diet was initiated after establishment of diabetes till the scarification day. Eight rats from each group were randomly sacrificed in the 4th week and the rest were sacrificed in the 8th week. Their eyes were enucleated, fixed, processed for Heamatoxylin and Eosin (H&E) and immunohistochemical staining for (glial fibrillary acidic protein) GFAP. **Results:** Diabetic rats showed a significant reduction in the thickness of the inner retinal layers , decrease in the retinal ganglion cells (RGC), reduction was increased in relation to the duration of diabetes . In group (3) there was a significant improvement in the thickness and in (RGC) numbers. The Glial fibrillary acidic protein (GFAP) immunoreactive processes in the diabetic groups were observed in the entire neural retina , while in the same curcumin treated group there was a significant reduction in the reactivity in the affected layers. **Conclusions:** Curcumin can inhibit diabetes-induced retinal abnormalities that are postulated in the development of early diabetic retinopathy. Thus, curcumin appears to be a useful therapy that may inhibit the development and progression of retinopathy, the main complication faced by diabetic patients.

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1. Introduction

Diabetic retinopathy (DR) is the leading cause of blindness in working age adults and it remains a major cause of morbidity in diabetic patients. DR is the 5th leading cause of blindness globally, while it is the first leading cause of blindness in the developed countries in adults at working age. The global prevalence of DR is predictable projected to exceed 200 million individuals by 2012⁽¹⁾. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030⁽²⁾.

Today retinopathy has been defined based on lesions that are clinically demonstrable, and all of those have been vascular in nature⁽³⁾, which become clinically obvious after 10-15 years of diabetes in humans⁽⁴⁾. In addition to the micro-vascular component, diabetic retinopathy also comprise both neurodegenerative and inflammatory features⁽⁵⁾.

The neural retina is a multilayered structure consists of 10 layers. These layers contain supporting glia (Müller cells and astrocytes) and neural cells (photoreceptor, bipolar, horizontal, amacrine & ganglion cells)⁽²⁾. The gradual loss of neurons in diabetic retina suggests that the progression of the disease is ultimately irreversible, since these cells cannot be replaced⁽⁴⁾. Some studies showed that retinal neurodegenerative changes in the early stage of diabetes in Sprague Dawley albino rats was markedly reduced⁽⁶⁾.

Streptozotocin and alloxan are the most frequently used chemicals to injure β -cells. It is a powerful alkylating agent that has been shown to interfere with glucose transport and glucokinase function and to induce multiple DNA strand breaks. As a result of Streptozotocin exposure, β -cells undergo destruction by necrosis⁽⁷⁾. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but is

not recognized by the other glucose transporters. This explains its relative toxicity to β -cells, since these cells have relatively high levels of GLUT2⁽⁸⁾. A single large dose of Streptozotocin can produce diabetes in rodents or alternatively, multiple small doses can be used⁽⁷⁾.

Diabetic retinopathy is characterized by blood-retinal barrier (BRB) breakdown and neurotoxicity, these pathological changes have been associated with oxidative stress. Experimental diabetes induced significant increases in oxidative stress, retinal neuronal cell death, and vascular permeability⁽⁹⁾. These effects were associated with increased levels of tumor necrosis factor α , vascular endothelial growth factor, intercellular adhesion molecule-1 and activation of p38 MAPkinase⁽¹⁰⁾.

Overstimulation of the N-methyl-D-aspartate (NMDA) and non-NMDA receptors by diabetes leads to excessive increase in the levels of intracellular calcium. This in turn leads to activation of nitric oxide synthase and excess accumulation of superoxides and nitric oxide (NO), causing lipid peroxidation, mitochondrial dysfunction, DNA damage, and eventual cell death⁽¹¹⁾. Excitotoxicity activation of NMDA receptors has been proposed as a pathogenic mechanism in severe retinal diseases such as glaucoma and diabetic retinopathy⁽¹²⁾.

Direct or indirect degeneration of pericytes and endothelial cells, thickening of basement membrane and alterations in tight junctions all contribute to the dysfunction of microvasculature in the retina. So when any of these three components of the retinal microvasculature is damaged, capillaries become leaky, occluded, and swollen leading to non proliferative diabetic retinopathy (NPDR). The more progressive proliferative diabetic retinopathy (PDR) occurs when new vessels formed (angiogenesis) from the existing vascular endothelial cells in the retina, to compensate for the ischemia caused by the impaired capillaries. However, the proliferating new vessels are weak and easily damaged. Once rupture, haemorrhage and fibrous scars can form within the vitreous cavity, leading to retinal detachment and eventual blindness at advanced stages⁽¹⁾.

