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Research Article

Effects of Quercetin Nanoparticles, An Antioxidant Bioflavonoid on the Retina of STZ-induced Diabetic Rats

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Abstract

The retina is susceptible to oxidative stress due to an increase in free radicals or deficiency in natural antioxidants, induced in diabetic retinopathy. So, this study is aimed to examine the beneficial effect of Quercetin Nanoparticles (QUNPs) to protect the retina against diabetic rats and its oxidative stress. Diabetic rats were treated with QUNPs (5 and 10 mg/kg BW) for 8 weeks, then the fasting blood sugar (FBS), lipid markers (TC, TG, LDL-c, and HDL-c), were determined at the end of the experiment. Superoxide dismutase; (SOD), Catalase; (CAT) activities; in addition to glutathione; (GSH) and malondialdehyde (MDA) were determined in the blood and retina. Histological changes in the retina were evaluated by a light microscopic (LM) study. Our results revealed that QUNPs treatment for diabetic rats caused a significantly decreased in FBS, TC, TG, LDL-c levels. Also, a highly significant increase in HDL-c level was noticed. QUNPs (10 mg/kg BW) +STZ (60 mg/kg BW) group results are the same more or less normal values in MDA, CAT, SOD, and GSH compared to a positive control (PC) and positive modulation of anti-oxidant enzyme activity was noticed. Moreover, QUNPs treatment which was an effective antioxidant did not have negative effects on the health of rats. Light microscopic study showed thickness increased in basement membrane in the diabetic retina, while relatively thin LM was observed in QUNPs 5 and 10 mg/kg BW- retina treated group. It can be concluded that QUNPs can be effective for the protection of diabetes-induced neurovascular complications such as diabetic retinopathy. Therefore, our study suggests that QUNPs can play an important role as natural nano-antioxidant of oxidative stress in diabetic retinopathy.

Keywords: Diabetic Retinopathy; Oxidative Stress; Quercetin Nanoparticles; Streptozotocin (STZ)

Introduction

Diabetes Mellitus (DM), defined as chronic hyperglycemia, is a metabolic disease that affects protein, fat, and carbohydrate metabolism because of the lack of insulin secretion in the pancreas or disruption of tissue response to insulin. Today it becomes an important health problem because it is approximately over a quarter (25.9%) of adults also, it reduces the quality of life. Long-term complications arise to from damage to blood vessels, nerves, and eyes [1]. Hyperglycemia not only raises reactive oxygen metabolites; but also weakens anti-oxidative mechanisms through nonenzymatic glycosylation of anti-oxidant enzymes [2]. Microvascular pericytes and endothelial cells in the retinal pathogenesis of diabetic retinopathy are lost selectively before another histopathology is detectable, or loss of vision is evident [3]. In STZ-induced diabetic mice, proliferative diabetic retinopathy occurs 5mo after the onset of diabetes. Furthermore, after the development of PDR, the mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR1 and VEGFR2), as well as matrix metalloproteinase (MMP2 and MMP9), increases [4].

ROS causes damage to retinal cells, which are essential for converting visual images into electrical impulses for the brain to interpret. In the case of preventing retinal degradation,

antioxidants can be used to protect against the damage caused by reactive oxidative species, thereby improving outcomes in terms of vision integrity and function [5].

Plant phenolic compounds such as flavonoids are strong antioxidant properties found in different dietary sources, such as apple, green beans, broccoli, onion, and garlic [6]. The chemical name of quercetin is 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one, the flavonol bioflavonoid aglycone subclass found in a variety of foods including onions, shallots, apples, berries, grapes, and tea leaves, as well as some medicinal botanicals including Ginkgo biloba [5]. Quercetin, a powerful antioxidant, directly scavenges free radicals [7], binds transition metal ions, increases the level of glutathione [6], inhibits lipid peroxidation, and xanthine oxidase [6], and alters anti-oxidant defense pathway *in vivo* and *in vitro* [9]. This enables it to reduce oxidative stress in the body, thereby protecting it from many neurodegenerative diseases such as atherosclerosis, chronic inflammation, and retinal degeneration. In diabetic rats, quercetin conjugated superparamagnetic iron oxide nanoparticles (QCSPIONs) can reduce miR-27a expression while increasing Nrf2 expression and responsive antioxidant genes, resulting in improved memory dysfunction [10]. Q/Zn therapy combined with MSC therapy had a synergistic effect against genotoxicity and oxidative stress, and it can be considered a potential ameliorative therapy for diabetes with pulmonary dysfunction that may benefit against COVID-19 [11]. However, its solubility in water is poor to nonexistent due to its hydrophobic nature. Many studies have been conducted using quercetin nanoparticles in various areas of the body and via various dosage routes, but there are no known studies aimed at delivery into the eye. Therefore, the current study was designed to prepare Quercetin nanoparticles and study its effect on oxidative stress in the retina in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Chemicals

