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DEPARTMENT OF ANATOMY

A Systematic Review of the Histopathological Effects of Ultraviolet Radiation Exposure on the Ocular structures in Human and Animal Studies.

A Project Paper (Anat. 749) Submitted to Department of Anatomy, Faculty of Medicine, College of Health Sciences, Addis Ababa University, for partial fulfillment of the requirement for MSc degree in Anatomy.

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List of abbreviations

ARVO- Association for Research in Vision and Ophthalmology

BCC - Basal cell carcinoma of the skin

BSS- Balanced Salt Solution

DNA- Deoxyribonucleic Acid

LED- Light Emitting Diodes

PBS - Phosphate Buffered Saline

RPE- Retinal Pigment Epithelium

SCC - Squamous cell carcinoma

SCL- Soft Contact Lenses

tEDC- transformed Equivalent Diazepam Concentration

UV -Ultraviolet

UVR - Ultraviolet Radiation

WHO - World Health Organization

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Summary

The transparent anterior segment of the human eye (cornea and lens), as well as neural retina are greatly affected by dose dependent UV radiation exposure.
The histopathological changes increased along with irradiation intensity and UV-B exposure.
The most severe corneal lesions were observed following eye exposure to some large doses of 0.72 J/cm^2 to 1.2 J/cm^2. The injuries caused by UV irradiation to cornea are named photokeratitis also known as ultraviolet keratitis. Photokeratitis is characterized by exfoliation of the corneal epithelium, diminished visual perception, inflammation, edema, eye redness, and burning-like pain from the ocular surface. Moreover, UV irradiation can also go deeper through the corneal epithelial layer and provoke inflammatory responses that involve the full corneal thickness

The radiation that hits the lens is first filtered by the lens capsule and at 300nm (range from 290 to 315 nm) wavelength, approximately 60% of the radiation is transmitted by the anterior capsule. The transmitted radiation induces apoptosis in the lens epithelial cells and thereafter the cortical fibers which contribute to the formation of lens cortical opacities. Because lens epithelial cells are responsible for maintaining much of the homeostasis of the underlying fibers, damage to lens epithelial cells may also result in abnormalities in lens fibers. For proteins, longevity is commonly assumed to be correlated with long-term retention of native structure.

The irradiations ranging from 380-520 nm contribute to degenerate outer nuclear layer area of the retina. Shorter wavelength light also is the most hazardous it is known to generate reactive oxygen species (ROS) in the retina. The retinal pigment epithelium is especially susceptible to oxidative stress because of its high light, oxygen tension, fluorophore and membrane lipid levels.
Acute and chronic exposure of the eyelid to UV radiation causes common types of skin lesions around the eyelids that frequently result in basal cell carcinoma, squamous cell carcinoma, sebaceous cell carcinoma, and malignant melanoma
In conjunctiva UVB at the dose of 0.72 J/cm^2 per day showed conjunctival epithelium metaplasia and loss of goblet cells thereby decreasing of tear quantity.

1. Introduction

1.1. General descriptions

Ultraviolet (UV) light is an electromagnetic radiation with a wavelength shorter than that of visible light, but longer than x-rays, in the range from 400 nm to 100nm (Tanito *et al*., 2006). The main sources of UV radiation are natural sunlight and some artificial sources such as fluorescent lamps in the voltaic arc welders, UV lamps used for sterilization in surgery rooms (Gallagher & Lee, 2006).

According to the international commission on illumination, UV radiation from sunlight is divided into three categories depending on the wavelength: Long wave UVA (315–400 nm), medium wave UVB (280–315 nm), and short wave UVC (100–280 nm) to analyze its capacity to cause damage in cell targets (Clydesdale *et al*., 2001; Hussein *et al*., 2005).

UVA radiation has a wavelength falling between 315–400 nm. It is the most commonly encountered type of UV light because atmospheric ozone absorbs very little of this part of the UV spectrum from the sun. Most phototherapy and tanning booths use UVA lamps (Diffey, 2002).

Ultraviolet B (UVB) radiation has a wavelength between 280–315 nm and it is typically destructive form of UV radiation to human and animal because it has enough energy to cause photochemical damage to cellular DNA, and not completely absorbed by the atmosphere thus individuals working outdoors are at the greatest risk of UVB effects (Godar *et al.,* 2011). UVB radiation is a key environmental mutagen, acting as both an initiator and promoter of skin cancer (Matsumura & Ananthaswamy, 2004), thus its effects on the epidermis are harmful (Tran *et al*., 2008), except for stimulation of vitamin D synthesis (Holick, 2008).

UVC radiation has a wavelength between 100-280 nm. It is almost absorbed completely by the atmosphere and has minimal effects on health. Germicidal lamps are designed to emit UVC radiation because of their ability to kill bacteria (Lucas *et al.*, 2008).

Ultraviolet radiation (UVR) carries the most potential for human harm despite it comprising of only 5% of the sun's energy, while solar UVR that reaches the earth's surface comprises approximately 95% UVA and 5% UVB (Oliva & Taylor, 2005).

The high levels of UVR are influenced by several geographical and environmental factors which include the high position of the sun, close proximity to the equator, increased altitude (increase by 5% with every 1000 meters altitude), depletion of the ozone layer, and by surfaces that reflect the sun's rays, such as water bodies, sea foam and snow (WHO, 2009).

Exposure to UV radiation is increasing as stratospheric ozone depletion and global climate changes influence surface radiation levels (Zepp *et al.*, 2007). As stratospheric ozone depletion and global climate changes influence surface radiation levels, human populations are increasingly exposed to solar radiation. The first and main target-structure for UV radiation in animals is the body surface, including the skin and eyes. An increased risk of ultraviolet radiation on the eye has been associated to the decrease in stratospheric ozone. These effects to the eye may be acute following long term exposure or chronic due to long periods of exposure or repeated exposure to high levels of UV radiation (Kabuyama *et al.*, 2002).

Solar UV radiation usually affects the anterior structures of the eye and those areas adjacent to the eye. The anterior structures of the eye (cornea and lens) absorb more than 99% of UV-B radiation, which has high energy, and can therefore damage the anterior structures easily (De Gruijl *et al.,* 2003).

UV light is absorbed by molecules known as chromophores, which are present in the eye cells and tissues. Chromophores absorb light energy from the various wavelengths at different rates a pattern known as the absorption spectrum. Broadband UV radiation can be detrimental to the eye and vision and cause damage to various ocular tissues (Oriowo *et al.*, 2002). If too much UV light is absorbed, eye structures such as the cornea, the lens, and the retina can be damaged (Di Girolamo, 2005).

