The Histological Changes of The Eyes of Rats after Exposure to UVB: Role of Vitamin E

Rana Mustafa Ahmad* , Harith Ali Hasan Al-Nauimi* , Omar Riyadh Hamdi*
***Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq**
Correspondence: harith.hasan@uomosul.edu.iq

(Ann Coll Med Mosul 2024; 46 (2):257-264).
Received: 17th April 2024; Accepted: 29th July 2024.

ABSTRACT

Background and Objectives: Eye exposure to UVB radiation can lead to photokeratitis. Antioxidant agents, such as Vitamin E, enhance corneal healing.

Aim of this work Study: the influence of a narrow band of UVB light on the eyes of rats and evaluate the preventive role of vitamin E on the eye following UVB radiation.

Methods: Twenty male Wistar albino rats were included in this work. They haphazardly distributed into four equal groups (n=5), **group A,** a negative control group, **group B** (exposed to different intensities of a single daily dose of UVB radiation extending from 240 mJ UVB/cm2 up to 960 MJ/1 week, **group C:** treated as group B with the oral ingestion of vitamin E, **group D:** ingested oral dosages of vitamin E for one week.
The rats were them sacrificed, and the eyes were removed and fixed in 10% buffered formalin for 24 hours to be followed by histological processing and tissue staining with hematoxylin and eosin stain as well as TUNEL stain to be ready for histopathological examination.

Results: The eyes of **group B** rats corneal damage in the form of irregular hypertrophy of the cornea, ulceration, stromal edema, hemorrhage, inflammatory cell infiltration, and increased apoptotic cells. All these changes were reduced following the use of vitamin E in **group C,** while **groups A** and **D** show normal histological pictures.

Conclusion: daily exposure of the eye to the progressive increasing intensity of UVB for one-week results in severe histological changes in the cornea that are alleviated by using a protective daily dose of vitamin E before exposate to radiation.

Keywords: UVB, rat, cornea, TUNEL stain.

التغيرات النسيجية لعيون الجرذان بعد التعرض للأشعة فوق البنفسجية: دور فيتامين E

رنا مصطفى احمد* ، حارث علي حسن النعيمي* ، عمر رياض حمدي*
*فرع التشريح، كلية الطب، جامعة الموصل، الموصل، العراق

الخلاصة

تعريض العين للإشعاع فوق البنفسجي من النوع B يمكن أن يسبب التهاب القرنية الضوئي، وهي حالة تؤثر على القرنية. لتعزيز الشفاء يتم استخدام مضادات الأكسدة، مثل فيتامين E.

تهدف هذه الدراسة إلى فهم كيفية تأثر أنسجة العين بعد التعرض للأشعة فوق البنفسجية وتأثير الاستخدام المتزامن لفيتامين E للوقاية

تتضمن الدراسة عشرين فأرًا قسمت عشوائيا إلى أربع مجموعات. المجموعة A كانت بمثابة مجموعة التحكم بينما تعرضت المجموعات B,C لجرعات من الأشعة فوق البنفسجية لفترات متفاوتة. تم فحص عينات من أنسجة العين باستخدام صبغة H&E وصبغة TUNEL.

النتائج: أظهرت فئران المجموعة B علامات الضرر صبغات في القرنية بما في ذلك نسيج مثل التضخم والتقرح والوذمة اللحمية والنزيف وتسلل الخلايا الالتهابية. وزاد معدل موت الخلايا المبرمج (موت الخلايا) في هذه المجموعة. ومع ذلك، تم تخفيف هذه التغييرات عندما تم إعطاء فيتامين E في المجموعة C. أظهرت المجموعتان A وD صورة نسيجية سليمة للقرنية.

نستنتج من الدراسة ان تعرض العينين لمستويات متزايدة من الأشعة فوق البنفسجية لمدة أسبوع يؤدي إلى تغيرات نسيجية كبيرة في القرنية. ومع ذلك، تم تخفيف هذه التغييرات عن طريق إعطاء جرعة من فيتامين E قبل التعرض للإشعاع.

الكلمات المفتاحية : الأشعة فوق البنفسجية، الفئران، القرنية، TUNEL.

INTRODUCTION

Ultraviolet light (UVL) is electromagnetic radiation with a short wavelength between X-ray and visible light; UVL wavelength is longer than X-rays and shorter than visible light. UVL wavelength is used to sterilize water and food [1]. Also, UVL is one of the best methods for skin disease treatment (vitiligo and psoriasis)[1,2].

The solar UVL consists of UVA, UVB as 90- 95% UVA and 5- 10% UVB[3]. Most people identify the UVL effects on skin and eyes as a painful sunburn[4]. The UVL wavelength ranges from 10-400 nm, particularly UVB light ranging from (300-320 nm) wavelengths, and it is usually absorbed by corneal stroma and lens[5,6]. Various works are studying the effect of UVL on the ocular lenses. The lens plays a main role in eye protection and avoiding the development of cataracts[7].