All of the chemicals were of the analytical grade. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits for measuring of serum levels of TG, TC, LDL-C, HDL-C, GSH, MDA and the activities of catalase and SOD were purchased from Bio-Diagnostic Company.

Preparation of quercetin nanoparticles (QUNPs)

QUNPs were prepared based on the technique of Nano participation [12]. Commercial QUE was dissolved by adding ethanol to water (1:35) at a predetermined concentration of 5mg/ml. The prepared solution was poured into the syringe, which was then attached to a syringe pump. The drug solution was quickly injected at a fixed flow rate (10 ml/min) while magnetic stirring was taking place into a definite volume of anti-solvent (deionized water) (1000 rpm). The QUNPs were vacuum dried after being filtered.

Diet

As a base diet, a commercial diet was used. The commercial diet consists primarily of not more than 64% carbohydrates, not less than 21% protein, not less than 6% fat, not less than 3% fiber, and not less than 6% vitamins and minerals mix, methionine, and choline chloride.

Experimental animals

Wistar albino rats (male and female) weighing 200 - 250 g were purchased and housed in the Research Institute of Ophthalmology's animal house facility (Giza, Egypt). The rats were fed a standard rat pellet diet (commercial diet) as well as tap water. The local ethical committee approved the experimental protocol, which followed ARVO (The Association for Research in Vision and Ophthalmology) animal-use guidelines for ophthalmic and vision research.

The doses of quercetin nanoparticles were used as previously described by Soheir and Suhailah, 2014 [12], and on the basis of our present study 5, 10 mg/kg body weight dose is reported in this scientific paper. In the treatment group (n = 12 per group), STZ-diabetic rats were dosed by stomach tube once per day for 8 weeks with quercetin nanoparticles (QUNPs) 5 and 10 mg/kg BW/day. A vehicle-treated group (n = 12 per group) of normal rats was treated with distilled water only over the same treatment period.

Induction of diabetes mellitus

To induce diabetes in rats, a single dose of streptozotocin (STZ 60 mg/kg BW) was injected intraperitoneally. The STZ was dissolved in 0.1 mL of sodium citrate buffer (pH 4.5). Blood glucose was measured on the third day after the STZ injection to ensure that diabetes was induced.

Six groups of twelve rats each were formed: G1: Normal Control Group (NC), G2: Diabetic Group (PC), injected with 60 g/kg BW STZ. G3 was a control group that received QUNPs (5 mg/kg BW/day), G4 was a diabetic group that received QUNPs (5 mg/kg BW/day), G5 was a control group that received QUNPs (10 mg/kg BW/day), and G6 was a diabetic group that received that QUNPs (10 mg/kg BW/day). All of the rats were fed a commercial diet. Blood glucose levels were measured on a regular basis. The eyes were removed after 8 weeks, and the retina was carefully excised and washed in 0.15 M isotonic sodium chloride solution.

Ophthalmological examinations

Both eyes of all groups were dilated using 2% tropicamide solution eye drops. The rat's eyes were examined for detecting symptoms of eye diseases. The progression of diabetic retinopathy in all groups was under observation.

Blood biochemical analyses

The rats fasted overnight at the end of the experiment. Blood was drawn from the eye canthus of all animals and placed in two separate tubes. The first tube contained EDTA, and the second tube was used to separate serum by centrifuging at 3500 r.p.m. for 10 minutes.

TG (Triglycerides), TC (Total Cholesterol), LDL-c (Low-density Lipoprotein), and HDL-c (High-density Lipoprotein) levels were measured using [13,14].

Fasting blood sugar (FBS) determination

The FBS in rats was measured at regular intervals with a glucometer and strips (AR-Med LTD, Runny Mede Malthouse, Egham TW209BD, UK, Prestige IQ® blood monitoring system). The strip was used to pot the drop blood, and the appropriate blood sugar concentration is displayed on the glucometer screen after 10–50 seconds.