The turmeric (*Curcuma longa*) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice. Curcumin is an orange–yellow crystalline powder practically insoluble in water at acidic and neutral pH, and soluble in alkali. The structure was first described in 1910 by **Lampe and Milobedeska**⁽¹³⁾. Curcumin can bind with heavy metals such as cadmium and lead, there by reducing the toxicity of these heavy metals. This property of curcumin

explains its protective action to the brain, it has been linked with suppression of inflammation, angiogenesis, tumorigenesis, depression, chronic fatigue, neuropathic pain and also diseases of cardiovascular, pulmonary, neurological, skin, liver, bone and muscle⁽¹⁴⁾.

Curcumin significantly increases intracellular antioxidant, Glutathione (GSH), regulates antioxidant enzymes, scavenges hyperglycemia induced reactive oxygen species (ROS) and it is at least 10 times more active as an antioxidant than vitamin E. Curcumin also decreases lipid peroxidation, mitochondrial dysfunction, and the apoptotic indices⁽¹⁵⁾. Its administration prevents the decrease in the antioxidant capacity that is induced by diabetes and It has been considered to have beneficial effects on the antioxidant defence system⁽¹⁶⁾.

In the present study, we have investigated the effect of administration of curcumin on early retinal neurodegenerative changes in diabetic albino rats.

2. Material and Methods

Animals:

Male Sprague-Dawley albino rats (wt.150-200 g) were used in this experimental study. All animals were housed in environmentally controlled rooms, in spacious wire mesh cages at room temperature. Prior to be utilized for experimental purpose, food and water were allowed *ad libitum*. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of National Research Centre in Egypt.

Animal grouping:

Rats were divided into three groups according to their treatment as following (16 rats in each group):

Group (1): Control group.

Group (2): Diabetic group.

Group (3): Diabetic and curcumin treated group. Each group was divided into 2 subgroups (4th and 8th weeks) according to the duration of diabetes.

Diabetes induction:

Diabetes was induced in rats after fasting overnight by a single intraperitoneal injection of streptozotocin (65 mg/kg/body wt) dissolved in 0.01 mol/L sodium citrate buffer pH4.5. After three days, fasting blood glucose level was measured using glucometer & strips (fasting for 16 hours). The rats with blood glucose level greater than 250mg/dl were confirmed as diabetic rats⁽⁴⁾. The diabetic rats were randomly selected to form group 2 (diabetic group) and group 3 (diabetic and curcumin treated). Glucose level and body weight of rats were measured before the induction of diabetes. Glucose level & body weight of rats were measured before the induction of diabetes.

Randomly eight rats were sacrificed in the 4th week and the rest were sacrificed in the 8th week in each group. Before sacrifice body weight was recorded.

Curcumin treatment

The rats in group (3) only received powdered diet supplemented with curcumin 0.5 g/kg. This diet was given after establishment of diabetes till the scarification day⁽¹⁶⁾.

Preparation of tissue specimens:

All rats were sacrificed by over dose of ether vapor anesthesia, the body weight and blood glucose level were measured just before scarification. Then their eyes were enucleated & fixed in 10% Of neutral buffered formalin up to 24 hours. Tissues were dehydrated using graded alcohols, cleared with xylene, and infiltrated with paraffin wax. Then the obtained sections were stained with Heamatoxylin & eosin (H&E) staining to measure the thickness of the retina. RGC counting and for Immunohistochemical staining for (glial fibrillary acidic protein) GFAP.

Morphometric study:

The thickness of the whole retina and the 5 layers were measured in micrometers by means of image analyzer using (Iecia quin) software, in the Histology Department, Faculty of Medicine, Cairo university. The thickness of each layer was measured using Haematoxylin & eosin stained sections from each group at two points 1 mm away from both sides of the optic nerve head of each eye (nasal and temporal sides), using low power magnification (x100). The thickness of retinal layers was measured as follows: The inner plexiform layer (IPL) is the distance between the ganglion cell layer (GCL) and the inner nuclear layer (INL) that consists of synapses. The (INL) is the distance between (IPL) and outer plexiform layer (OPL) that consists of cell bodies. The (OPL) is the distance from (INL) and outer nuclear layer (ONL) that also consists of synapses. The (ONL) is the distance from the innermost to the outermost extent of the layer excluding the photoreceptor segments. The photoreceptor segment layer (PSL) is the distance from the outermost limit of photoreceptor nuclei to the tip of the segments. The retina (R) is the distance from (GCL) to the apical surface of retinal pigment epithelium (RPE)⁽⁶⁾.

Retinal ganglion cells (RGC) count:

H&E stained sections from each group were served for the examination of the number of (RGC). in the area between the nasal and temporal ora serrata of retinal tissue sections at two points 1 mm away from both sides of the optic nerve head of each eye using magnification (x 100) in a length of 100 mm. The mean of the two values from every animal was used as the number of (RGC) of the animal and the mean it was defined as the group result.