The UVL phototoxicity relies on different factors such as energy, exposure time, and wavelength. Result in exposure to UVL makes photokeratitis. Chronic or long-lasting exposure causes keratopathy, endothelial dystrophy, pterygium, carcinoma, squamous metaplasia, cataracts, and macular degeneration[1,3,8,9].The beginning of symptoms occurs 6-12 hours after exposure. It is characterized by photokeratitis, photophobia, severe bilateral ocular pain, blepharospasm, chemosis and erythema of the face. In contrast, a sign of photokeratitis includes superficial keratitis, erosion, epithelial desquamation, corneal edema, and haze-clinical resolution from symptoms usually within 72 hours[10].The histological changes that result from UVL exposure are characterized by the separation of superficial cell epithelium, damage of nuclei and cytoplasm, edema, and apoptosis in all corneal layers, relining on the wavelength and dose of UVL[11].

Vitamin E is the most effective lipid-soluble antioxidant, preventing oxidative stress on cell membranes[12]. Free radicals contribute to many diseases, but vitamin E acts as a protective agent against their damage, including damage related to cataracts, aging, cancer, circulatory conditions, pollution, arthritis, and active exercise. Using vitamin E improved the fight of glutathione-depleted epithelial cells of the lens to peroxide-induced cell apoptosis[13]. So, an adequate intake of antioxidants like vitamin E can protect from increased concentrations of free radicals caused by air pollution and recent lifestyle designs[14].

This work aims to study the influence of UVB light narrow band on the rat's eye and evaluate the vitamin E protective effect on the eye following UVB radiation.

MATERIAL AND METHODS

The Ethical Committee of the College of Medicine at Mosul University approved this study. The experiment was conducted in an animal house the related to the College of Veterinary Medicine at Mosul University. The experimental animals used in this study were twenty adult Wistar albino rats. Rats were maintained under standard conditions and diet. They were haphazardly separated into four equal groups, including group A (negative control) without UVB exposure, group B (positive control) (exposed to UVB as single daily progressive increase doses mJ/cm^2 for one week), group C (exposed to single daily progressive increase dose mJ UVB/cm^2 for one week with the administration of oral dose of vit E), group D (received oral doses of vit. E for one-week).

Vit. E 400 IU (Adrian Gagnon) was mixed with olive oil to attain a suitable dose of Vit E (100 IU/kg body weight), then ingested by gavage syringe according to previous studies[15]. Anesthesia was done with intraperitoneal injection of ketamine hydrochloride 80 mg/kg and xylazine hydrochloride 10 mg/kg.

The device used in this study was a 311narrow band UVB KN-4003LB with a comb. The plastic translucent comb gets a standard distance of 3cm between the rat's eye and the source of the device light.

Each animal in groups B and C was given a progressive increasing dose as follows: on the first day, the dose was 240 mJ/cm$^{2;}$ on the second day, 360 mJ/cm^2; on the third day, 480 mJ/cm^2; fourth day 600 mJ/cm^2, fifth day 720 mJ/cm^2, sixth day 840 mJ/cm^2, and seventh day 960 mJ/cm^2.

The protocol of UVB radiation was achieved by giving the rats daily gradual increasing doses, the calculation of dose using the following formula [16]:

Radiation time = dose * 1000 ÷ intensity * 60 [min. = J/cm^2 * 1000 / (mW/cm^2) * 60]

The rats were recovered for 24 hours, and then the animals were killed by cervical displacement. The eyes of rats were removed and fixed with 10% buffered formalin. The eye samples underwent processing techniques followed by sectioning of eye samples and staining with hematoxylin and eosin stain in preparation for histopathological check and TUNEL stain as an immunohistochemical stain to descript apoptotic cell signaling. TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay with an Apoptag Plus Peroxidase in Situ Apoptosis Detection Kit (S7101; MilliporeSigma, Burlington, MA, USA).

RESULTS

In this work, the histological manifestations of rat's eyes in group A (without exposure to UVB light) and group D (rat received Vitamin E only) showed normal histological architecture, including normal corneal epithelium, stroma and thickness (Figures 1, 2). TUNEL stain is positive in both groups with a small number of apoptotic cells and normal corneal epithelium, intraepithelial, and regular cells (Figures 3,4).

In comparison, the histological changes of eyes in group B (exposed to UVB) exhibited corneal injuries characterized by irregular hypertrophy of the cornea along its length with hyperplasia of epithelium, ulceration in the central surface of the cornea while Bowman's membrane remains uninterrupted continuously over the stroma in the ulcerative corneal areas along with stromal edema (Figure 5). In addition to the above changes, there is a hemorrhage in the corneal epithelium and stroma (figure 6). Massive infiltration of inflammatory cells (polymorphonuclear leukocytes and mononuclear cells) in all corneal stroma and sclerocorneal areas (figure 7). Additionally, the TUNEL stain showed a positive increase in apoptotic cells in corneal stroma and epithelium and irregular cells with pyknotic nuclei (Figure 8).