1.2. Anatomical and histological features of ocular structures

The anterior segment of the human eye is comprised of the cornea, lens, iris, ciliary body, and highly specialized tissue at the iridocorneal angle. The main functions of the anterior ocular segment are to focus incoming light onto the neural retina and to regulate intraocular pressure. Refraction of light entering the eye is accomplished by both the transparent cornea and lens (Soules *et al*., 2005).

The lens is a transparent, avascular and biconvex structure situated behind the cornea and iris, suspended between the edges of the ciliary body by the zonular fibers, and is supported by the vitreous body. Zonular fibers are suspensory ligaments of the lens, primarily provide support of the lens and they extend from the equatorial rim of the lens to the surrounding muscle of the ciliary body. When the ciliary muscles relax, the pull of the zonular fibers keeps the lens in a flattened condition, losing some of its convexity. This variation of lens convexity allows a clear image of an object to be focused on the retina (Robinson *et al*., 2004).

The crystalline lens refracts light in the vitreous humor and contains crystalline proteins. These proteins are a large family of proteins that constitute 90% of the soluble protein in lens fiber cells (Bloemendal *et al*., 2004). Crystallin is a protein that is essential for maintaining the transparency of the eye's lens by forming a crystalline array. Lens crystallines are expressed at high concentrations in lens cells to achieve the high index for refraction required for normal optical function. Since most proteins would aggregate and strongly scatter light long before accumulating such high concentrations, the crystallines are believed to have a number of special properties that allow the lens to be transparent. Crystalline proteins are arranged in short-range, glass-like order in the cytoplasm and are vital for the development and maintenance of lens transparency (Greiling *et al*., 2009).

The alpha, beta and gamma-crystallins are the major protein components of the crystalline lens. The predominant type is α-crystallin, which accounts for

about 40% of the protein content of the mammalian lens, contributes structurally to the transparency and refractive index of the lens necessary for clear vision (Bloemendal *et al*., 2004).

Alpha-crystallin is found as a heteroaggregate of two proteins in the lens, αA-crystallin and αB-crystallin proteins which are members of a small heat shock protein family, are typically, stress inducible and prevent the aggregation of denaturing proteins. The alpha-crystallins prevent the aggregation of aging and stressed proteins that would otherwise lead to lens opacity, known commonly as cataract (Dahlman *et al*., 2005).

Beta- and gamma-crystallins serve only as structural proteins that are related by sequence and structure of lens (Sharma & Santhoshkumar, 2009). Since a primary function of crystallins is to focus light on the retina by maintaining the necessary refractive characteristics of the lens, modifications of the crystallins can have adverse effects on the clarity of vision. Ultraviolet radiation damages the lens through several mechanisms, leading to formation of protein cross-linking, damage to the membrane transport system, and changes in cellular DNA. These alterations have a major impact on the metabolic pathways in the lens (Risa *et al*., 2005). The crystalline lens readily absorbs UV-A (36% at 320 nm and 48% at 340 nm) and the remaining 2% of UV-B (at 300 nm) not absorbed by the cornea and aqueous humor. The wavelength range between 295 nm and 320 nm has been shown to be efficient in producing UV-induced cataracts in rabbits (WHO, 1994).

According to Cullen, (2002) the cornea is an important mechanical and chemical barrier, which limits the access of exogenous substances into the eye and protects the intraocular tissues. The cornea is avascular and transparent structure with average thickness of 0.5 mm at its center and about 1 mm thick peripherally. It is composed of five layers: the corneal epithelial layer which is the most superficial followed by Bowman's membrane, stroma, Descemet's membrane and corneal endothelium (Young & Heath, 2000).

The corneal stroma which constitutes 85–90% of the total corneal mass, composed of several hundred sheets of highly organized collagen lamellae, stacked on each other preferably in parallel with respect to the corneal surface (Winkler *et al*., 2011).The cornea contains relatively few cells in the stroma, and most of these are so called keratocytes (fibrocytes); there are no lymphoid cells or other apparent protective elements, apart from some dendritic cells and their precursors (Hamrah *et al*., 2003).

The corneal endothelial mono-layer maintains an effective barrier between the stroma and aqueous humor. Bowman's membrane imparts some strength to the cornea, but more significantly it acts as a barrier to the spread of infections. Active ion and fluid transport mechanisms in the endothelium are responsible for maintaining cornea transparency (Cullen, 2002).

The corneal epithelium is responsible for protecting deeper corneal structures in the eye from physical and chemical damage, like ultraviolet exposure (Podskochy, 2004). It also serves to focus light onto the lens and retina (Reid *et al*., 2005). Two-thirds of the total refractive power of the eye is provided by the cornea (Kenchegowda & Bazan, 2010). Because of its exterior location, physiological and morphological changes in the cornea may occur through mechanical, heat, chemical or radiation damage (Bazan, 2005; Kenchegowda & Bazan, 2010). Among ultraviolet radiation, type B (UVB), particularly UVB having 300 to 320 nm wavelengths, has gained interest, since it is mostly absorbed by corneal stroma (Yin *et al*., 2008).

The neural retina is the most complex structure in the eye, processing light signals from the environment into patterns in the visual cortex through the optic nerve (Dillon, 1991). The energy of light is related to the wavelength, and a discriminate point appears to lie at 510 nm. Below this point there appears to be a direct relationship between retinal damage and the wavelength of light, with lower wavelengths causing greater damage (Kuijk, 1991). Only a small proportion of light reaching the retina is required for the

visual signal, and the excess light energy is thought to be absorbed mainly by the retinal pigment epithelium (RPE) (Bok, 1993).

The retinal pigment epithelium is a polarized monolayer of epithelial cells, separates the neural retina and the choroidal blood supply and forms a highly selective barrier fundamentally important for maintaining the health and integrity of the photoreceptors. This epithelium is derived from neural ectoderm and forms a close anatomical relationship with the photoreceptors, like the neuronal – glial relationship observed in the central nervous system. In the eye, light – dark transitions and circadian rhythms modulate the retinal pigment epithelium transport of nutrients, metabolic waste products, ions and fluid between the choroidal blood supply and the subretinal space surrounding the photoreceptor outer segments (Adijanto *et al.*, 2009).

