Original Article

Effect of thiamine pyrophosphate on retinopathy induced by hyperglycemia in rats: A biochemical and pathological evaluation

Emine Cinici, Ibrahim Ahiskali[1], Nihal Cetin[2], Bahadir Suleyman[2], Osman Kukula[3], Durdu Altuner[2], Abdulkadir Coban[4], Hilal Balta[5], Mehmet Kuzucu[6], Halis Suleyman[2]

Purpose: Information is lacking on the protective effects of thiamine pyrophosphate (TPP) against hyperglycemia-induced retinopathy in rats. This study investigated the biochemical and histopathological aspects of the effect of TPP on hyperglycemia-induced retinopathy induced by alloxan in rats. **Materials and Methods:** The rats were separated into a diabetic TPP-administered group (DTPG), a diabetes control group (DCG) and a healthy group (HG). While the DTPG was given TPP, the DCG and HG were administered distilled water as a solvent at the same concentrations. This procedure was repeated daily for 3 months. At the end of this period, all of the rats were euthanized under thiopental sodium anesthesia, and biochemical and histopathological analyses of the ocular retinal tissues were performed. The results of the DTPG were compared with those of the DCG and HG. **Results:** TPP prevented hyperglycemia by increasing the amount of malondialdehyde and decreasing endogen antioxidants, including total glutathione, glutathione reductase, glutathione S-transferase and superoxide dismutase. In addition, the amounts of the DNA oxidation product 8-hydroxyguanine were significantly lower in the retinas of the DTPG compared to the DCG. In the retinas of the DCG, there was a marked increase in vascular structures and congestion, in addition to edema. In contrast, little vascularization and edema were observed in the DTPG, and there was no congestion. The results suggest that TPP significantly reduced the degree of hyperglycemia-induced retinopathy. **Conclusions:** The results of this study indicate that TPP may be useful for prophylaxis against diabetic retinopathy.

Access this article online

Website: www.ijo.in

DOI: 10.4103/0301-4738.187666

Quick Response Code:

Key words: Alloxan, antioxidants, diabetic retinopathy, oxidants, rat, thiamine

Hyperglycemia in diabetes mellitus is due to a lack of insulin secretion by pancreatic cells or decreased insulin sensitivity of target cells (insulin resistance).[1] Diabetes mellitus often causes microvascular complications in the kidneys, heart, brain and eyes.[2] Diabetic retinopathy is a specific complication of diabetes mellitus and is the most important microvascular complication.[3] Diabetic retinopathy is caused by microangiopathy of the arterioles, capillaries and venules of the retinal layer of the eye, and is responsible for 12% of all cases of blindness in the United States.[4] Retinopathy develops in more than 90% of diabetic patients and confers a 25-fold increased risk of blindness when compared to nondiabetic patients.[5] Although the mechanisms underlying the development of retinopathy in diabetes-related hyperglycemia are known,[6,7] the mechanisms underlying hyperglycemia-induced microvascular events in the retina are not clear.[8] One study implicated the overproduction of free oxygen radicals in the development of diabetic retinopathy.[9] Another reported that hyperglycemia caused microvascular stress.[10] During prolonged hyperglycemia, glucose was reported to bind to the amino groups of proteins and to cause auto-oxidation of proteins and free-oxygen-radical production.[10] The resulting increase in oxidants and decrease in antioxidant defense systems caused oxidation of lipids and nucleic acids, as well as oxidative stress in cells. Lipid peroxidation involves the oxidation of unsaturated fatty acids in the cell membrane, and ends with transformation into cytotoxic aldehydes, such as malondialdehyde (MDA).[11] Lipid oxidation causes cellular DNA damage. A previous study showed that 8-hydroxyguanine (8-OH/Gua) was an indicator of oxidative damage in DNA, and that it caused microangiopathic complications.[12] In addition, animal studies demonstrated that an accumulation of pyruvate and lactic acid may play a role in the pathogenesis of retinopathy induced by experimental hyperglycemia.[13]

The focus of the present study was on the protective effect of the active form of thiamine, thiamine pyrophosphate (TPP), against hyperglycemia-induced retinopathy.[14] TPP was reported to have antioxidant characteristics,[15] to play a role in