In contrast, the microscopical changes of rat's eye in group C (exposed to UVB and received Vit. E) were showed amelioration to all microscopical changes that seen in group B as mild edema in corneal stroma and mild infiltration of inflammatory cells with normal epithelial structure (Figure 9, 10). The number of apoptotic cells markedly decreased compared with group C (Figure 11).

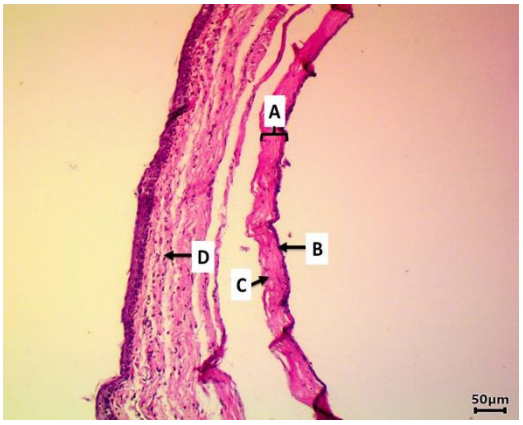

Fig. 1: Histological section of rat eye in group A showing normal thickness of cornea (A) consisting of epithelium (B) and stroma(C), with choroid and retina (D). H&E stain, 100X.

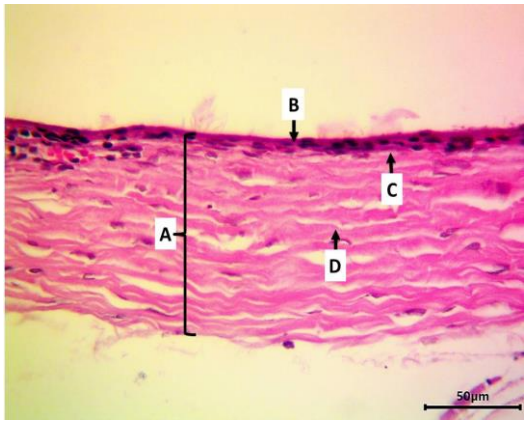

Fig. 2: The histological section of the rat eye of group D shows the normal thickness of the cornea (A) consisting of the epithelium (B), Bowman's membrane (C), and stroma (D). H&E stain, 400X.

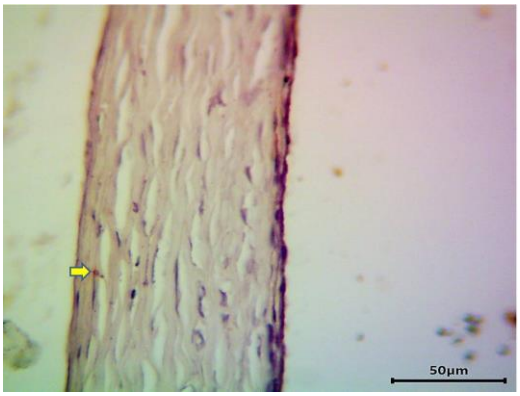

Fig. 3: Histological section of TUNEL assay for apoptotic cells in the cornea of group A shows weak positive stained apoptotic cells (arrow). H&E stain, 400X.

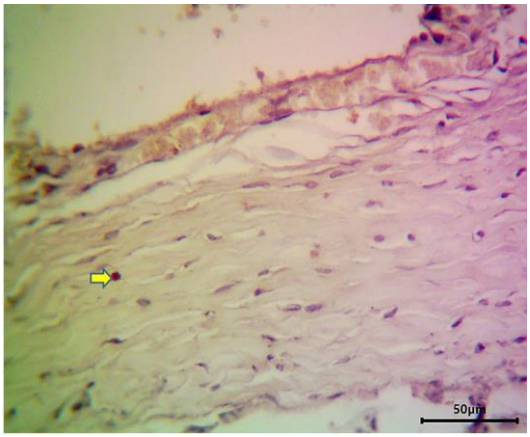

Fig. 4: The histological section of the TUNEL assay for apoptotic cells in the cornea of group D shows weak positive stained apoptotic cells (arrow). H&E stain, 400X.

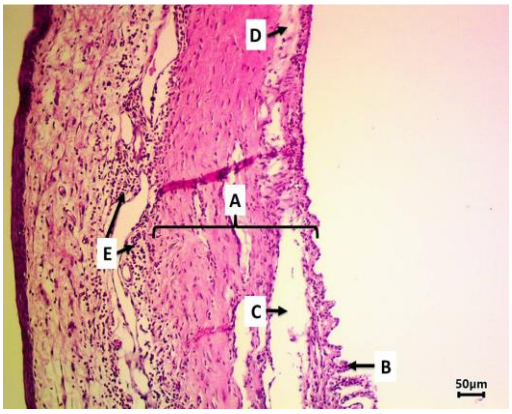

Fig. 5: The histological section of the rat eye of group B shows irregular corneal hypertrophy along its length (A), hyperplasia of the epithelium (B), ulceration (C), edema in the stroma (D), and inflammatory cells infiltration (E). H&E stain, 100X.