High metabolic activity and ongoing exposure to light makes the retinal pigment epithelium particularly vulnerable to oxidative damage. Abnormalities in RPE phagocytosis of rods and cones or in the maintenance of the visual cycle can lead to retinal degeneration and photoreceptor cell death. Thus, agents that damage the retinal pigment epithelium may have adverse effects on the photoreceptors and ultimately, on vision (Remè *et al.,* 1996).

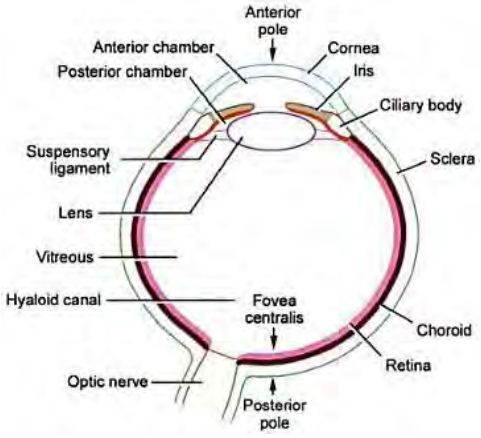


Figure A

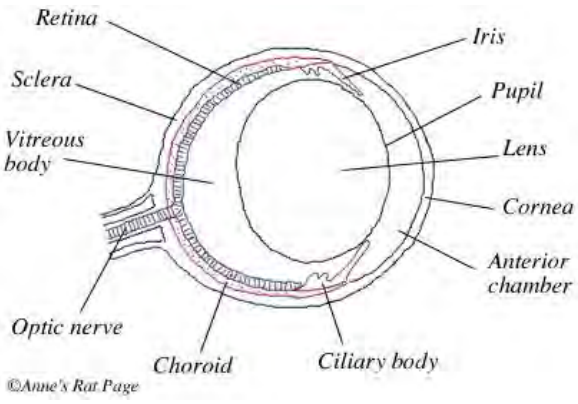


Figure B

Figure 1. Anatomical drawing at different scales of (A) human eye (Adopted from Inderbir, (2010), and (B) rat eye adopted from Vanea *et al.* (2013).

2. Review & analysis of published articles on histopathological effects of ultraviolet radiation exposure on eye

2.1. Histopathological changes of ultraviolet radiation exposure on the cornea

Mureşan *et al.* (2013) used a rat model to assess the histological injuries induced in the intact rat cornea following its exposure to UV-B radiation. A total of 15 Wistar rats weighted 100-120 grams and were fed *ad libitum* and kept under standard conditions with a 12 hours light/dark cycle and acclimatized to the laboratory for one week before the experiments. They were randomly split into five groups (I, II, III, IV & V) and each group contained three rats with group I serving as control (no UVB exposure) and II-V as experimental group. Group II (exposed to 45 mJ UVB/cm^2 for 47 seconds), group III (exposed to 90 mJ UVB/cm^2 for one minute and 57 seconds), group IV (exposed to 180 mJ UVB/cm^2 for three minutes and 57 seconds), and group V (exposed to 360 mJ UVB/cm^2 for five minutes and 26 seconds).

The corneal UV-B irradiation was by using a lamp (UVB Waldmann) with the wavelength range of light 280–360 nm. After a single UVB exposure of rats' cornea, the animals were allowed to recover (for about 24 hours) and then sacrificed to extract eyes, harvested and fixed in 10% buffered formalin for 48 hours. The eye samples were then embedded in paraffin, cut at 5μm thickness, and mounted on glass slides.

The result demonstrated that the control group showing normal histology indicating a relatively uniform corneal thickness (fig.2).

The rat eyes from group IV exhibited serious corneal damages as represented by the irregular hypertrophy of the cornea in all its length (fig.4). Ruptures in the central corneal surface, leading to ulceration, were often observed. Both superficial (i.e., epithelial) and stromal corneal edema were noticed. Several large clefts were noticed throughout the whole corneal stroma suggesting edema.

Significant histological changes were observed in cornea of the rats from the fifth group. Severe irregular thickness in all the corneal length occurred because of some large spaces (i.e., edema induced clefts) separating and displacing the stromal collagenous fibers (fig.5). Similarly to the previous group, the cornea suffered epithelial ulceration in its central region. Critical epithelial changes were detected in the remaining corneal epithelium.

Quantitatively, major differences were found between group V than the other groups. Thickness of the cornea increased considerably (especially in its central region) about three times in contrast to the control group. In this group, the inflammatory infiltrate was diffuse and abundant in comparison to groups III and IV. The inflammation extended not only to epithelial–stromal junction, but also all through the corneal stroma depth.

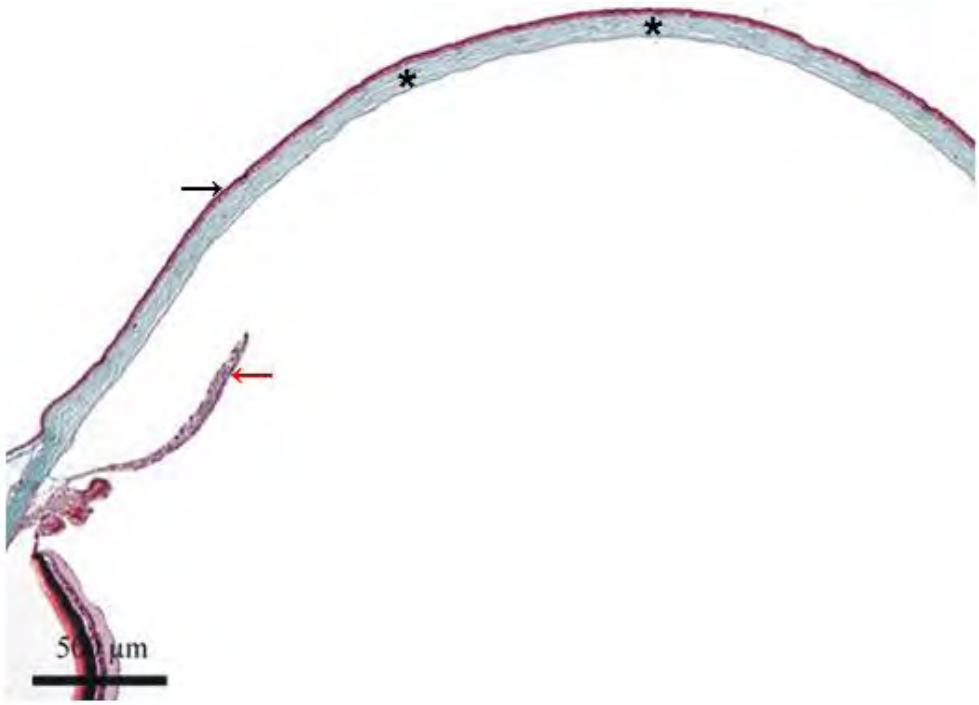


Figure 2. Normal aspect of the rat cornea from the control group; corneal epithelium (arrow), corneal stroma (asterisks) and iris (red arrow). Goldner's trichrome staining. (Adopted from Mureşan *et al.,* 2013).