Immunohistochemistry:

Immunohistochemistry was carried out by using the peroxidase labeled streptavidin biotin method⁽¹⁷⁾.

Primary antibody was Glial fibrillary acidic protein (GFAP), Ab-1 mouse monoclonal antibody, Ab-1 stains astrocytes, glial cells, ependymal cells of the CNS and their corresponding tumours.

Immunohistochemical staining procedure:

All steps are carried at room temperature in humidified chamber. Staining dishes or coplin jars were used and excess liquid was tapped off using a lintless tissue (gauze pad), carefully wiped around the specimen to remove any remaining liquid then endogenous peroxidase activity was blocked by incubating the sections in peroxidase quenching solution for 30 minutes.rinsed gently with distilled water for 5minutes then washed by phosphate buffer saline (PBS) for 5 minutes. suction was used to remove reagents after each step, but drying of specimens between steps avoided. Then slides were covered by none specific binding protein .The Primary Antibodies (Glial Fibrillary Acidic Protein) Ab-1 mouse monoclonal Antibody was applied to cover specimen. The slides were incubated in humidified chamber, at room temperature overnight. In the next morning Slides were washed in PBS (2 times for 5minute each). secondary antibody was applied on the slides and Incubated for 30 minutes in humidified chamber Slides were rinsed as before. Enough drops of the enzyme conjugated Streptavidin peroxidase were applied to cover specimen and Incubated for 30 minutes in humidified chamber. Slides were rinsed as before.

Two drops of the DAB Substrate-Chromogen solution was applied to cover specimen. Incubated for 2-4 minutes depending on the desired stain intensity. Then Rinsed gently with distilled water. Slides were immersed in a bath of hematoxylin, for nuclear counting staining, and then they were washed in taped water .The slides were kept in PBS until they became blue (approximately 30 seconds) then rinsed in distilled water. Slides were dehydrated in a graded series of ethanol (2changes, 5 minutes for each); 70% ethanol, 90% ethanol, & 100% ethanol. The slides were cleared in xylene, then covered with slips using a permanent media⁽¹⁷⁾.

Control slides :In addition to the slides to be tested control slides were included in each staining sections as a positive control tissue ,they were processed in the sequence which we mentioned above but we did not use the primary antibody instead we used PBS.then examined under light microscope. Slides were scored by using Nordic immunohistochemical quality control for evaluation of Immunohistochemical staining results (*Table-1*). On a scale from 0 to 4 as the following:

- 0- means No staining,
- 1- means poor staining
- 2- means borderline staining .

3- means good staining.
4- means very good staining.

Table-1. Scale for scoring the staining Immunohistochemistry⁽¹⁸⁾.

Score	Classification	Definition
0	No staining	No specific staining
1	Poor staining	Very insufficient staining: presenting a very weak staining signal, false-negative staining of cells or tissue components or false-positive staining reaction in cells or tissue components. Incompatible with cell counting.
2	Borderline staining	Insufficient staining: presenting too weak staining, false-negative staining of cells, or false-positive staining of cells. Incompatible with cell counting
3	Good staining	Staining is acceptable visualizing the appropriate cells and tissue components. The staining still could be optimized for improving the staining intensity Suitable for cell counting.
4	Optimal staining	Perfect or close to perfect staining result visualizing the appropriate cells. Suitable for cell counting.

Statistical analysis:

Statistical evaluation of the numerical data was performed to estimate the mean values and the standard deviation in each group (means±SD). Data were analyzed for the difference between means of the different groups by the student's (t-test).The immunohistochemistry GFAP Statistical differences between groups were tested using Chi Square test. The difference was considered significant when (P ≤ 0.05).

3. Results

Body weight changes:

There was a very significant weight gain in both control groups in the 4th and 8th weeks by (p<0.001 and p≤0.0001) respectively but in the diabetic groups the both showed a significant decrease by (P≤0.0001) compared to the control respective groups. There was a significant reduction in the body weight of group (3), (p≤0.001) in 4th weeks and (p≤0.0001) in 8th weeks (Fig.1).