Departments of Ophthalmology and [5]Pathology, Erzurum Region Education and Research Hospital, [1]Department of Ophthalmology, Palandoken State Hospital, Erzurum, Departments of [2]Pharmacology and [4]Biochemistry, Faculty of Medicine, Erzincan University, [6]Department of Biochemistry, Faculty of Arts and Sciences, Erzincan University, Erzincan, [3]Department of Pharmacology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey

Correspondence to: Prof. Halis Suleyman, Department of Pharmacology, Faculty of Medicine, Erzincan University, Erzincan 24030, Turkey. E-mail: halis.suleyman@gmail.com

Manuscript received: 29.02.16; Revision accepted: 13.05.16

Cite this article as: Cinici E, Ahiskali I, Cetin N, Suleyman B, Kukula O, Altuner D, *et al*. Effect of thiamine pyrophosphate on retinopathy induced by hyperglycemia in rats: A biochemical and pathological evaluation. Indian J Ophthalmol 2016;64:434-9.

the intermediate metabolism of carbohydrates, and to facilitate oxidative decarboxylation of alpha-keto acids (pyruvate and alpha-ketoglutarate).[16] It has been stated that pyruvate and lactic acid accumulate in the blood and tissues in TPP deficiency.[17] Thus, based on the current literature, TPP may be useful in the treatment of diabetic retinopathy. A literature search found no information on the effects of TPP on hyperglycemia-induced retinopathy caused by alloxan in rats. Thus, the purpose of this study was to investigate the biochemical and histopathological aspects of the effects of TPP on alloxan-induced hyperglycemia and the resulting retinopathy in rats.

Materials and Methods

Animals

The experimental animals were obtained from the Recep Tayyip Erdoğan University Experimental Practices and Research Centre. Thirty-six male albino Wistar rats with similar weights (295–300 g) were randomly selected for use in the experiment. The rats were housed in a laboratory at room temperature (22°C) for 1 week for acclimatization to the environment. The animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals, and were approved by Recep Tayyip Erdoğan University, Animal Experiments Local Ethics Committee (Number 2014/63, date October 30, 2014).

Chemicals

Thiopental sodium was obtained from IE Ulagay (Turkey), alloxan was obtained from Sigma (USA), and TPP was obtained from Biopharma (Russia). Phosphate buffer, hexadecyl trimethyl ammonium bromide, potassium chloride, Tris, ethylenediaminetetraacetic acid (EDTA), 2-mercaptoethanol, Triton XI00, phenol, ethanol and acetonitrile were obtained from Merck (Germany), and the deoxyguanine (dG) and 8-OHdG standards were obtained from Sigma (USA).

Induction of hyperglycemia

Hyperglycemia was induced by alloxan, which was dissolved in distilled water and administered to the rats intraperitoneally (i.p.) in 120 mg/kg doses for 3 consecutive days. Fasting glucose levels were measured in blood samples obtained from the tail 3 days after alloxan administration. A commercial device was used to measure the blood glucose levels. Animals with blood glucose levels of 250 mg/dL and higher were included in the trial, as these levels are accepted as diabetes.[18]

Experimental groups

The diabetic rats were separated into a TPP-administered group (DTPG) and a control group (DCG). The healthy group (HG) was not administered any drugs except for distilled water as a solvent.

Experimental procedure

In the DTPG (n = 12), the hyperglycemic rats were injected with TPP (20 mg/kg i.p.). In the DCG (n = 12) and HG (n = 12), distilled water was administered as a solvent at the same concentrations and via the same route. This procedure was repeated daily for 3 months. At the end of this period, all of the rats were euthanized under high-dose thiopental sodium anesthesia, and the retinal layer of the eye was removed under sterile conditions. Biochemical parameters, such as MDA, total glutathione (tGSH), glutathione reductase (GSHRd), glutathione S-transferase (GST), superoxide dismutase (SOD) and 8-OHdG, were quantified in retinal samples from the three rat groups. Histopathological studies of the retinal layers were performed. The results of the DTPG were compared with those of the DCG and HG.

Biochemical experimental procedure

Preparation of the samples

A phosphate buffer with a pH of 6 and consisting of 0.5% hexadecyl trimethyl ammonium bromide was used to identify myeloperoxidase in the retinal tissue, and 1.15% potassium chloride solution was used to identify MDA. For the other measurements, a phosphate buffer with a pH of 7 was used. Two milliliters of medium were homogenized and stored in a freezer until use. Afterward, the samples were centrifuged at + 4°C, 10,000 rpm for 15 min. The supernatant was removed and used in the analysis.