Assessment of lipid peroxidation in the blood serum and retina

Retinal (10% w/v in 0.1 M Tris-HCl, pH 7.4) and blood serum were used for the estimation of moalonaldehyde. Ohkawa., *et al*. [15] method was used to measure the malonaldehyde content. 20% glacial acetic acid (1.5 ml) at pH = 3.5, 0.8 ml of sample (1 mg), 8.1% SDS (0.2 ml), and 0.8% TBA aqueous solution (1.5 ml) were mixed, then added distilled water to complete to 4 ml and heated the mixture at 95°C for 60min using condenser. Distilled water (1 ml) and mixture of pyridine and n-butanol (5 ml) (1: 15) were added after cooling the mixture. Then, the solution was shaken vigorously and centrifuged at (2000 xg) for 10 min. After that, the organic layer extracted absorbance was measured at 532 nm. We used the standard curve (1, 10, 3, 30-tetramethoxy propane) to calculate the thiobarbituric reacting substances amount.

Retinal antioxidant enzymes assay

Retinas from both eyes of each rat were pooled as one sample of all rats, and then were homogenized at pH = 7.4 in ice-cold 0.1 M Tris-HCl and centrifuged for 30 minutes at 12,000 rpm and 4ºC. The supernatant was collected and used for following experiments as described below. The SOD activity; (Cat. No. ab65354) was measured as described at [16]. Catalase; (Cat. No. ab118184) was assayed by the method of Luck [17], and also GSH; (Cat. No. ab65322) was assayed by the method of Sedlak and Lindsay [18]. An all-glass Ten-Broeck homogenizer was used to homogenize the retina in 0.02 M EDTA (5 mg/ml) in an ice bath. These homogenizing medium to tissue ratios were chosen to obtain 0.1 to 0.8 absorbance when using 5.0 ml for the GSH estimation. 5 ml of homogenates solution was mixed with (1.0 ml) trichloroacetic acid 50% and (4.0 ml) distilled water. For 10-15 min and intermittently the tubes were shaken, then centrifuged at 3000 xg for 15 min. 4.0 ml Tris buffer at pH = 8.9 and 0.1 ml DTNB were mixed with 2 ml of supernatant and the sample was shaken. 412 Am within 5 min was used to read the absorbance against a reagent blank.

Histological studies

The rats were sacrificed at the end of the experiment, and retinal specimens were taken. The retina specimens were labeled and fixed for six hours in 2.5 percent buffered glutaraldehyde before being washed in phosphate buffer. The retina was dissected and then fixed in phosphate buffer pH 7.3 with 1.3 percent osmium tetroxide. After processing, the samples were embedded in Araldite cy212 semi-thin sections of one-micron thickness were obtained and stained with toluidine blue (TB) stain before being exposed to light. Glauert's [19] method was used to prepare the retina for histological examination.

Statistical analysis

The data were presented in the form of the mean standard deviation (SD). The analysis of variance is used to compare multiple

groups (ANOVA), mean and standard deviation were descriptive measures of data. Using a commercially available software package, the least significant difference (LSD) multiple comparison test was then performed (SPSS- 10 for windows, SPSS Inc, Chicago, IL, USA). At P values less than 0.05, the results were considered significant (2-sided).

Results and Discussion

Table 1 shows the result of BG (Blood glucose), TG, TC, LDL-c, and HDL-c. BG, TG, and TC are decreased significantly ($P \leq 0.05$) in groups administrated STZ (60 mg /kg BW) + QUNPs (5 and 10 mg/kg BW), it was found (126.62 ± 5.87, 77.02 ± 1.94 mg/dl), respectively, compared to PC, it found (193.18 ± 4.41 mg/dl), after 8 weeks of treatments. HDL-c significantly ($P \leq 0.05$) decreased in PC (33.31 ± 3. 60 mg/dl) and increased of treatments of STZ (60 mg/kg BW) + QUNPs (5 and 10 mg/kg BW) after 8 weeks, it found (45.18 ± 2.16, 42.36 ± 1.99 mg/dl), respectively, while LDL-c levels significantly decreased (130.67 ± 3.85 and 78.36 ± 7.45 mg/dl), respectively than PC (310.94 ± 4.53 mg/dl). STZ is a diabetogenic agent that contains a nitrous substance that releases nitric oxide during metabolism, rendering beta-cells toxic, that STZ-induced insulin deficiency [20-22].