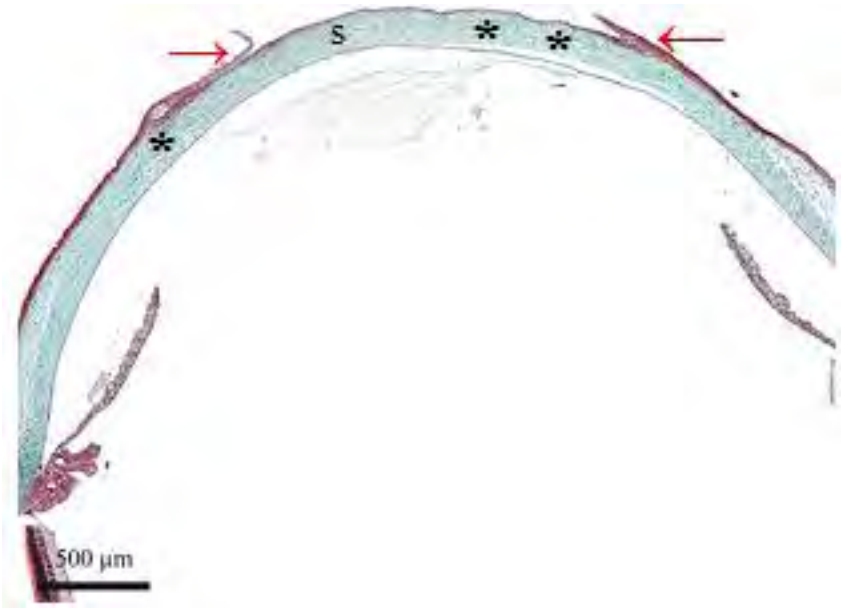


Figure 3. Irregular hypertrophy of the rat corneal stroma (asterisks), central epithelial ulceration (red arrows) from stroma, S (experimental group III). Goldner's trichrome staining. (Adopted from Mureşan *et al.,* 2013).

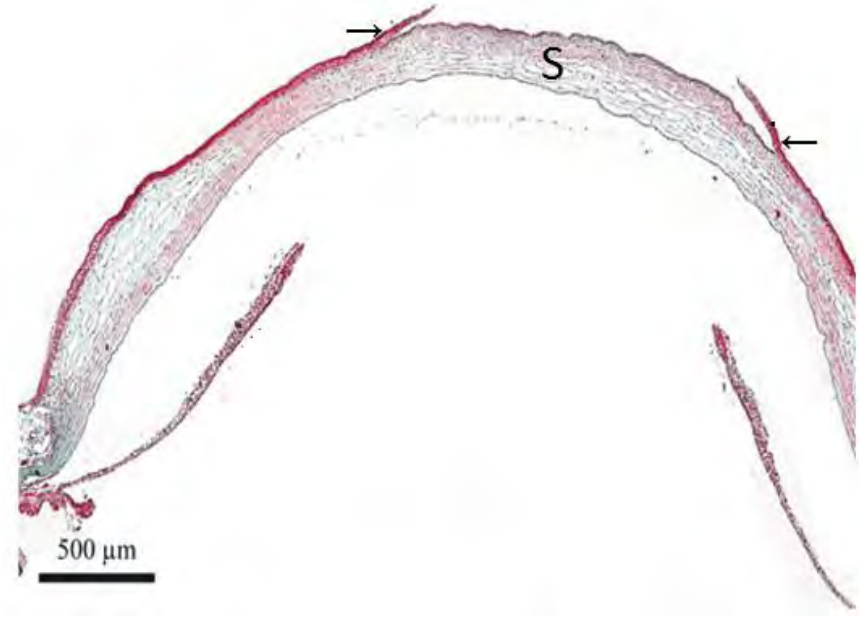


Figure 4. Marked diffuse irregular hypertrophy of the rat cornea, central epithelial ulceration and severe stromal (S) edema represented by large clefts (arrows) displacing the stromal collagenous fibers (group IV). Goldner's trichrome staining. (Adopted from Mureşan *et al.,* 2013).

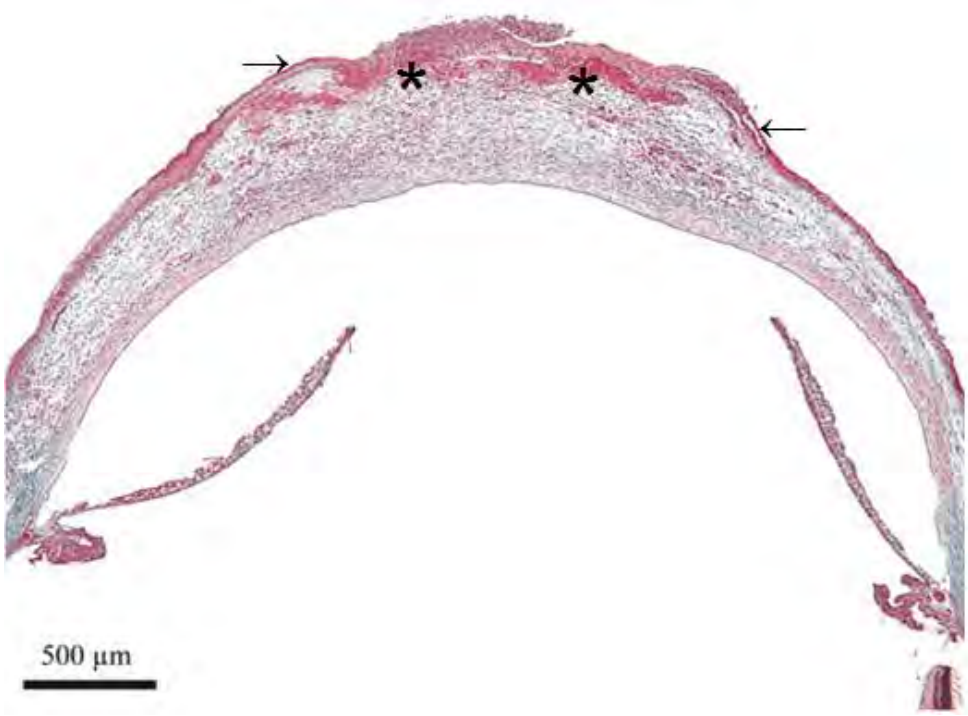


Figure 5. Marked diffuse irregular hypertrophy of the rat cornea, central epithelial necrosis (arrows), diffuse inflammatory infiltrate, hemorrhage and severe edema in the stroma (asterisks) (group V). Goldner's trichrome staining. (Adopted from Mureşan *et al.,* 2013).