Malondialdehyde analysis

The amount of MDA was calculated according to the method of Ohkawa *et al*.[19]

Total glutathione analysis

The amount of GSH in the total homogenate was measured according to the method of Sedlak and Lindsay, with some modifications.[20]

Glutathione reductase analysis

The GSHRd activity was determined spectrophotometrically by measuring the rate of nicotinamide adenine dinucleotide phosphate oxidation at 340 nm, according to the Carlberg and Mannervik method.[21]

Glutathione S-transferase activity

GST activity was determined according to the method of Habig and Jakoby.[22]

Superoxide dismutase analysis

SOD was measured according to the method of Sun *et al*.[23]

DNA oxidation analyses

A total of 50–100 mg of tissue was homogenized by a mechanical homogenizer in ice at 4°C with 1 ml of homogenization buffer (30 mM Tris, pH = 8, 10 mM EDTA, 10 mM 2-mercaptoethanol, 0.5% [v/v] Triton XI00). The mixture produced was centrifuged at 1000 g for 10 min. The supernatant was discarded, and the 1 ml pellet that was obtained was resuspended in an extraction buffer (0.1 M Tris pH = 8, 0.1 M NaCl, 20 mM EDTA). The suspension was vortexed for 30 s, homogenized and then centrifuged at 1000 g for 2 min. The pellet that was obtained was resuspended in the extraction buffer. The suspension was vortexed and mixed thoroughly. Next, 400 μL of phenol was added to the mixture, and it was vortexed strongly for 1 min. The mixture was left to sit at room temperature for 10 min for the phases to separate. The top phase was removed and placed in a clean tube, and 400 μL of chloroform-isopropanol was added to this portion (for a ratio of 24:1). The mixture was centrifuged at 10,000 g for 10 min, and the top phase was once again placed in a clean tube. Subsequently, 40 μL of 3M sodium acetate and 800 μL of ethanol (pH = 5) at ice temperature were added to the mixture obtained from the last centrifuge, then mixed slowly by shaking. The mixture was then centrifuged at 10,000 g for

15 min. The upper portion was removed completely, and 1 ml of 70% ethanol was added to the lower portion. Finally, 0.5 ml of 60% formic acid was added to the final 1 ml of the mixture. The tubes were sealed at 150°C and left to cool for 60 min at room temperature, for the formic acid in the tubes to disappear. The mixtures (approximately 1 ml volumes) were stored at −20°C until the study date.

The levels of 8-OHdG and dG were measured in predefined systems at various wavelengths by high-performance liquid chromatography (HPLC) with HPLC-ultraviolet and HPLC- electrochemical detectors (ECD). Before the HPLC analysis, the hydrolysed DNA samples were redissolved with HPLC eluent. The final volume of 1 ml consisted of 20 ml of the final hydrolysate, HPLC-ECD (HP, HP 1049A ECD, Agilent 1100 modular systems HP 1049A ECD, Germany) reverse-phase C18 column (250 mm × 4.6 mm × 4.0 µm, Phenomenex, CA, USA) and a 0.05 M potassium phosphate (pH = 5.5) tampon containing acetonitrile (97:3, v/v), with 1 ml flow velocity per minute as the mobile phase. The concentration of dG was quantified by measuring the absorbance at 245 nm, and the level of 8-OHdG was observed using electrochemical readings (600 mV). The amounts of dG and 8-OHdG were determined according to Sigma brand dG and 8-OHdG standards, and 8-OHdG/10^5 was used as a marker of DNA damage.[24]

Histopathological procedures

The retinal tissue removed from the rats was fixed in a 10% formalin solution. Following routine processing of tissue-embedded paraffin sections, 5 µm slices were cut from the paraffin blocks. After deparaffinization and rehydration, the slices were stained with hematoxylin and eosin. All of the slices were coded and evaluated with a light microscope (Olympus CX 51, Tokyo, Japan) by the same pathologist, who had no knowledge of the treatment protocols.

Statistical analyses

The results of the experiments are expressed as the mean value ± standard deviation (x ± SEM). The degree of importance of the variance between the groups was determined using one-way ANOVA, followed by Fisher's *post hoc* least significant difference test. All statistical calculations were done with SPSS for Windows 22.0 (IBM, Armonk, New York, USA), and a $P < 0.05$ was accepted as statistically significant.