Parameters Groups	Glucose mg/dl	TC mg/dl	TG mg/dl	HDL-c mg/dl	LDL-c mg/dl
G_1 (NC)	79.72 ± 1.6^{b}	119.45 ± 3.47^{b}	57.72 ± 1.90^{bc}	41.85 ± 3.55^{ac}	77.70 ± 2.59^{b}
G_2 (PC)	193.18 ± 4.41^{a}	339.28 ± 6.66^{a}	180.99 ± 7.99^{a}	33.31 ± 3.60^{bc}	310.94 ± 4.53^{a}
QUNPs (5 mg/kg bw)	79.37 ± 5.19^{b}	117.98 ± 3.60^{b}	61.87 ± 1.65^{b}	41.59 ± 1.42^{ac}	75.19 ± 1.04^{b}
QUNPs (5 mg/kg bw+ STZ)	126.62 ± 5.78^{c}	176.43 ± 3.50^{bc}	98.89 ± 1.34^{bc}	45.18 ± 2.16^{a}	130.67 ± 3.85^{bc}
QUNPs (10 mg/kg bw)	88.32 ± 5.32^{b}	116.16 ± 2.30^{b}	57.84 ± 1.13^{bc}	43.87 ± 2.63^{ac}	74.41 ± 2.77^{b}
QUNPs (10 mg/kg bw + STZ)	77.02 ± 1.94^{b}	121.02 ± 4.30^{b}	62.91 ± 1.05^{bc}	42.36 ± 1.99^{ac}	78.36 ± 7.45^{b}

Table 1: QUNPs effect on glucose and lipid profiles (TC, TG, HDL-c, and LDL-c) in control and experimental blood serum.

Each value represents the mean S.D. of twelve rats in each group. Values with different superscript letters (a, b, c) differ significantly from one another ($p \leq 0.05$). NC = Negative Control, PC = Positive Control, QUNPs = Quercetin Nanoparticles, STZ = Streptozotocin.

The main problem associated with (DM) are the elevation of blood glucose levels due to impaired metabolism, and the generation of harmful free radicals as a result of the use of lipids for energy production. In cases of diabetes, the auto-oxidation of glucose increases, and during the conversion of oxidized glucose into glucose acid, free radicals are generated [23,24]. At the end of 5 weeks, in diabetic rats, the levels of blood glucose were significantly higher than in normal rats. However, when compared to untreated diabetic rats, the blood glucose level was significantly lower in quercetin-treated rats (50 mg/kg body weight) [25]. When STZ diabetic rats were given QUE, it was found to have a hypoglycemic effect. Our findings revealed a decrease in QUNPs + STZ; this hypoglycemic effect of QUENPs could be attributed to its antioxidant properties [22,26]. In addition, in STZ-induced diabetic rats, QUE supplementation is beneficial for lowering blood glucose levels, promoting pancreatic islet regeneration, and increasing insulin release, all of which have anti-diabetic properties [27]. Diabetic animals had higher blood glucose levels four weeks after STZ injection (465.37 14.27 and 107.82 4.11 mg/dL for diabetic rats and control, respectively) (P 0.05). At the end of 8 weeks, diabetic rats had significantly higher plasma glucose levels than control rats. Quercetin (10 mg/kg per day) administration had no effect on plasma glucose levels in either control or diabetic rats from 4 to 8 weeks [28].

Table 2 shows the oxidative stress biomarkers of NC, PC, QUNPs, and QUNPs + STZ groups. MDA level significantly ($P \leq 0.05$) increased, while SOD, GSH, and CAT significantly ($p \leq 0.05$) decreased in the PC group; than NC group. On the other hand, QUNPs (5 and 10 mg/kg BW) + STZ decreased the level of MDA and increased the activity of CAT, SOD, and GSH than PC after 8 weeks. QUNPs (10 mg/kg BW) + STZ group showed an increase in the level of GSH and CAT activities, comparable to the NC group (P 0.05). However, there was no statistically significant difference in MDA, CAT, or SOD activities between NC and QUNPs (5 and 10 mg/kg BW) alone. These results could be attributed to QUE's ability to