Giblin *et al.* (2011) investigated the effects of UVB radiation in exfoliation of the corneal epithelium and the ability of UV-blocking soft contact lens to protect against UV-B induced ocular tissues *in vivo* rabbit.

Rabbits (New Zealand White, 2.0 –2.5 kg) were obtained from Kuiper rabbit ranch (Indianapolis, IN). All studies conformed to the association for research in vision and ophthalmology (ARVO) statement for use of animals in ophthalmic and vision research and were approved by the Oakland university animal care and use committee. The animals were first anesthetized with xylazine and ketamine HCl, followed by injection of an overdose of pentobarbital sodium. Eyes of rabbits were exposed to UVB irradiation. Before irradiation, the animals were tranquilized with an intramuscular

injection of xylazine (20 mg/kg) and ketamine HCl (5 mg/kg), and the eyes were fully dilated with 1% tropicamide. During irradiation, the rabbits were confined in an adjustable retaining cage that protected most of the animal, except the head from the UV light. One eye of each animal was exposed to UV radiation from a bank of UV lamps at a distance of 18 cm. Contralateral control eyes were patched. UV levels were determined with a radiometer equipped with either a UVA sensor at 365 nm or a UVB sensor at 312 nm.

The maximum intensity of light from the UV lamps was at a wavelength of 310 nm. The UVB irradiance on the corneas of the animals was 1.7mW/cm^2 which is approximately 34 times the maximum exposure of the human cornea to UVB contained in sunlight. The eyes were irradiated for 30 minutes to produce a total of 3 J/cm^2.

The eyes were exposed to UV radiation in the presence of either a senofilcon A contact lens (absorbs 99% of incident UVB and 90% of UVA), a lotrafilcon A contact lens (absorbs 30% of incident UVB and 15% of UVA) or no contact lens at all. Contact lenses were applied to the corneas of tranquilized rabbits. A drop of saline was added to the contact lens (to prevent air being trapped between the lens and the cornea) and using the tip of the index finger, the lens was applied to the cornea. The eyes of the animals were examined with a slit lamp photo microscope using blue light illumination.

Loss of corneal epithelia was also evaluated in enucleated eyes 15 hours after UVB irradiation. The eyes were fixed with 4% formalin and embedded in wax. Sections were stained with hematoxylin and eosin, and photographs were taken under light microscopy. Histologic examination of the corneas showed nearly complete exfoliation of the epithelium in the central region of the cornea after UVB irradiation in those with no contact lens (fig. 6B). The senofilcon A lens provided complete protection (fig. 7A), whereas the lotrafilcon A lens offered no protection (fig. 7B).

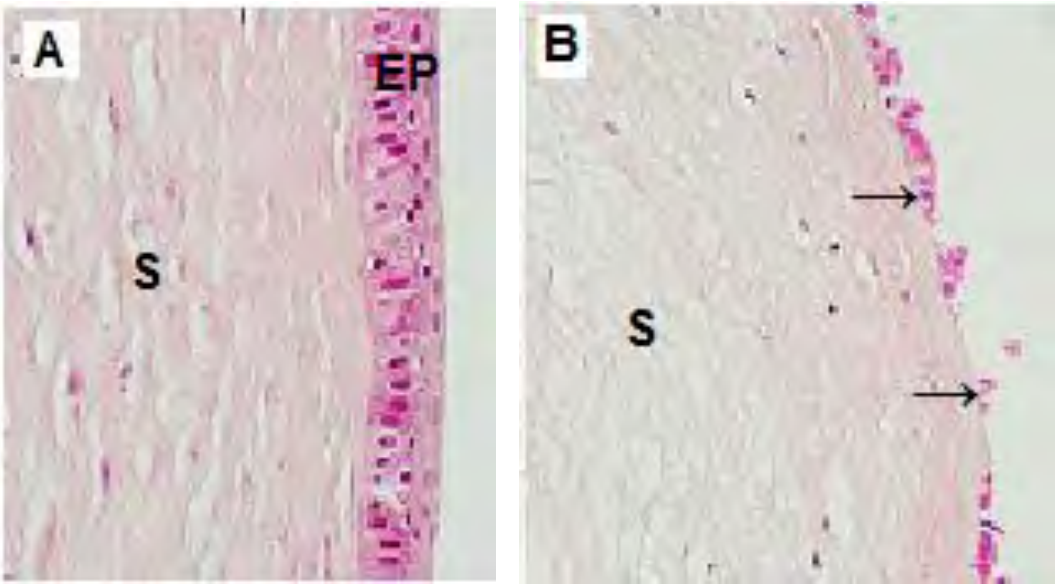


Figure 6. Light micrographs of the central region of the rabbit corneal epithelium without contact lens protection. (A) Showing normal epithelium EP, and stroma S, (B) no contact lens showing exfoliation of the epithelium. Magnification, X 100, (Adopted from Giblin *et al.,* 2011).