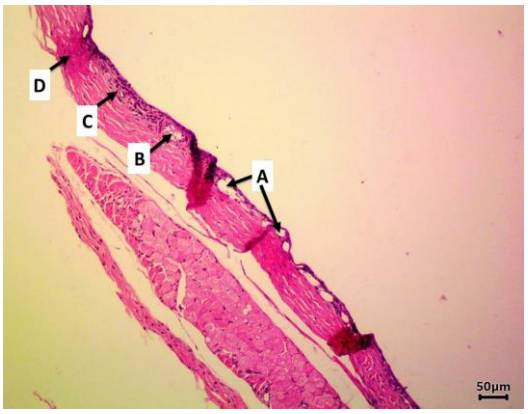

Fig. 6: The histological section of the rat eye of group B shows ulceration of epithelium with continuous Bowman's membrane (A), edema in the stroma (B), infiltration of inflammatory cells (C), and hemorrhage (D). H&E stain, 100X.

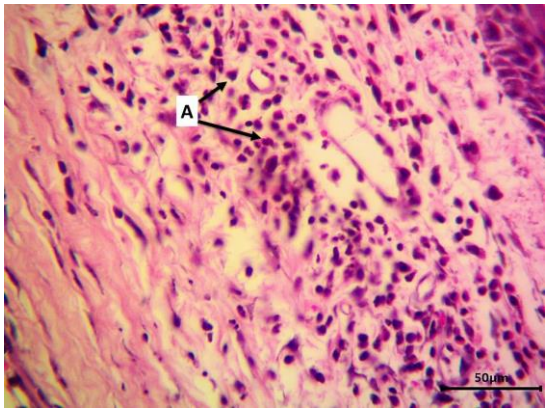

Fig. 7: The histological section of the rat eye of group B shows inflammatory cell infiltration (polymorph nuclear leukocytes and mononuclear cells) in all sclerocorneal areas (A). H&E stain, 400X.

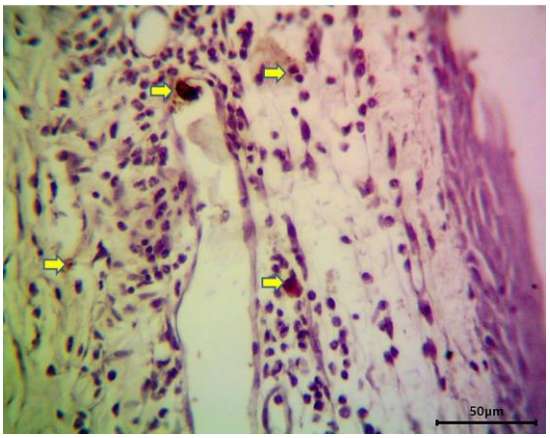

Fig. 8: The histological section of the TUNEL assay for apoptotic cells in the cornea of group B shows highly positive stained apoptotic cells (arrows). H&E stain, 400X.

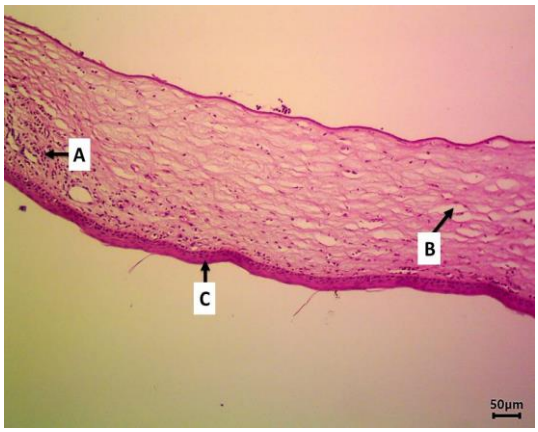

Fig. 9: The histological section of the rat eye of group C shows mild infiltration of inflammatory cells (A) and mild edema (B), with normal structure of the epithelium (C). H&E stain, 100X.

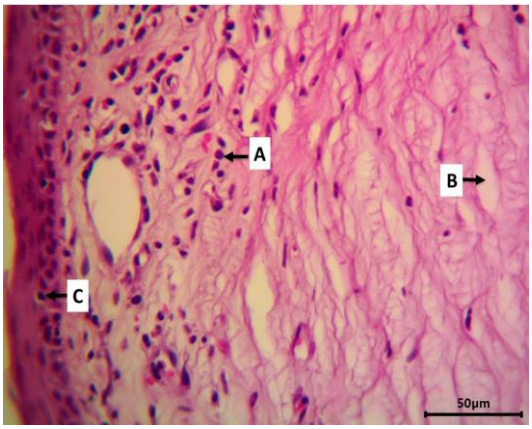

Fig. 10: The histological section of the rat eye of group C shows mild infiltration of inflammatory cells (A) and mild edema (B), with normal structure of the epithelium (C). H&E stain, 400X.