Results

Biochemical findings

As shown in Fig. 1, TPP prevented hyperglycemia-induced MDA increases in the rats' retinas. Hyperglycemia decreased the retinal levels of endogen antioxidants, such as tGSH, GSHRd, GST, and SOD, while TPP increased them [Figs. 2 and 3]. In addition, the amounts of the DNA oxidation product 8-OH/Gua were significantly lower in the DTPG than in the DCG [Fig. 4].

Histopathological findings

The histopathological analysis of the retinas in the HG revealed a ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, and ganglion cells [Fig. 5a]. As shown in Fig. 5b, there was a statistically significant increase in vascular structure (arrow), congestion (arrow), and edema (star) in the DCG, as well as a loss of ganglion cells. In contrast, as presented in Fig. 5c, there was very little increase in vascularization (arrow), minimal edema (arrow), and no congestion or loss of ganglion cells in the retinal tissue of the DTPG.

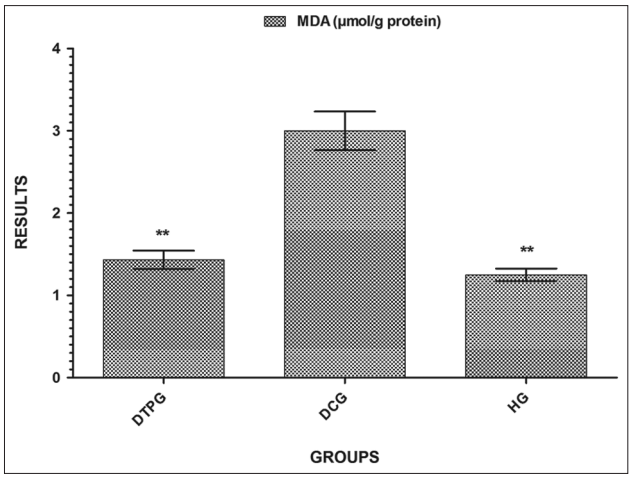

Figure 1: Effects of thiamine pyrophosphate on malondialdehyde levels in hyperglycemic rats (DTPG: Diabetic thiamine pyrophosphate-administered group, DCG: Diabetes control group, HG: Healthy group, $^{**}P < 0.0001$, $n = 12$)

Discussion

This study investigated the biochemical and histopathological effects of TPP on retinopathy in rats with alloxan-induced hyperglycemia. The results of the biochemical analyses showed that the amount of the oxidant MDA increased significantly, whereas the levels of endogen antioxidants, such as tGSH, GSHRd, GST, and SOD, decreased significantly in the retinal tissue of the rats with alloxan-induced hyperglycemia when compared to the HG. These findings showed that the oxidant/antioxidant balance shifted in favor of oxidants in the retinal tissue of the DCG. In healthy tissues, the oxidant/antioxidant balance was maintained due to the predominance of antioxidants.

Disruption of the oxidant/antioxidant balance is known to cause oxidative stress in tissues,[25] and the role of oxidative stress in the development of diabetic retinopathy is accepted.[9] Lipid peroxidation is known to play a role in the occurrence of diabetes-related late complications.[26] In the present study, the increased MDA levels in the DCG were the end-product of lipid peroxidation caused by oxidative stress.[11] A previous study reported that MDA was increased in the retinal tissues of diabetic rats.[27] The findings of that study are consistent with those of the present study.

In the current study, the level of tGSH decreased in the retinal tissues of the alloxan-induced hyperglycemic rats. As a nonenzymatic endogen antioxidant molecule, tGSH is present in cells at high concentrations and protects biological membranes from lipid peroxidation.[28] A previous study showed that nonenzymatic tGSH levels decreased in animals with induced diabetes.[27] In addition, the activity of certain enzymatic antioxidants, such as GST, GSHRd, and SOD, is reportedly decreased in diabetic rats.[29-31] It has been suggested that decreased GSHRd plays a role in the development of