transfer free radicals, electrons, chelate metal catalysts [29], and activate antioxidant enzymes [30]. Furthermore, the decrease in GSH concentrations in the kidney, brain, and liver is most likely due to GSH consumption in peroxide removal or NADPH depletion [31]. When compared to the NC group, GSH and SOD levels in the PC group decreased. After treating diabetic rats with QUNPs (10 and 20 mg/kg BW/day), the levels in the NC group returned to near-normal levels ($p \leq 0.05$). The PC group, on the other hand, had higher levels of MDA than the NC group. The QUNPs (20 mg/kg BW/day) + STZ group reduced serum MDA levels in the same way that the NC group did ($p \leq 0.05$) [32]. The serum antioxidant enzyme activities revealed that the diabetic group treatment significantly reduced SOD activity when compared to the control group. QUE prevents STZ-induced oxidative stress and protects b-cells by scavenging free radicals and inhibiting lipid peroxidation, resulting in increased insulin secretion and lower plasma glucose levels [29,33]. Treatment with QUE increased the levels of these antioxidants, with the QUE group showing the greatest increase among the individual QUE group treatment of diabetic rats reduced SOD levels to levels lower than controls. In the diabetic rat liver, QUE inhibits antioxidant enzyme activation by scavenging ROS [21]. The different QUE doses gradually restored the elevated levels of MDA in serum in diabetics and completely recovered the increased MDA levels when compared to the diabetic group [29]. QUE, which is a natural flavonoid that has found common use owing to its strong antioxidant effect, restored MDA, insulin and glucose levels, and SOD, GSH,activities in STZ- induced diabetic rats, and did not cause any adverse effect in rats administered with quercetin alone [34].

Parameters Groups	MDA nmol/ml	CAT U/ml	SOD U/ml	GSH mg/dl
G_1(NC)	1.18 ± 0.11^{b}	12.93 ± 1.87^{a}	107.39 ± 1.39^{ac}	57.13 ± 1.59^{a}
G_2 (PC)	11.05 ± 0.16^{a}	3.36 ± 0.32^{b}	42.34 ± 3.46^{b}	22.53 ± 2.60^{b}
QUNPs (5 mg/kg bw)	1.16 ± 0.21^{b}	12.97 ± 2.04^{a}	107.14 ± 3.99^{ac}	61.48 ± 3.97^{a}
QUNPs (5 mg/kg bw + STZ)	1.52 ± 0.13^{b}	11.72 ± 1.06^{a}	79.71 ± 2.84^{c}	52.22 ± 2.26^{a}
QUNPs (10 mg/kg bw)	1.17 ± 0.23^{b}	12.88 ± 0.78^{a}	108.74 ± 2.71^{ac}	58.35 ± 1.13^{a}
QUNPs (10 mg /kg bw+ STZ)	1.33 ± 0.14^{b}	12.84 ± 0.52^{a}	105.80 ± 2.47^{ac}	56.44 ± 2.81^{a}

Table 2: QUNPs effect on MAD, CAT, SOD, and GSH levels in control and experimental blood serum.

Each value represents the mean S.D. of twelve rats in each group. Values with different superscript letters (a, b, c) differ significantly from one another ($p \leq 0.05$). NC = Negative Control, PC = Positive Control, QUNPs = Quercetin Nanoparticles, STZ = Streptozotocin.

Table 3 shows the MDA, CAT, SOD, and GSH levels of experimental rats. Following STZ injection, retina MDA levels were significantly ($p \leq 0.05$) higher in the PC group and significantly ($p \leq 0.05$) lower in the NC group. In STZ-treated rats, QUNPs (5 and 10 mg/kg BW) administration significantly ($p \leq 0.05$) decreased retina MDA levels while significantly ($p \leq 0.05$) increasing retina GSH, CAT, and SOD activities. Table 3 also revealed that the GSH, MDA, SOD, and CAT activities in the QUNPs (10 mg/kg BW) + STZ (60 mg/kg BW) group were comparable to the NC group. Quercetin's free radical scavenging properties, inhibition of lipid peroxidation, and subsequent decrease in the production of vasoactive mediators may all play a role in improving diabetic renal dysfunction. Natural antioxidant quercetin inhibits lipid peroxidation by directly scavenging hydroxyl, peroxy, and superoxide radicals [35]. MDA levels, a lipid peroxidation marker, were 2.65 0.04-fold higher in diabetic rats compared to control rats and significantly reduced by quercetin. GSH levels were significantly reduced (nearly 1.62 0.09-fold) in diabetic rats, but increased after quercetin treatment. Streptozotocin-injected rats had lower SOD and catalase levels than control rats, which were significantly reversed by quercetin treatment [28]. CAT, GPx and fasting blood glucose concentration measurements, it was ascertained that, while MDA, GPx, and the diabetic group had significantly higher glucose levels, the same parameters had drawn closer to the values of the control animals in the diabetic group administered with quercetin [36]. The increased serum MDA levels, the significant decrease detected in the activities of SOD and GSH, which are antioxidants that play an important role in the prevention of oxidative stress, in the diabetic rats, was also consistent with the results of previous research. Additionally, the MDA, SOD and GSH activities having been found to be similar in