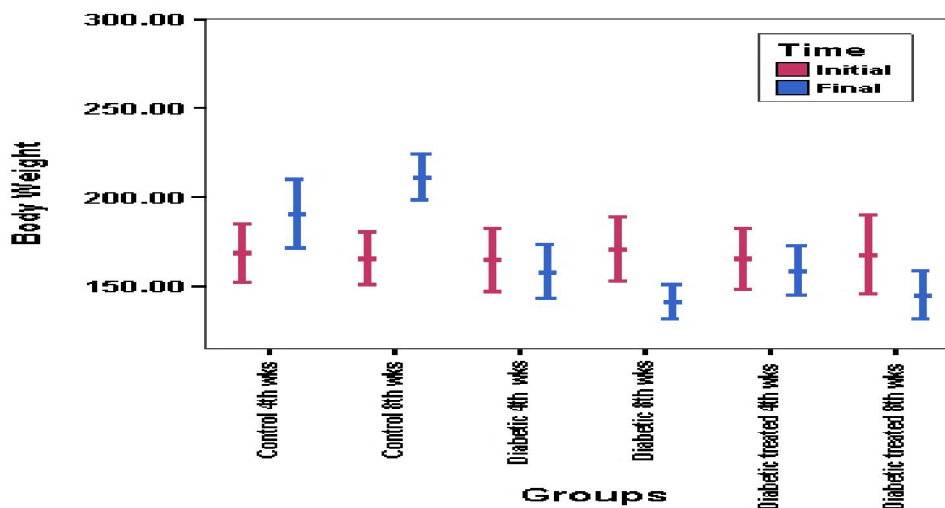


Figure (1): Body weight of the three groups and their two sub groups. Values are expressed as the mean ±SD in gram in the initial & final time (initial time before induction of diabetes and final time just before scarification).

Morphometric measurements of the retinal thickness:

The measurement of IPL indicated that there was a significant reduction in the thickness with the increase in the duration of diabetes when compared it with the Control retina (Fig. 2). There was a

reduction in the 4th week diabetic group by 10.7 % (p<0.034) and in 8th week by 19 % (p<0.002). In diabetic curcumin treated groups there was no a significant improvement in the thickness of the 4th week group 8.6% (p<0.101) and with a significant

improvement in the 8th week group 11.5% ($p < 0.046$). (Fig. 3).

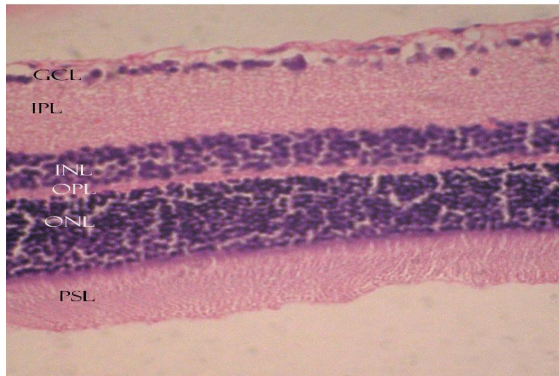


Figure (2): Photomicrograph of the layers in control retina. (GCL) ganglion cell layer, (IPL) inner plexiform layer, (INL) inner nuclear layer, (OPL) outer plexiform layer, (ONL) outer nuclear layer, (PSL) The photoreceptor segment layer (H&E, 40X).

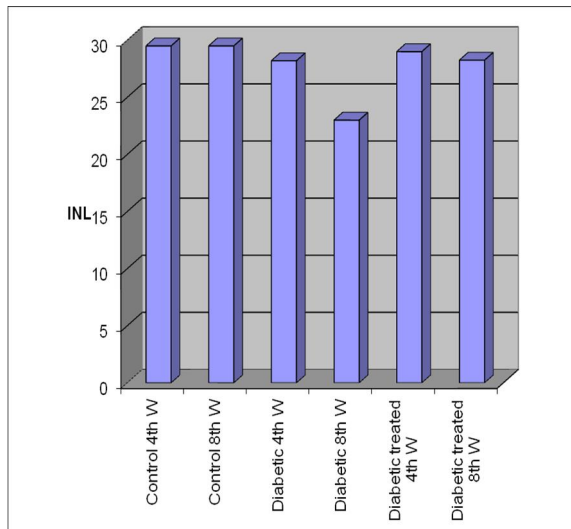


Figure (3): Morphometric measurement of the Inner plexiform layer (IPL). Values were expressed as the mean \pm SD length in μm .

In INL there was a reduction in the thickness of diabetic rats in 4th weeks by 4% ($p < 1$) and with significant reduction in 8th weeks 8.7% ($p < 0.021$) compared to the Control rats. There was a significant improvement in the 8th weeks diabetic curcumin treated group ($p < 0.04$) (fig. 4).

In the outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor segment layer (

PSL) and also in the total retinal thickness there were no remarkable significant changes among the groups.