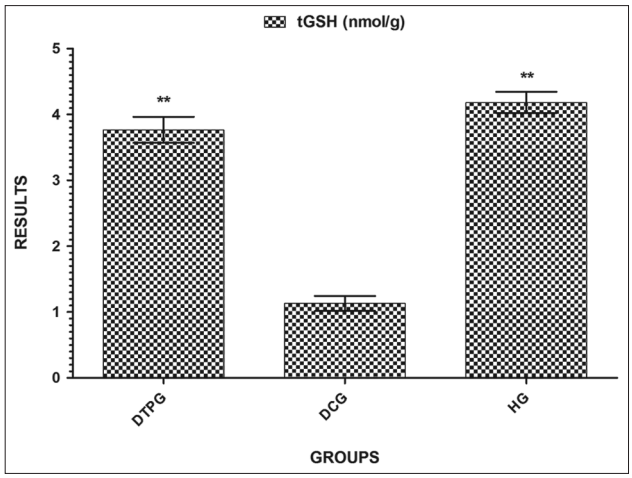

Figure 2: Effects of thiamine pyrophosphate on total glutathione levels in hyperglycemic rats (DTPG: Diabetic thiamine pyrophosphate-administered group, DCG: Diabetes control group, HG: Healthy group, $^{**}P < 0.0001$, $n = 12$)

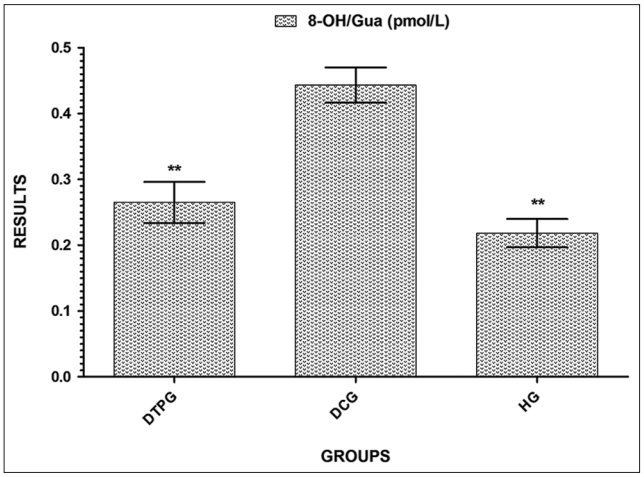

Figure 4: Effects of thiamine pyrophosphate on glutathione S-transferase, superoxide dismutase and glutathione reductase levels in hyperglycemic rats (DTPG: Diabetic thiamine pyrophosphate-administered group, DCG: Diabetes control group, HG: Healthy group, $^{*}P < 0.05$, $^{**}P < 0.0001$, $n = 12$)

retinopathy, one of the major complications of diabetes.[32] GST polymorphisms have been reported as risk factors for the development of diabetic retinopathy.[33] The importance of decreased SOD levels in the pathogenesis of diabetic retinopathy has also been emphasized.[32] The literature points to dysfunction of the antioxidant defense system in diabetes.[34]

Increased oxidant production and decreased activity of the antioxidant defense system have severe consequences, not only for lipids and proteins but also for nucleic acids,[11] leading to increased levels of 8-OH/Gua,[35] a DNA oxidation product that is accepted as an indicator of oxidative stress.[25,36] Increased 8-OH/Gua in diabetic patients was shown to lead to the development of microangiopathic complications.[12] Diabetic retinopathy is the most important microvascular complication of diabetes.[3] In the present study, the level of 8-OH/Gua was significantly higher in the DCG than in the HG. The findings of this study are consistent with those in the literature.

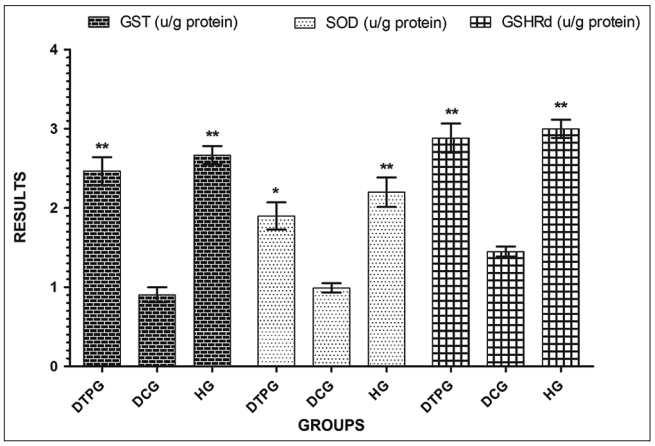

Figure 3: Effects of thiamine pyrophosphate on 8-hydroxyguanine levels in hyperglycemic rats (DTPG: Diabetic thiamine pyrophosphate-administered group, DCG: Diabetes control group, HG: Healthy group, $^{**}P < 0.0001$, $n = 12$)