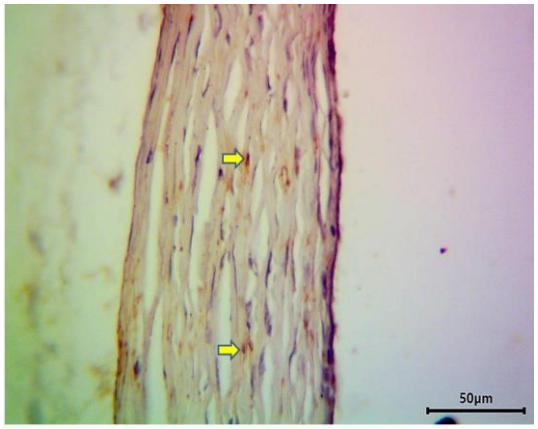

Fig. 11: The histological section of the TUNEL assay for apoptotic cells in the cornea of group C shows mild positive stained apoptotic cells (arrows). H&E stain, 400X.

DISCUSSION

The eye is a unique sense organ that relies on noticeable light energy and is affected by exposure to ultraviolet plus infrared wavelength. The available data suggested that the corneal tissue affected by UV radiation is dangerous in case of prolonged exposure[17]. Most of these researchers related these injuries to the involvement of the ROS in the induction of corneal damage[18].

The irradiated group B that received a progressively increasing dose of UVB showed damage to the corneal epithelium and stroma in irregular hypertrophy of corneal epithelium with ulceration in the central surface of it. At the same time, Bowman's membrane remains uninterrupted continuously over the stroma in the ulcerative corneal areas and polymorph nuclear cells infiltration in corneal lamellae, As the corneal epithelium is the most sensitive layer to UVB than other corneal layers and such structural changes can be explained by UVB -induced DNA cumulative damage with changes in permeability and membrane transport and ROS damage that stimulate the manufacture of pro-inflammatory molecules[19].

Similar histopathological changes, mainly irregular hypertrophy, were detected by Muresan and his team [20], and complete exfoliated corneal epithelium was seen in corneal rabbits by Giblin et al. after UVB exposure[21]. At the same time, other studies show identical changes to the above in addition to discontinuation and separation of the anterior epithelium (Bowman's membrane) in the affected corneal stroma[22]. Such photokeratitis resulted in our work, which was reported by similar previous studies[11,23,24]. This photokeratitis is also proved by Muresan et. al. who stated that the more UVB exposure with more duration, the more corneal tissue is affected[19].

The other change seen in our study in those exposed to UVB is corneal stroma edema related to endothelial dysfunction[11].

TUNEL assay for apoptotic cells in rat's cornea of the irradiated group B showed highly positive stained apoptotic cells in comparison to the negative control group; such increase in apoptotic cells is due to UVB has been stated to rise the making of interleukins as IL -1, IL-6 and IL-8 [25], adding to TNF-α, and NF-κB in stromal cells of the cornea[26-28].

The translocated NF-κB will stimulate the transcription of pro-inflammatory cytokines, containing inducible nitric oxide iNOS and cyclooxygenase COX-2 which are considered the main mediators for inflammation and cell death or apoptosis[29-32], or this highly positive apoptotic cells can be explained as exposure to UVB stimulates apoptosis in epithelial cells of rats' cornea[33,34].

This means continuous UVB irradiation makes an organic injury in absorbing tissues, mostly involving DNA damage, ROS increase, and reduction in enzymes that have antioxidant activity[35-37]. Such significant TUNEL-positive apoptotic cells were noticed in corneal mice with thinning of corneal epithelium after exposure to 400 mJ/ cm^2 of UVB[32]. Similar TUNEL-positive apoptotic cells were seen in rats' corneal stroma after a single exposure to UV injury[11]. In other words, the histological changes in the cornea of the irradiated B group result from the oxidative stress of cellular membranes, including proteins, plus direct DNA oxidative damage[38-40], such declining in mitochondrial jobs and reduced antioxidants with protective mechanisms, leading to increase apoptosis in the tissues of the cornea [41,42].

The histopathological changes of irradiated group C, which was treated with vitamin E as a precaution, showed a reduction in all changes that were seen in group B, which was treated with UVB only; this means that the given antioxidant will replace the reduced protective factors which are the leading cause of corneal injury and damage as these antioxidants with its defense tools are susceptible to increase exposure to UVB[43]. Such findings run in agreement with similar findings in previous reports as Palazzo and her team, who found using local application of eye drops having riboflavin and D- α-tocopherol polyethylene glycol succinate (TPGS) vitamin E lessens the corneal injury in rabbits exposed to UV[44]. Vizzari et al. presented a similar result: topical application of the antioxidant considerably lessened the oxidative stress in rabbits' eyes induced by UV[38].