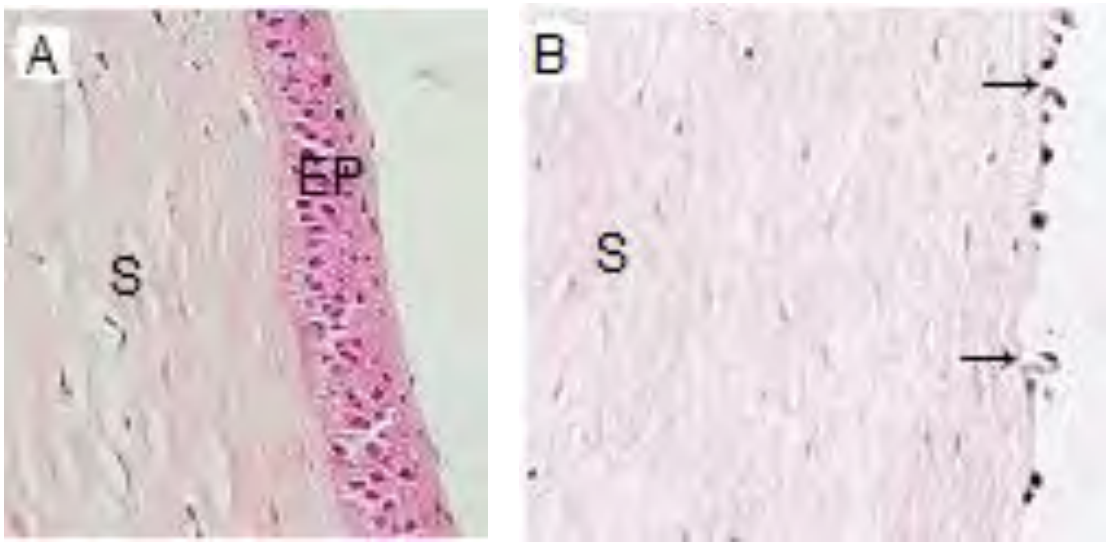


Figure 7. Light micrographs of the central region of the rabbit corneal epithelium with contact lens protection. (A) Senofilcon A lens with normal epithelium (Ep) and stroma(S). (B) Lotrafilcon A lens showing exfoliation of the epithelium. Note: nearly complete exfoliation of the epithelium in both (6B) and (7B); Magnification, X 100, (Adopted from Giblin *et al.,* 2011).

2.2. Histopathological changes of ultraviolet radiation exposure on the lens

Newkirk *et al*. (2007) evaluated the histopathological changes resulted from exposure of UVA and UVB radiation describing the clinical and histologic lesions in the lens of mice after chronically exposed.

Twenty-one mice (14 females, 7 males) were randomly obtained from a breeding colony of 129 mice from Jackson Laboratory (Bar Harbor, USA). Mice were exposed to 3200 J/m^2 of UV radiation three times a week for up to 50 weeks. UV radiation was provided by Kodacel filtered westinghouse FS-40 sunlamps that emitted wavelengths between 280 and 400 nm.

The emitted light contained approximately 60% UVB and 40% UVA; UVC wavelengths were removed by Kodacel filters. A single mouse was sacrificed after 9 weeks of UV radiation exposure. A total of 14 mice were alive at the end of the study (50 weeks). For comparison, eyes from 13 untreated mice maintained in the breeding colony were examined. Six of these control mice were 10–12 weeks old; and 7 of the mice were 13–17 months of age, to match the ages of the chronically exposed mice at the end of the study. All mice were housed under the same conditions. Mice were sacrificed by carbon dioxide asphyxiation. Following euthanasia, the globes were removed and fixed in 10% neutral-buffered formalin for histologic examination. Eyes were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

The results demonstrate that, of the lenses examined microscopically, 92% of UV radiation exposed lenses had evidence of cataract formation.

Cataracts were characterized by minimal to moderate swelling and degeneration of lens fibers at the posterior pole and in some cases, by vacuolation of nucleated lens fibers at the equator (fig. 8B) in response to UV radiation exposure compared to unexposed group (fig. 8A).

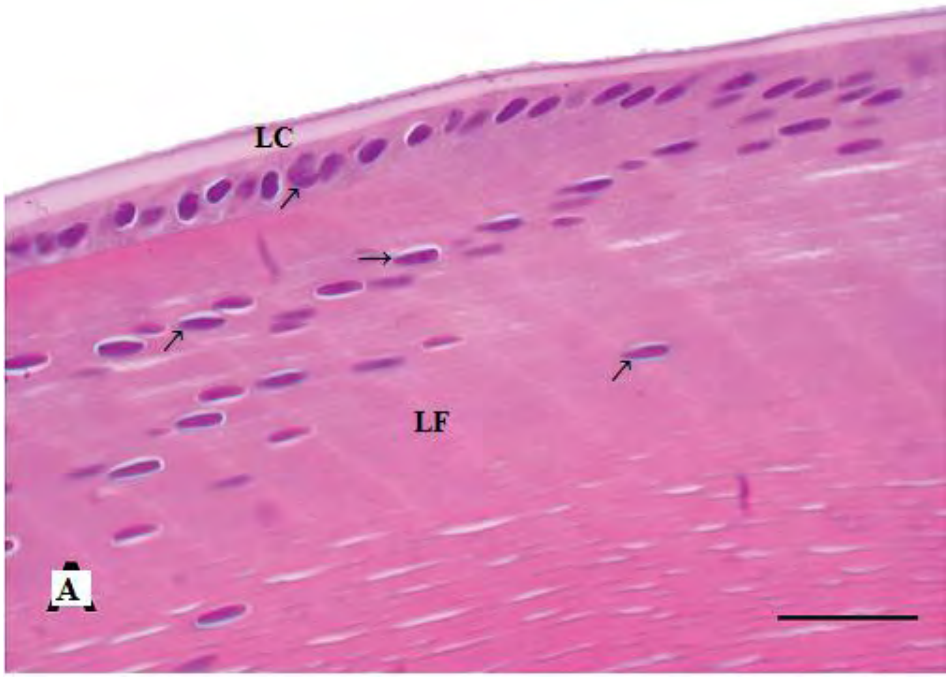


Figure 8A. Histopathology of the lens of unexposed mice showing normal lens equator with cuboidal epithelium (arrows), lens capsule LC, and lens fibers LF, (hematoxylin & eosin stain), (scale bar: 30 μm), (Adopted from Newkirk *et al*., 2007).

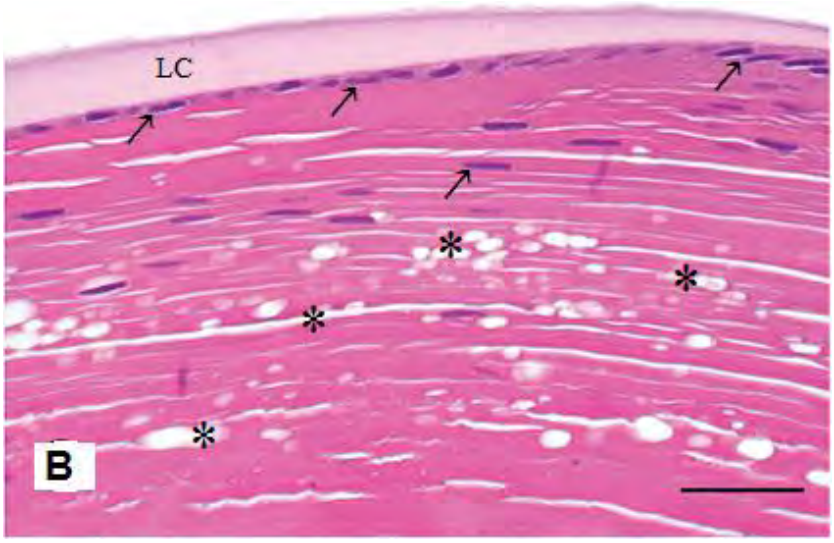


Figure 8B. Histopathology of the lens equator of UVR-exposed mice showing vacuolization of the lens fibers (asterisks) and necrosis of cuboidal epithelium (arrows). (Hematoxylin & eosin stain), (scale bar: 30 μm), (Adopted from Newkirk *et al*., 2007).