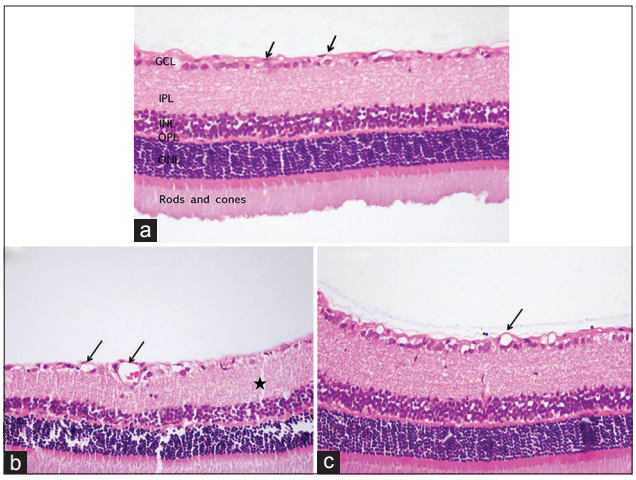

Figure 5: (a) Light microscope image of a healthy group rat retina (H and E, ×40). (b) Light microscope image of a diabetes control group rat retina (H and E, ×40). (c) Light microscope image of a diabetic thiamine pyrophosphate-administered group rat retina (H and E, ×40)

In the present study, there was a statistically significant increase in vascular structures, congestion, and edema, and a decrease in ganglion cells in the retinal tissue of the DCG, in which the oxidant/antioxidant balance was shifted in favor of oxidants. Neovascularization is the main finding in diabetic retinopathy. For a diagnosis of proliferative diabetic retinopathy, new vessel formation on the retinal surface and fibrous tissue proliferation must be present.[37] In addition, venous congestion is an important indicator of diabetic retinopathy.[38] Edema is seen in 3% of mild nonproliferative retinopathy cases, in 38% of moderate and severe nonproliferative cases, and in 71% of proliferative cases.[39] In the present study, the loss of ganglion cells in the retina was observed on the histopathological analysis. A previous experimental study showed that diabetes led to the loss of retinal ganglion cells.[40] A recent study also reported the reduced numbers of ganglion cells in the retinal tissues of diabetic mice.[41] Based on the current literature, hyperglycemia-induced oxidative stress was likely responsible in the present study for the severe pathological signs in the retinas of the DCG, in which no treatment was administered.

The findings also suggest that elevated oxidative stress plays a role in the development and progression of retinopathy.[34]

For this reason, the importance of antioxidants in the treatment of diabetic patients has been emphasized.[42] As shown in the present study, hyperglycemia shifted the oxidant/antioxidant balance in the DCG in favor of oxidants, whereas the balance shifted in favor of antioxidants in the DTPG. In addition, hyperglycemia-induced histopathological changes were minimal in the retinas of the DTPG compared to those of the DCG. TPP was previously reported to have antioxidant properties.[43] Another study reported that although the antioxidant activity of TPP was lower than that of melatonin and mirtazapine, TPP prevented infertility in diabetic rats caused by ischemia-reperfusion, whereas melatonin and mirtazapine did not.[44] The findings of that study suggest that the protective effects of TPP against oxidative retinal injury are not solely due to its antioxidant activity. In TPP deficiency, pyruvate and lactic acid accumulate in blood and tissue[17] and damage the retinal tissue of the eye.[45] Organic and functional tissue disorders occur when thiamine is not transformed into its active form, TPP, in cells.[46] Some medications and substances were reported to inhibit the expression of the thiamine pyrophosphokinase enzyme and to prevent the formation of TPP from thiamine.[47] Doxorubicin was reported to inhibit thiamine pyrophosphokinase and to have cardiotoxic effects.[48] Furthermore, although treatment with TPP reversed these cardiotoxic effects, treatment with thiamine did not.[48]

Conclusions

In the present study, alloxan-induced hyperglycemia caused oxidative retinal damage in rats. This retinal damage was histopathologically defined as diabetic proliferative retinopathy. TPP significantly decreased the degree of hyperglycemia-induced retinopathy. It is believed that the hyperglycemia-induced oxidative stress was caused by TPP deficiency in the retinal tissue, and retinopathy consequently developed. The results of this study show that TPP may be useful for prophylaxis against retinopathy in diabetic patients.