The role of vitamin E in reducing TUNEL-positive cells in this group is clearer than in group B. This run is consistent with a previous study using astaxanthin as a potent antioxidant activity that helps in lowering TUNEL-positive cells and decreases photokeratitis in mice eyes, where they explained this as astaxanthin reducing NF-$_K$B expression in the corneal epithelium [32], this run with the fact that UVB radiation trigger the oxidative damage related transcription factors, for example nuclear factor-κB (NF-κB), and encouraged the making of several cytokines as TNF-α, IL-1, and IL-6 [45], and using antioxidant will facilitate production of pro-inflammatory factors. Another work using Fullerenols as eye drops following irradiation in the rat's eye helps decrease corneal injury, where it acts as an exogenous antioxidant[46]. The effect of antioxidants such as Vitamin C was obvious. Another, when given intraperitoneally for infant rats, was to alleviate the acute UVB irradiation on corneal structure[47].

This antioxidant role provided by Vitamin E in group C is due to its facility to pass through the cell membrane as it has lipid-soluble properties that preserve the membrane against lipid peroxidation and scavenge free radicals, occluding the radical chains and generating a low-reaction derivative incapable of fighting against the lipid substrates[48,49].

CONCLUSION

The eye rats exposed to the progressive increasing intensity of UVB once daily for one week resulted in severe histological changes in the cornea, the epithelium, and st, alleviated by using a protective daily dose of oral vitamin E before radiation.

Conflict of Interest

None declared.

REFERENCES

1. Hamill MB. Corneal and scleral trauma. In: Krachmer JH, Mannis MJ, Holland EJ, editors. Cornea. 3rd ed. Saint Louis: Mosby Elsevier; 2010. P. 1176–8. DOI: 10.1016/s0896-1549(02)00018-4.
2. Tanito M, Takanashi T, Kaidzu S, Yoshida Y, Ohira A. Cytoprotective effects of rebamipide and carteolol hydrochloride against ultraviolet B-induced corneal damage in mice. Invest Ophthalmol Vis Sci, 2003, 44(7):2980–2985. doi:https://doi.org/10.1167/iovs.02-1043.
3. Jacobs DS. Photokeratitis. In: Trobe J, Wilterdink J, editors. UpToDate. Available at:https://021081217-y-https-www-uptodate-com.proxy.kirikkale-elibrary.com/contents/ photokeratitis. Accessed Aug 10, 2018.
4. Gallagher RP, Lee TK, Adverse effects of ultraviolet radiation:a brief review, ProBiophys Mol Biol, 2006, 92(1):119–131. https://doi.org/10.1016/j.pbiomolbio.2006.02.011.
5. Merriam JC, Löfgren S, Michael R, Söderberg P, Dillon J, Zheng L, Ayala M. An action spectrum for UV-B radiation and the rat lens. Invest Ophthalmol Vis Sci, 2000, 41(9): 2642–2647. https://arvojournals.org/article.aspx?articleID=2162374
6. Yin J, Huang Z, Wu B, Shi Y, Cao C, Lu Y, Lornoxicam protects mouse cornea from UVB-induced damage via inhibition of NF-kB activation, Br J Ophthalmol, 2008, 92(4): 562–568. http://dx.doi.org/10.1136/bjo.2007.129064.
7. Risa O, Saether O, Kakar M, Mody V, Löfgren S, Söderberg PG, Krane J, Midelfart A, Time dependency of metabolic changes in rat lens after in vivo UVB irradiationanalysed by HR-MAS 1H NMR spectroscopy, Exp Eye Res, 2005, 81(4):407–414. https://doi.org/10.1016/j.exer.2005.02.012
8. Taylor HR, The biological effects of UV-B on the eye, Photochem Photobiol, 1989, 50(4):489–492. https://doi.org/10.1111/j.1751-1097.1989.tb05553.x
9. Young RW, The family of sunlight-related eye diseases, Optom Vis Sci, 1994, 71(2):125–144. https://doi.org/10.1097/00006324-199402000-00013
10. Johnson GJ, The environment and the eye, Eye (Lond), 2004, 18(12):1235–1250. https://doi.org/10.1038/sj.eye.6701369.
11. Filizay MC, Gokcinar NB, Sahinturk V, Oner S, Kacar S, Onaran Z, Yumusak E. Evaluation of retinol palmitate treatment of photokeratitis in rat eyes exposed to ultraviolet B radiation. Beyoglu Eye J. 2019 Aug 5;4(2):55-61. DOI:10.14744/bej.2019.51523.