the control group and group QUE as well as in group diabetes + QUE and the groups other than group diabetes (D), whereas having been observed to significantly differ between Groups DQ and D was attributed to the strong antioxidant effect of quercetin [34,37] and Adewole., *et al*. [38] told the levels of GSH, GPx, SOD, and CAT activities were significantly reduced as a result of oxidative stress, while MDA levels increased, and it was reported that quercetin administration normali ed all values and preserved the integrity of pancreatic -cells. In further research on the antioxidant activity of quercetin, it was determined that the administration of this substance increased SOD, CAT and GPx activities and decreased MDA levels.

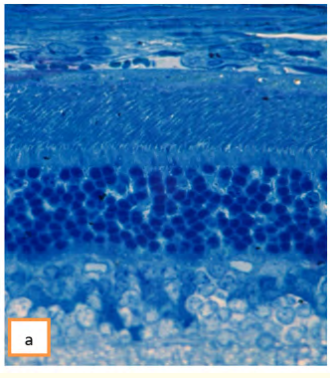
Parameters Groups	MDA nmol/mg	CAT U/mg	SOD U/mg	GSH mg/g
G_1(NC)	1.35 ± 0.09^{b}	5.84 ± 0.37^{a}	9.15 ± 1.00^{a}	490.63 ± 6.79^{a}
G_2 (PC)	16.63 ± 1.80^{a}	1.44 ± 0.29^{b}	4.46 ± 0.80^{b}	277.39 ± 11.82^{b}
QUNPs (5 mg/kg bw)	3.42 ± 0.02^{b}	5.07 ± 0.04^{a}	9.21 ± 1.00^{a}	508.33 ± 11.75^{a}
QUNPs (5 mg/kg bw+ STZ)	2.24 ± 0.07^{b}	5.41 ± 0.16^{a}	7.50 ± 0.70^{ab}	404.22 ± 10.21^{a}
QUNPs (10 mg/kg bw)	1.25 ± 0.10^{b}	5.13 ± 0.07^{a}	9.25 ± 0.57^{a}	506.56 ± 11.93^{a}
QUNPs (10 mg/kg bw + STZ)	1.78 ± 0.35^{b}	5.73 ± 0.49^{a}	8.99 ± 0.31^{a}	484.76 ± 10.38^{a}

Table 3: QUNPs effect on lipid peroxidation (MDA, CAT, SOD and GSH) in retinal in control and experimental groups.

Each value represents the mean S.D. of twelve rats in each group. Values with different superscript letters (a, b, c) differ significantly from one another ($p \leq 0.05$). NC = Negative Control, PC = Positive Control, QUNPs = Quercetin Nanoparticles, STZ = Streptozotocin.

Histopathological investigations of retinal layers in different experimental groups:

Light micrograph (L.M) of G1: normal control (NC) retina. Figure 1 (a) shows its ten layers as follows: Light microscopic of control group showing the pigmented epithelium cell layer blue nine layers. There are There are (1) The photoreceptor layer, (2) The outer limiting membrane, (3) the Outer nuclear layer, (4) the outer plexiform layer, (5) the inner nuclear layer, (6) the inner plexiform layer, and (7) the ganglion cell layer, (8) Layer of nerve fibers, (9th) The inner limiting membrane Figure 1 (b): Light micrograph (L.M) of G2: Positive Control (PC) retina group injected with a single dose of STZ (60 g/kg BW), showing: All the layers are severely changed (1): Vacuolated ph layer. (2): decreased cell density in the O.N. layer (3): the TN layer showed dissolute chromatin.