Acknowledgment

This manuscript was supported by the Erzincan University Scientific Research Projects Centre (Project No: SAG-B-080715-0157).

Financial support and sponsorship

This study received the financial support from Erzincan University Scientific Research Projects Centre.

Conflicts of interest

There are no conflicts of interest.

References

1. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules 2015;5:194-222.
2. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev 2013;93:137-88.
3. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: A systematic review. JAMA 2007;298:902-16.
4. Chew EY, Ferris FL 3rd. Nonproliferative diabetic retinopathy. In: Ryan SJ, Ogden TE, Hinton DR, Schachat AP, editors. Retina. 3rd ed. St. Louis: Mosby; 2001. p. 1295-308.
5. Kahn HA, Hiller R. Blindness caused by diabetic retinopathy. Am J Ophthalmol 1974;78:58-67.
6. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: A review. Diabetologia 2001;44:129-46.
7. Stitt AW. The role of advanced glycation in the pathogenesis of diabetic retinopathy. Exp Mol Pathol 2003;75:95-108.
8. Chistiakov DA. Diabetic retinopathy: Pathogenic mechanisms and current treatments. Diabetes Metab Syndr 2011;5:165-72.
9. Arden GB, Sivaprasad S. Hypoxia and oxidative stress in the causation of diabetic retinopathy. Curr Diabetes Rev 2011;7:291-304.
10. Bursell SE, Clermont AC, Aiello LP, Aiello LM, Schlossman DK, Feener EP, *et al.* High-dose Vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. Diabetes Care 1999;22:1245-51.
11. Turk HM, Sevinc A, Camci C, Cigli A, Buyukberber S, Savli H, *et al.* Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. Acta Diabetol 2002;39:117-22.
12. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, *et al.* Oxidative damage to DNA in diabetes mellitus. Lancet 1996;347:444-5.
13. Choudhuri S, Mandal LK, Paine SK, Sen A, Dutta D, Chowdhury IH, *et al.* Role of hyperglycemia-mediated erythrocyte redox state alteration in the development of diabetic retinopathy. Retina 2013;33:207-16.
14. Sica DA. Loop diuretic therapy, thiamine balance, and heart failure. Congest Heart Fail 2007;13:244-7.
15. Turan MI, Cetin N, Turan IS, Ozgeris FB, Suleyman H. Effects of thiamine and thiamine pyrophosphate on oxidative stress by methotrexate in the rat brain. Lat Am J Pharm 2013;32:203-7.
16. Jurgenson CT, Begley TP, Ealick SE. The structural and biochemical foundations of thiamin biosynthesis. Annu Rev Biochem 2009;78:569-603.
17. Brown G. Defects of thiamine transport and metabolism. J Inherit Metab Dis 2014;37:577-85.
18. Jaouhari JT, Lazrek HB, Jana M. The hypoglycemic activity of *Zygophyllum gaetulum* extracts in alloxan-induced hyperglycemic rats. J Ethnopharmacol 2000;69:17-20.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
20. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
21. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985;113:484-90.
22. Habig WH, Jakoby WB. Assays for differentiation of glutathione S-transferases. Methods Enzymol 1981;77:398-405.
23. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
24. Shigenaga MK, Aboujaoude EN, Chen Q, Ames BN. Assays of oxidative DNA damage biomarkers 8-oxo-2'-deoxyguanosine and 8-oxoguanine in nuclear DNA and biological fluids by high-performance liquid chromatography with electrochemical detection. Methods Enzymol 1994;234:16-33.
25. Kisaoglu A, Borekci B, Yapca OE, Bilen H, Suleyman H. Tissue damage and oxidant/antioxidant balance. Eurasian J Med 2013;45:47-9.
26. Peuchant E, Delmas-Beauvieux MC, Couchouron A, Dubourg L, Thomas MJ, Perromat A, *et al.* Short-term insulin therapy and normoglycemia. Effects on erythrocyte lipid peroxidation in NIDDM patients. Diabetes Care 1997;20:202-7.
27. Zuo ZF, Zhang Q, Liu XZ. Protective effects of curcumin on retinal Müller cell in early diabetic rats. Int J Ophthalmol 2013;6:422-4.