12. L Packer, Protective role of vitamin E in biological systems, The American Journal of Clinical Nutrition, Volume 53, Issue 4, April 1991, Pages 1050S–1055S, https://doi.org/10.1093/ajcn/53.4.1050S.
13. Shang F, Lu M, Dudek E, Reddan J, Taylor A. Vitamin C and vitamin E restore the resistance of GSH-depleted lens cells to H2O2. Free radical biology and medicine. 2003 Mar 1;34(5):521-30. https://doi.org/10.1016/S0891-5849(02)01304-7
14. Abdelazim SA, Darwish HA, Ali SA, Rizk MZ, Kadry MO. Potential antifibrotic and angiostatic impact of idebenone, carnosine and vitamin E in nano-sized titanium dioxide-induced liver injury. Cellular Physiology and Biochemistry, vol. 35, no. 6, pp. 2402–2411, 2015.View at: Publisher Site | Google Scholar https://doi.org/10.1159/000374041.
15. OECD Guidelines for Testing of Chemicals. In-Vitro 3T3 NRU Phototoxicity Test. 432 Adopted 13 April 2014. Available online: https://ntp.niehs.nih.gov/sites/default/files/iccvam/suppdocs/feddocs/oecd/oecdtg432-508.
16. Wittenberg S, Solar radiation and the eye: a review of knowledge relevant to eye care, Am J Optom Physiol Opt, 1986; 63(8):676–689. PMID: 3532811.
17. Čejková J, Ardan T, Čejka Č, Kovačeva J, Zídek Z,Irradiation of the rabbit cornea with UVB rays stimulates the expression of nitric oxide synthases-generated nitric oxide and the formation of cytotoxic nitrogen-related oxidants, Histol Histopathol, 2005, 20(2):467–473.
18. Kennedy M, Kim KH, Harten B, Brown J, Planck S, Meshul C, et al. Ultraviolet irradiation induces the production of multiple cytokines by human corneal cells. Invest Ophthalmol Vis Sci 1997;38:2483–91.
19. Mureşan SI, Filip A, Mureşan AD, Şimon V, Moldovan R, Gal AF, Miclăuş V. Histological findings in the Wistar rat cornea following UVB irradiation. Rom J Morphol Embryol. 2013;54(2):247-52. https://rjme.ro/RJME/resources/files/540213247252.
20. Giblin JF,Lin RL, Leverenz RV, Dang L. A class I (senofilcon A) soft contact lens prevents UVB induced ocular effects, including cataract, in the rabbit in VIVO. Invest. Ophthalmol. Vis. Sci. 2011;52 (6): 3667–3675. https://doi.org/10.1167/iovs.10-6885.
21. Golu A, Gheorghişor I, Bălăşoiu AT, Baltă F, Osiac E, Mogoantă L, Bold A. The effect of ultraviolet radiation on the cornea - experimental study. Rom J Morphol Embryol. 2013;54(4):1115-20. PMID: 24399010.
22. Yin J, Huang Z, Wu B, Shi Y, Cao C, Lu Y. Lornoxicam protects mouse cornea from UVB-induced damage via inhibition of NF- {kappa}B activation. Br J Ophthalmol 2008;92:562–8.
23. T Fujihara, T Nagano, K Endo, M Nakamura, K Nakata. Lactoferrin protects against UV-B irradiation-induced corneal epithelial damage in rats. Cornea 2000;19(2):207–11. http://doi:10.1097/00003226-200003000-00015.
24. Hamill MB, Corneal trauma, in: J.H. Krachmer, M.J. Mannis, E.J. Holland (Eds.),Cornea, third ed., Mosby Elsevier, Saint Louis, 2010. https://doi.org/10.1016/j.tria.2020.100086.
25. Di Girolamo N, Coroneo MT, Wakefield D, UVB-elicited induction of MMP-1 expression in human ocular surface epithelial cells is mediated through the ERK1/2 MAPK dependent pathway, Invest Ophthalmol Vis Sci, 2003, 44(11):4705-4714. https://doi.org/10.1167/iovs.03-0356.
26. Lee DH, Kim JK, Joo CK, Translocation of nuclear factor kappaB on corneal epithelial cells induced by ultraviolet B irradiation, Ophthalmic Res, 2005, 37(2):83–88. DOI: 10.1159/000084249.
27. Pauloin T, Dutot M, Joly F, Warnet JM, Rat P. High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation in human epithelial corneal cells. Mol Vis. 2009;15:577-83. Epub 2009 Mar 23. PMID: 19325714; PMCID: PMC2660376.
28. Chen BY, Lin DP, Wu CY, Teng MC, Sun CY, Tsai YT, Su KC, Wang SR, Chang HH. Dietary zerumbone prevents mouse cornea from UVB-induced photokeratitis through inhibition of NF-κB, iNOS, and TNF-α expression and reduction of MDA accumulation. Mol Vis. 2011 Apr 6;17:854-63. PMID: 21527993; PMCID: PMC3081794.
29. Hur S, Lee YS, Yoo H, Yang JH, Kim TY. Homoisoflavanone inhibits UVB-induced skin inflammation through reduced cyclooxygenase-2 expression and NF-kappaB nuclear localization. J Dermatol Sci. 2010 Sep;59(3):163-9. https://doi.org/10.1016/j.jdermsci.2010.07.001.
30. Golu T, Mogoantă L, Streba CT, Pirici DN, Mălăescu D, Mateescu GO, Muţiu G. Pterygium: histological and immunohistochemical aspects. Rom J Morphol Embryol. 2011;52(1):153-8. PMID: 21424047.
31. Livezeanu C, Crăiţoiu MM, Mănescu R, Mocanu C, Crăiţoiu S,Angiogenesis in the pathogenesis of pterygium, Rom J Morphol Embryol, 2011, 52(3):837–844. PMID: 21892527.