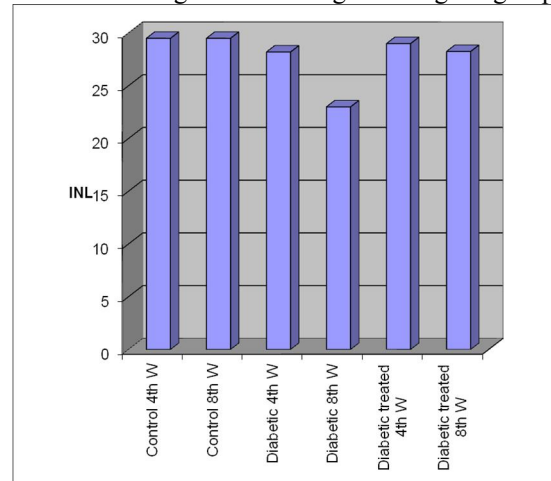


Figure (4): Morphometric measurement of the Inner nuclear layer (INL). Values were expressed as the mean \pm SD length in μm .

There was a significant decrease in the number of retinal ganglion cells (RGC) in diabetic rats of 4th week group 5.3% ($p < 0.049$) and in 8th week group 13% ($p < 0.0001$). In group three there was only a significant improvement in 8th week group by 7.5% ($p < 0.033$). (Fig. 5).

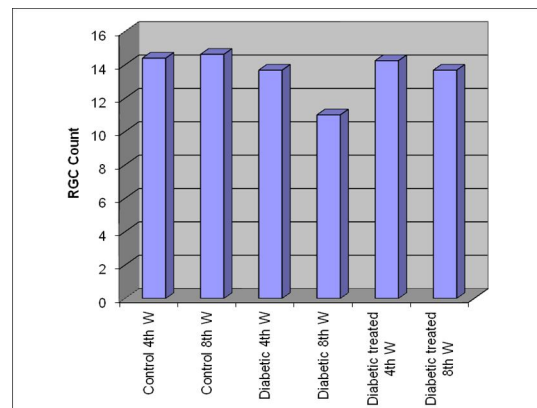


Figure (5): Retinal ganglion cell count (RGC). Values were expressed as the mean \pm SD number of cells.

GFAP Immunohistochemistry:

A very significant difference of GFAP expression was observed among the 3 groups and their two sub-groups using Chi-square test. Expression of GFAP was confirmed by the presence of the stained vertical brown strips in the retinal tissue. GFAP immunohistochemistry reactivity of the 4th and 8th weeks of control groups, their staining 100% within the poor staining grade (1) (Figure 8), In

which the GFAP immunoreactivity is restricted primarily to GCL (Figure 6).

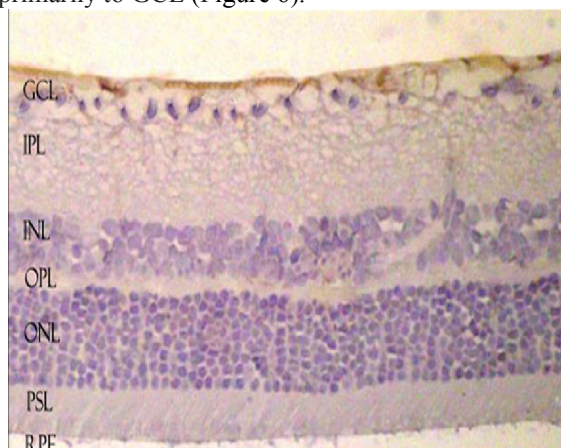


Figure (6): Glial fibrillary acidic protein (GFAP) immunostaining of control retina. Glial fibrillary acidic protein immunoreactivity was observed in the GCL layer. (GCL) ganglion cell layer, (IPL) inner plexiform layer, (INL) inner nuclear layer, (OPL) outer plexiform layer, (ONL) outer nuclear layer, (PSL) The photoreceptor segment layer, (RPE) retinal pigment epithelium (40X).

Diabetic groups of 4th weeks GFAP reactivity was observed by 50% no staining, 37.5% in grade(1) and 12.5% in grade(2) (Table-2) & (figure 8). The reactivity was seen in GCL and extended to the outer retinal layers OPL & ONL (Figure7,A). In Which a significant difference was seen compared to the control group ($p > 0.002$). In the same weeks curcumin treated group their reactivity was significant ($p > 0.002$) compared to diabetic group, 62.5% in grade(1) and 37.5% in grade(2). (Table-2) & (Figure 8). Reactivity processes was with slight extension to the outer layer OPL (Figure7,B).

In the 8th weeks diabetic group the GFAP immunoreactive processes were observed in the entire neural retina excluding the PSL (Figure7,C). In which a very significant difference was seen compared to the control group ($p > 0.0001$). 66.7% in grade(3) and 33.3% in grade(4). In the same weeks curcumin treated group the ($p > 0.0001$) which was also very significant compared to diabetic group, 50% in grade (1) and 50% grade (2) (Table-2) & (Figure8) with a reduction in the reactivity in the affected layers, (Figure7,D).