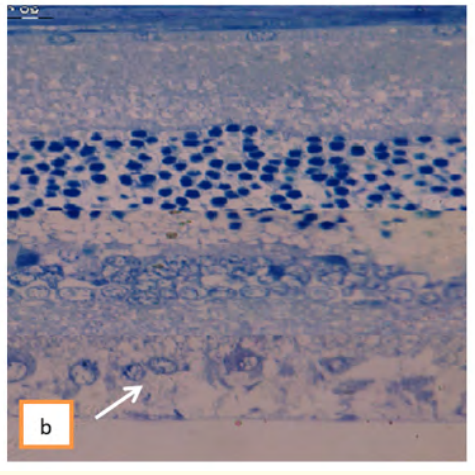


Figure 1: (a): Light micrograph (L.M) of G1: normal control (NC) retina and (b): Light micrograph (L.M) of G2: The PC retina group was injected with a single dose of STZ (60 g/kg BW).

Light micrograph (L.M) of G3; control group that received QUNPs (5 mg/kg BW/day) retina. Figure 2 (a) showing: There is thickened blood vessel in the inner plexiform layer. Figure 2 (b): G4; the group that was given QUNPs (5 mg/kg BW/day) + one dose of STZ (60 g/kg BW), showing: the gial cells in the ganglion cell layers are less proliferated.

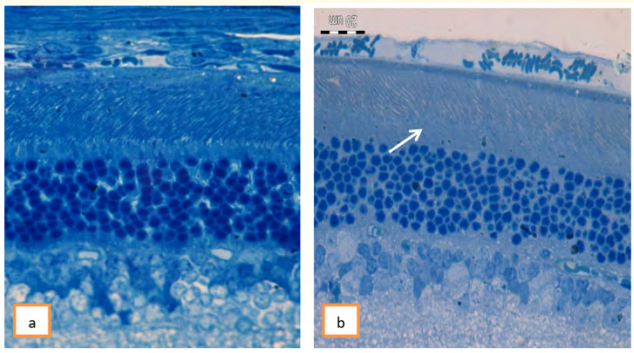


Figure 2: (a): Light micrograph (L.M) of G3; The control group received QUNPs (5 mg/kg BW/day) in the retina. (b): G4; The treated group received QUNPs (5 mg/kg BW/day) + a single dose of STZ (60 g/kg BW).

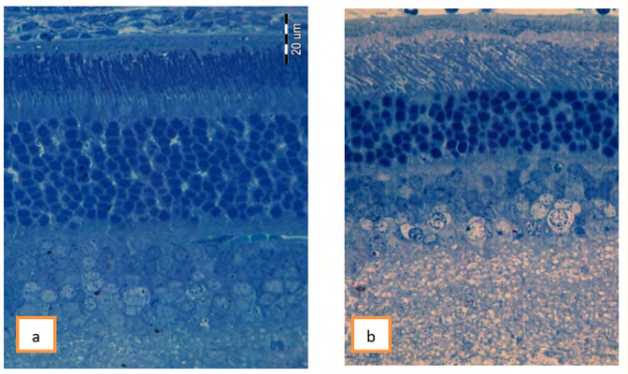


Figure 3: (a): G5; control group that received QUNPs (10 mg/kg bw/day) showing normal appearance of all retinal layers. (b): G6; The treated group received QUNPs (10 mg/kg BW/day) + a single dose of STZ (60 g/kg BW) showing reduced vascular changes of PH layer. The most probably made the situation change dramatically to the better.

An improvement of the cytoplasm of inner nuclear layer (IN).

QUNPs-treated retina demonstrated a return to normalcy of the retina's antioxidant defence system, resulting in fewer retinal changes (Figure 2b and 3b). As previously stated, QUNPs, as a bioflavonoid, has strong anti-oxidant activity and effectively modulates enzymatic activity. Similarly, positive modulation of GSH and antioxidant enzymes in the blood and retina was observed in our study.

Conclusion

Based on the findings of this study, it is possible to conclude that diabetes has a significant degeneration effect on all retinal layers. QUNPs is a natural flavonoid that can reduce diabetic retinal damage and, to a large extent, prevent most of the negative effects of diabetes on retinal layers. It can also be used as a dietary supplement to treat/prevent diabetic complications and oxidative stress, most likely through its anti-oxidative action. As a result, QUNPs may prove to be an effective flavonoid/drug and a promising adjuvant agent in the treatment of diabetic retinopathy. The authors recommended conducting more studies on the human benefits of QUNPs as supplementary food or drugs for the protection of diabetic retinopathy.

Conflict of Interests

There has been no declaration of a conflict of interest by the authors.

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