32. Lennikov A, Kitaichi N, Fukase R, Murata M, Noda K, Ando R, Ohguchi T, Kawakita T, Ohno S, Ishida S. Amelioration of ultraviolet-induced photokeratitis in mice treated with astaxanthin eye drops. Mol Vis. 2012;18:455-64. Epub 2012 Feb 14. PMID: 22393271; PMCID: PMC3291518.
33. Podskochy A, Gan L, Fagerholm P. Apoptosis in UV-exposed rabbit corneas. Cornea. 2000;19:99–103. doi: 10.1097/00003226-200001000-00019.
34. Cejková J, Stípek S, Crkovská J, Ardan T, Pláteník J, Cejka C, Midelfart A. UV Rays, the prooxidant/antioxidant imbalance in the cornea and oxidative eye damage. Physiol Res. 2004;53(1):1-10. PMID: 14984308.
35. Hammond BR Jr, Renzi-Hammond L. Individual variation in the transmission of UVB radiation in the young adult eye. PLoS ONE. 2018;13:e0199940. https://doi.org/10.1371/journal.pone.0199940.
36. Cejková J, Stípek S, Crkovská J, Ardan T, Midelfart A. Reactive oxygen species (ROS)-generating oxidases in the normal rabbit cornea and their involvement in the corneal damage evoked by UVB rays. Histol. Histopathol. 2001;16:523–533.
37. Cejková J, Ardan T, Filipec M, Midelfart A. Xanthine oxidoreductase and xanthine oxidase in human cornea. Histol. Histopathol. 2002;17:755–760.
38. Vizzarri F, Palazzo M, Bartollino S, Casamassima D, Parolini B, Troiano P, Caruso C, Costagliola C. Effects of an antioxidant protective topical formulation on eye exposed to ultraviolet-irradiation: a study in rabbit animal model. Physiol Res. 2018 Jul 17;67(3):457-464. https://doi.org/10.33549/physiolres.933759.
39. Cejka C, Cejkova J. Oxidative stress to the cornea, changes in corneal optical properties, and advances in treatment of corneal oxidative injuries. Oxid. Med. Cell Longev. 2015;2015: 1-10. https://doi.org/10.1155/2015/591530.
40. Wakamatsu T H, Dogru M, Ayako I, Takano Y, Matsumoto Y, Ibrahim O M A, Okada N, Satake Y, Fukagawa K, Shimazaki J, et al. Evaluation of lipid oxidative stress status and inflammation in atopic ocular surface disease. Mol. Vis. 2010, 16, 2465–2475. PMID: 21139696; PMCID: PMC2994734.
41. Saccà SC, Cutolo CA, Ferrari D, Corazza P, Traverso CE. The Eye, Oxidative Damage and Polyunsaturated Fatty Acids. Nutrients. 2018 May 24;10(6):668. https://doi.org/10.3390/nu10060668.
42. Seen S, Tong L. Dry eye disease and oxidative stress. Acta Ophthalmol. 2018 Jun;96(4): e412-e420. https://doi.org/10.1111/aos.13526.
43. Hepel M, Stobiecka M, Peachey J, Miller J. Intervention of glutathione in pre-mutagenic catechol-mediated DNA damage in the presence of copper(II) ions. Mutat Res. 2012, 1;735(1-2):1-11. https://doi.org/10.1016/j.mrfmmm.2012.05.005.
44. Palazzo M, Vizzarri F, Ondruška L, Rinald M, Pacente L, Guerra G, Merolla F, Caruso C, Costagliola C. Corneal UV Protective Effects of a Topical Antioxidant Formulation: A Pilot Study on In Vivo Rabbits. Int. J. Mol. Sci. 2020, 21, 5426. https://doi.org/10.3390/ijms21155426.
45. Delic NC, Lyons JG, Di Girolamo N, Halliday GM. Damaging Effects of Ultraviolet Radiation on the Cornea. Photochem Photobiol.2017;93(4):920-929. https://doi.org/10.1111/php.12686.
46. Chen X, Yang J, Li M, Zhu S, Zhao M, Yang C, Liu B, Gao H, Lu A, Ge L, Mo L, Gu Z, Xu H. Fullerenol protects cornea from ultraviolet B exposure. Redox Biol. 2022; 54:102360. https://doi.org/10.1016/j.redox.2022.102360.
47. Chen W, Guo J, Guo H, Kong X, Bai J, Long P. Protective Effect of Vitamin C against Infancy Rat Corneal Injury Caused by Acute UVB Irradiation. Biomed Res Int.2020:8089273. https://doi.org/10.1155/2020/8089273.
48. Caruso C, Porta A, Tosco A, Eletto D, Pacente L, Bartollino S, Costagliola C. A Novel Vitamin E TPGS-Based Formulation Enhances Chlorhexidine Bioavailability in Corneal Layers. Pharmaceutics. 2020;12(7):642. https://doi.org/10.3390/pharmaceutics12070642.
49. Hajibabaei K. Antioxidant properties of vitamin E. Ann Res Antioxid. 2016;1(2):e22.