Risa *et al*. (2004) also investigated changes in the metabolic concentration of intact rat lenses after UVB irradiation of the eyes. Six weeks old female albino rats (n= 28) with average weight of 150g were anesthetized with 11 mg/kg xylazine and 80 mg/kg ketamine intraperitoneally.

Before irradiation one drop of tropicamide was instilled in both eyes to dilate the pupils. The animals were divided into three groups. In each group, one eye of each animal was exposed to UV radiation doses of 2.5 (n= 9), 5.0 (n= 11), and 7.5 (n= 8) kJ/m^2 for 15 minutes of exposure time. The contralateral eye served as a non-exposed control. The UV-B source was a high pressure mercury lamp equipped with water filter, set to maximum 300 nm. The irradiance was measured with a thermopile calibrated by the Swedish national bureau of standards. All animals were kept and treated according to the ARVO.

One week after exposure the rats were killed by CO_2 asphyxiation, the eyes enucleated, and the lenses dissected from remnants of ciliary body and zonular fibers. The isolated lenses were then put in room-tempered physiologic saline solution (balanced salt solution, BSS) and photographed, and the lens forward light-scattering (resulted from disturbance or fluctuations any content of lens) was measured with a light-dissemination meter. The scattering standard was a lipid emulsion of diazepam and the unit was therefore expressed as transformed equivalent diazepam concentration (tEDC). Finally, the samples were frozen and stored at 80°C before nuclear magnetic resonance spectroscopy was used to investigate changes in the metabolic profile of intact rat lenses after UV-B irradiation of the eyes. Analysis of the spectra was performed with special software for analysis of complex mixtures.

The result showed all three groups of exposed lenses showed a significant increase in light scattering compared with nonexposed lenses at one week after UVB exposure of 2.5, 5.0, or $7.5kJ/m^2$. The mean forward light scattering increased with increasing UV radiation dose.

The dose response relationship between UVB irradiation and subsequent lens opacities was quantified in this study (fig.9). In addition to the amino acids, water soluble organic metabolites were analyzed and showed significant decrease ($P < 0.05$ reduction) in concentration compared with unexposed lenses after one week UVB irradiation.

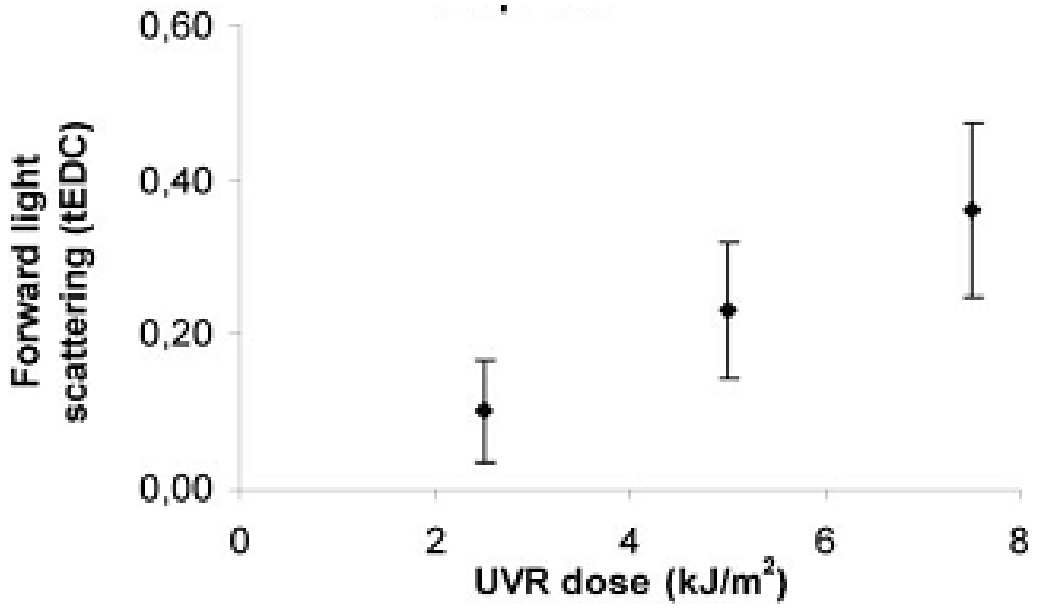


Figure 9. Difference in intensity of forward light scattering between exposed and nonexposed rat lenses one week after a UV dose of (2.5, 5.0 & 7.5) kJ/m^2. All three groups of exposed lenses showed a significant increase in light-scattering compared with nonexposed lenses. tEDC represents the transformed equivalent diazepam concentration, (Adopted from Risa *et al.*, 2004).

Ayala *et al*. (2000) conducted a study on the influence of UV radiation exposure time for induced cataract, on the experimental rat having average age of 6 weeks. Two experiments were conducted with the same experimental procedure with different exposure times. The first experiment, 100 rats were divided into five groups according to exposure time (7.5, 15, 30, 60, and 120 minutes) and in the second experiment, 80 rats divided in four groups according to exposure time (5, 7.5, 11, and 15 minutes). All the animals in both experiments received the same dose (8 kJ/m^2 UVR in the 300nm) to unilateral eye.

The radiation from a high pressure mercury lamp was passed through a water filter and finally projected on the cornea of the exposed eye. One eye was exposed *in vivo* to UV radiation. Intensity of forward light scattering in the lens was measured after *in vitro* isolation of the lens.

The spectral irradiance in the corneal plane was recorded with a spectrometer and the total UV radiation dose at the corneal plane was checked with a calibrated standard traceable United States National Bureau of Standards. The intensity of light was measured before and after each UV radiation exposure. The intensity of forward light scattering was measured with a light dissemination meter.