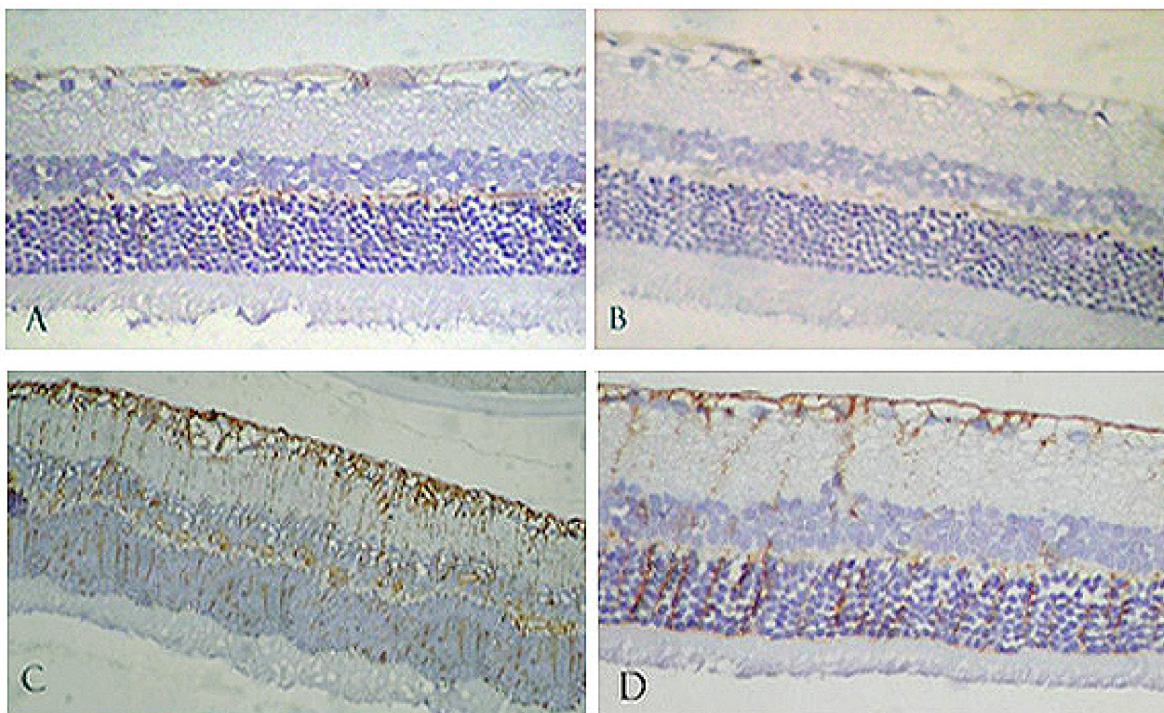


Figure (7): Glial fibrillary acidic protein (GFAP) immunostaining of diabetic retina, (A) in 4th week group immunoreactivity was observed by the presence of the stained vertical brown strips in the retinal tissue in GCL, OPL, ONL layers. (B) 4th week curcumin group the reactivity processes was with slight extension to OPL. (C) in 8th week immunoreactive processes were very strong in the entire neural retina excluding the PSL. (D) 8th week curcumin group the reactivity showed severe reduction after treatment (40X).

Table (2): Percentage of staining grades of the GFAP immuno-histochemistry in the three groups and their two sub-groups.

	Control 4 th week (%)	Control 8 th week (%)	Diabetic 4 th week (%)	Diabetic 8 th week (%)	Diabetic treated 4 th week (%)	Diabetic treated 8 th week (%)
No Stain	-	-	50	-	-	-
Poor Stain Grade (1)	100	100	37.5*	-	62.5*	50**
Border line Stain Grade (2)	-	-	12.5	-	37.5	50**
Good Stain Grade (3)	-	-	-	66.7**	-	-
Very good Stain Grade (4)	-	-	-	33.3*	-	-

*P Value ≤ 0.05 is significant. ** P Value ≤ 0.001 is highly significant.