28. Di Mascio P, Murphy ME, Sies H. Antioxidant defense systems: The role of carotenoids, tocopherols, and thiols. Am J Clin Nutr 1991;53 1 Suppl: 194S-200S.
29. Halim EM, Mukhopadhyay AK. Effect of *Ocimum sanctum* (Tulsi) and Vitamin E on biochemical parameters and retinopathy in streptozotocin induced diabetic rats. Indian J Clin Biochem 2006;21:181-8.
30. Kamuren ZT, McPeek CG, Sanders RA, Watkins JB 3rd. Effects of low-carbohydrate diet and Pycnogenol treatment on retinal antioxidant enzymes in normal and diabetic rats. J Ocul Pharmacol Ther 2006;22:10-8.
31. Thiraphatthanavong P, Wattanathorn J, Muchimapura S, Thukham-mee W, Lertrat K, Suriharn B. The combined extract of purple waxy corn and ginger prevents cataractogenesis and retinopathy in streptozotocin-diabetic rats. Oxid Med Cell Longev 2014;2014:789406.
32. Kumawat M, Kharb S, Singh V, Singh N, Singh SK, Nada M. Plasma malondialdehyde (MDA) and anti-oxidant status in diabetic retinopathy. J Indian Med Assoc 2014;112:29-32.
33. Moasser E, Azarpira N, Shirazi B, Saadat M, Geramizadeh B. Genetic polymorphisms of glutathione-s-transferase M1 and T1 genes with risk of diabetic retinopathy in Iranian population. Iran J Basic Med Sci 2014;17:351-6.
34. Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong D. Serum markers of oxidative stress and severity of diabetic retinopathy. Diabetes Care 2000;23:234-40.
35. Ates O, Alp HH, Caner I, Yildirim A, Tastekin A, Kocer I, *et al.* Oxidative DNA damage in retinopathy of prematurity. Eur J Ophthalmol 2009;19:80-5.
36. Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: An enemy within. Trends Genet 1993;9:246-9.
37. Engerman RL. Pathogenesis of diabetic retinopathy. Diabetes 1989;38:1203-6.
38. Nesterov AP. Role of local factors in the pathogenesis of diabetic retinopathy. Vestn Oftalmol 1994;110:7-9.
39. Bandello F, Battaglia Parodi M, Lanzetta P, Loewenstein A, Massin P, Menchini F, *et al*. Diabetic macular edema. Dev Ophthalmol 2010;47:73-110.
40. Szabadfi K, Atlasz T, Kiss P, Reglodi D, Szabo A, Kovacs K, *et al.* Protective effects of the neuropeptide PACAP in diabetic retinopathy. Cell Tissue Res 2012;348:37-46.
41. Kim J, Kim CS, Lee YM, Sohn E, Jo K, Kim JS. Litsea japonica extract inhibits neuronal apoptosis and the accumulation of advanced glycation end products in the diabetic mouse retina. Mol Med Rep 2015;12:1075-81.
42. Battioni JP, Fontecave M, Jaouen M, Mansuy D. Vitamin E derivatives as new potent inhibitors of microsomal lipid peroxidation. Biochem Biophys Res Commun 1991;174:1103-8.
43. Coskun R, Turan MI, Turan IS, Gulapoglu M. The protective effect of thiamine pyrophosphate, but not thiamine, against cardiotoxicity induced with cisplatin in rats. Drug Chem Toxicol 2014;37:290-4.
44. Yapca OE, Turan MI, Borekci B, Akcay F, Suleyman H. Bilateral ovarian ischemia/reperfusion injury and treatment options in rats with an induced model of diabetes. Iran J Basic Med Sci 2014;17:294-302.
45. Nyengaard JR, Ido Y, Kilo C, Williamson JR. Interactions between hyperglycemia and hypoxia: Implications for diabetic retinopathy. Diabetes 2004;53:2931-8.
46. Soukoulis V, Dihu JB, Sole M, Anker SD, Cleland J, Fonarow GC, *et al.* Micronutrient deficiencies an unmet need in heart failure. J Am Coll Cardiol 2009;54:1660-73.
47. Subramanian VS, Subramanya SB, Tsukamoto H, Said HM. Effect of chronic alcohol feeding on physiological and molecular parameters of renal thiamin transport. Am J Physiol Renal Physiol 2010;299:F28-34.
48. Polat B, Suleyman H, Sener E, Akcay F. Examination of the effects of thiamine and thiamine pyrophosphate on doxorubicin-induced experimental cardiotoxicity. J Cardiovasc Pharmacol Ther 2015;20:221-9.