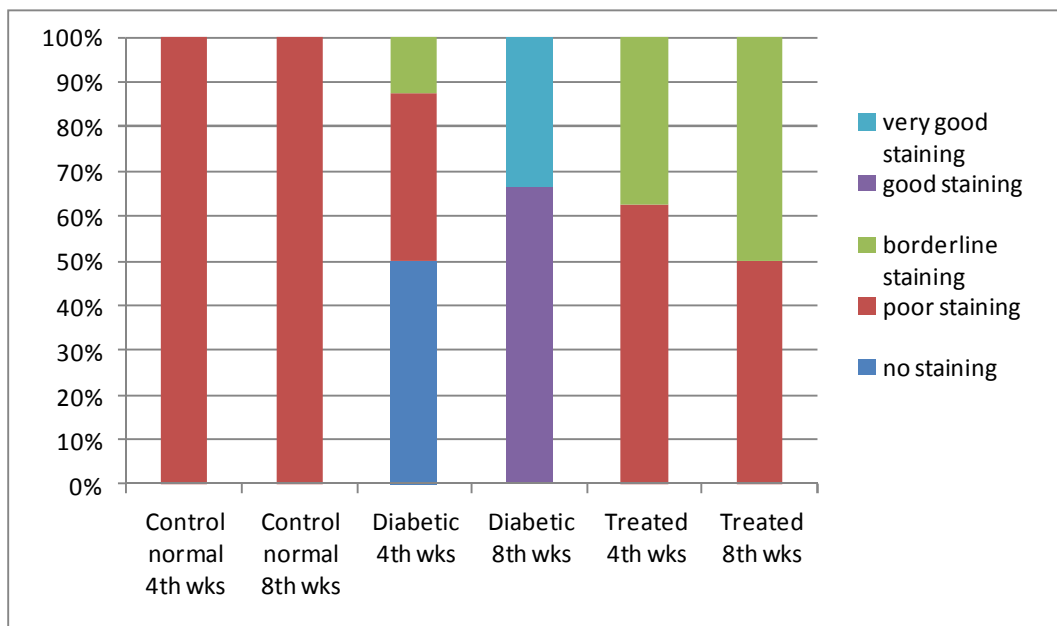


Figure (8): Percentage of the Glial fibrillary acidic protein GFAP staining in the three groups & their two subgroups.

4. Discussion

Early in the 1960s, **Bloodworth *et al.*** ⁽¹⁹⁾ suggested that the acute stages of diabetes are characterized not only by impaired retinal vasculature, but also by structural and functional changes in retinal neural cell. Previous Clinical ⁽²⁰⁾ and animal studies ⁽²¹⁾ have showed that the pathological changes in retinal neural cells precede retinal microvascular abnormalities and to explain the early visual dysfunction mechanism of DM patients, the present study tried to determine the effect of curcumin in the early diabetic neurodegenerative changes in rats. Rats are usually used as the subjects of STZ diabetic experiments ⁽²²⁾. Some studies showed that retinal neurodegenerative changes in the early stage in diabetes like; thickness of the internal plexiform layer (IPL) and photoreceptor segment layer (PSL) of diabetic rats in 1 month and 6 months after the onset was markedly reduced ⁽⁶⁾. Diabetic retina in mice for 10 weeks

were observed in the whole retina with significant differences, the inner and outer nuclear layers and The number of retinal ganglion cells were also decreased ⁽²³⁾. Some vascular characteristic features such as the loss of pericytes and thickening of capillary basement membrane demonstrated with the STZ model of diabetic rats resemble those in humans ⁽²²⁾. For instance, significant reductions in the thickness of the inner retinal layer and number of ganglion cells in 7.5-month-old STZ rats were observed ⁽⁶⁾.

In addition, a reduction in the number of the nerve fibers, increases in the number of glial cells in the optic nerve ⁽¹²⁾ and glial fibrillary acidic protein (GFAP) expression have been observed in diabetic rats 3 months after the onset of diabetes. However, only a few reports dealt with the early changes in the neural elements in the retina of diabetic rats ⁽¹⁸⁾.

The photoreceptor outer segments of the PSL are dynamic structures that continually exchange and

renew their membrane and protein components. The retinal pigment epithelium (RPE) plays an active role in this process by digesting old materials to make room for the new and, in doing so, conserves vitamin A and other needed compounds within the eye. The visual dysfunction of diabetic retinopathy is considered to be a defect at the post-receptor level, which is a level above the photoreceptor-RPE cell complex in the inner retina⁽³⁾. As the disruption of photoreceptor-RPE cell complex is accompanied by the disappearance of lectin-binding sites on the retinal interphotoreceptor matrix (IPM)⁽⁶⁾. The thickness of IPL & PSL were markedly reduced in SD rats 1 or 6 months after the onset of diabetes. These results are consistent with the report of **Barber et al.**,⁽²⁴⁾.

The fact that neither the bipolar cells nor the photoreceptor cells showed morphological changes in the cell body in diabetic rats optically and electron microscopically leads to the speculation that the axon and processes of retinal neurons are more sensitive than the cell body in DM. That is why the IPL was shortened, as it consists of synapses formed by the axons of the bipolar cells and the dendrites of ganglion cells⁽⁶⁾. In our study there was also a significant reduction in the thickness of the IPL & INL and this reduction increases with the increase in the duration of diabetes in 8th weeks group more than in 4th weeks group. In diabetic curcumin treated groups there was only an improvement in 8th week groups in both affected layers. The outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor segment layer (PSL) and also in the total retinal thickness there were no remarkable significant changes among the groups.

Neuronal apoptosis is believed to be one of the underlying causes responsible for lesions in early retinal neural cells observed in diabetic retinopathy, which are typically represented by reduced numbers of ganglion cells and thinned ganglion cell layer, inner plexiform layer, and

inner and outer nuclear layer⁽²⁴⁾. **LI Yong-hao et al.**, found that two weeks after STZ induction of diabetes in Wistar rats, apoptosis of retinal neural cells was observed, so hypothesis that apoptosis of retinal ganglion cells occurs mainly through a caspase-dependant pathway in the early stages of diabetes⁽²⁵⁾. In our study there was a decrease in the number of retinal ganglion cells (RGC) in diabetic rats of 4th & 8th weeks after induction of diabetes with an improvement in the curcumin treated 8th week group.

Lieth et al., reported that one of the early histological alterations in the retina of diabetic rats was the increase in GFAP expression in astrocytes of the nerve fiber layers and processes of Müller cells in 3-month STZ diabetic rats. GFAP immunoreactivity

was observed in the nerve fiber layer of SD rats 1 month & 6 months after the onset of diabetes. The GFAP immunoreactivity showed no difference between diabetic for 1 month and age-matched control rats. GFAP immunoreactive processes were observed in the entire neural retina excluding the PSL⁽¹⁸⁾. To conclude, Müller cells remained inactive even though degeneration of the retinal neuronal processes and RPE had already begun 1 month after the onset of diabetes, as the Müller cell processes did not reach the PSL, where only rods/cones degenerated at the period. Retinal pigment epithelium is the structure in retina that degenerates in the early stage of diabetic rats. It suggests the destruction of the transport pathway between the outer retina and choroid and a possible breakdown of the blood-retinal barrier (BRB)⁽²⁴⁾. we found that GFAP immunohistochemistry reactivity was restricted primarily to GCL in the 4th and 8th weeks in control groups, in 4th week diabetic groups the reactivity was seen in GCL and extended to the outer retinal layers OPL & ONL. In which a significant difference was seen compared to the control group. In the same weeks curcumin treated group their reactivity was significant compared to diabetic group. Reactivity processes was in the inner layers of the retina & with slight extension to the outer layers mainly seen in OPL.

In the 8th week diabetic group the GFAP immunoreactive processes were observed strongly in the entire neural retina excluding the PSL. In the same weeks curcumin treated group, the reactivity showed a severe reduction after treatment in the affected layers.

Curcumin was tested as protective agent against excitotoxicity in rat retinal cultures. It reduces N-methyl-D-aspartate (NMDA) - mediated excitotoxic cell damage, estimated as decrease of cell viability and increase in apoptosis⁽²⁶⁾. In 2007 **Renu and Mamta** were found that the effects of curcumin on the metabolic abnormalities in diabetic retinopathy could have potential benefits in inhibiting the development of retinopathy⁽²⁷⁾. In recent studies, treatment with curcumin showed significant hypoglycemic activity compared with the diabetic group. Retinal glutathione levels were decreased by 1.5 folds, and antioxidant enzymes, superoxide dismutase and catalase, showed >2 fold decrease in activity in the diabetic group; on the other hand, curcumin positively modulated the antioxidant system, proinflammatory cytokines, tumor necrosis factor- α and vascular endothelial growth factor, were elevated >2 fold in the diabetic retina, and prevented by curcumin. Transmission electron microscopy showed degeneration of endothelial cell organelles and increase in capillary basement membrane

thickness in diabetic retina, while curcumin prevented the structural degeneration and increase in capillary basement membrane thickness of the diabetic rat retina⁽²⁸⁾.

Curcumin administration prevented the reduction in the antioxidant capacity, and increase in 8-hydroxydeoxyguanosine (8-OHdG) and nitrotyrosine of the diabetic rats, It also inhibited diabetic elevation in the levels of interleukin-1 β , vascular endothelial growth factor VEGF and nuclear transcription factor NF- κ B. The effects of curcumin were achieved without amelioration of the severity of hyperglycemia. It has beneficial effects in experimental studies of the diabetic retinopathy that are characterized by increased oxidative stress and inflammatory reactions supporting its clinical use⁽²⁷⁾.

In conclusion, we report here that the beneficial effects of curcumin on the metabolic abnormalities postulated to be important in the development of diabetic retinopathy suggest that curcumin could have potential benefits in inhibiting the development of retinopathy in diabetes.

These findings may provide novel targets for future early diabetic retinopathy intervention and management.

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