The animal was anesthetized with 94mg/kg ketamine plus 14mg/kg xylazine intraperitoneally ten minutes before the exposure. Tropicamide was instilled in both eyes, and after 5 minutes, one eye was exposed to UVR. The corneas were moistened every 15 minutes with Ringer solution. The animals were killed one week after the exposure with an overdose of CO_2 followed by cervical dislocation.

The eyes were enucleated, and both lenses were extracted and placed in balanced salt solution (BSS). Vestiges of the ciliary body were removed from the lens under a microscope. The animals were kept and treated according to the ARVO.

The light scattering data were first analyzed with the Kolmogorov–Smirnov test for normality. Analysis of variance (ANOVA) was then used to test for significant differences between groups and between the exposed and nonexposed specimens. Thereafter, a multiple comparisons test was performed to reveal differences among the groups. Statistical procedures were the same for both experiments, but each experiment was analyzed separately.

The result demonstrates morphologic changes in the exposed lenses were evaluated with a stereomicroscope and identified different patterns of cataract.

Superficial cataract (epithelial cataract) was a common finding with incidence of more than 60% in all groups. Lenses exposed for 5 and 7.5 minutes showed the highest number (n =18) of superficial cataracts (fig. 10A).

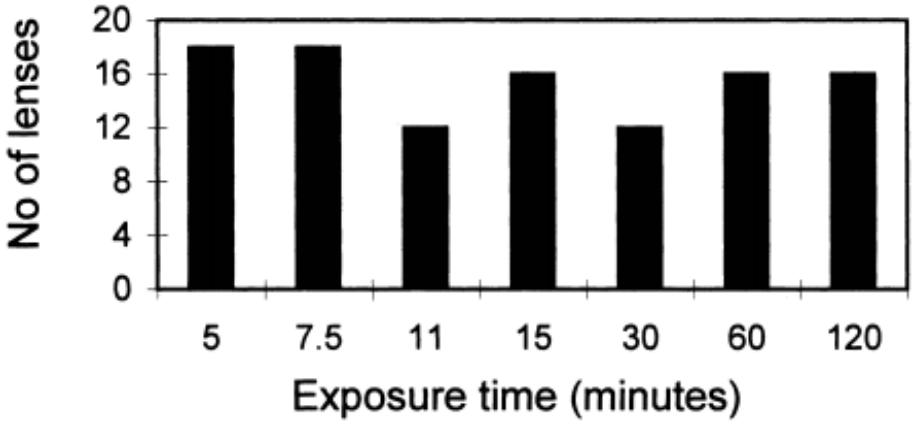


Figure 10A. The incidence of superficial cataract in the different exposure time groups (n = 20). (Adopted from Ayala *et al*., 2000).

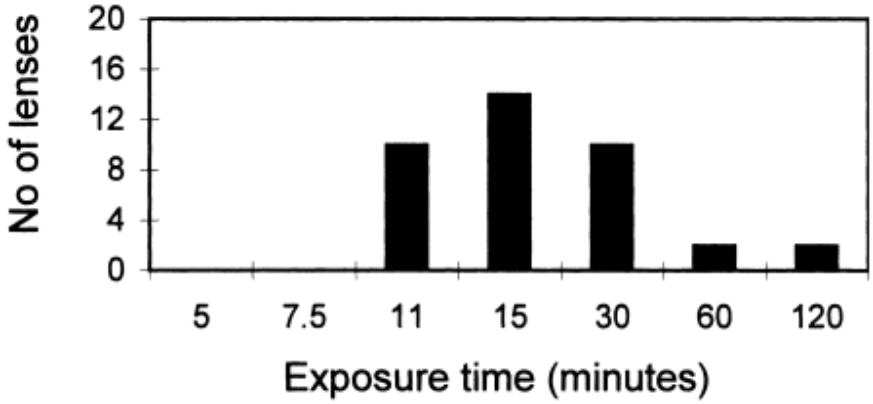


Figure 10B. The incidence of equatorial cataract in the different exposure time groups (n = 20). (Adopted from Ayala *et al*., 2000).

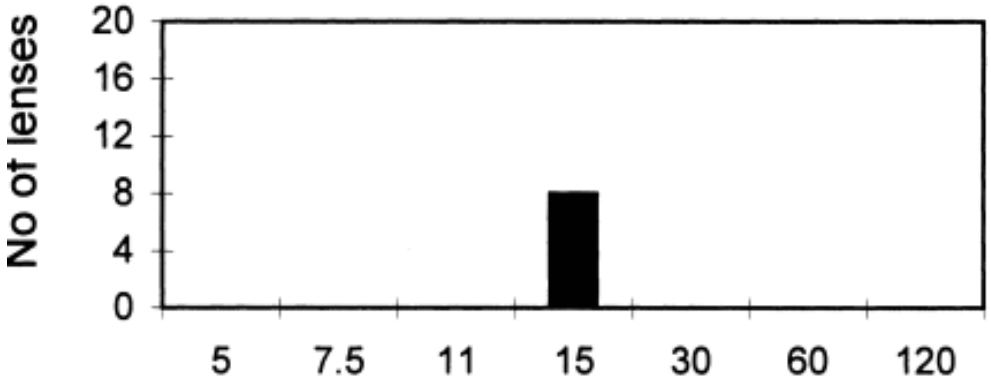


Figure 10C. The incidence of cortical cataract in the different exposure time groups (n = 20). (Adopted from Ayala *et al*., 2000).

Most of the equatorial cataracts were found in the lenses exposed for 15 minutes (n = 14), whereas no equatorial cataract was detected in the lenses exposed for 5 and 7.5 minutes (fig. 10B). Cortical cataract was found only in lenses exposed for 15 minutes (fig. 10C). Cortical, equatorial opacities (a special geographical cortical cataract) and vacuoles could be seen in lenses exposed for 15 minutes.

2.3. Histopathological changes of ultraviolet radiation and blue light exposure on the retina

Ibrahim *et al*. (2012) conducted a research on the lipid and DNA oxidative stress in retinal effects after ultraviolet B exposure in mice using with or without UV-absorbing silicone hydrogel soft contact lenses (SCL). The study was performed on twenty eight C57BL6 strain mice dividing into four groups (seven mice in each group) as follows: Group I control group with (no soft contact lenses and no UVB exposure), group II (with senofilcon A soft contact lenses), group III (with lotrafilcon A soft contact lenses) and group IV (with no soft contact lenses).

All mice except group I received UVB (312 nm) for 5 days (a total dose of 2.73 J/cm^2). All mice were anesthetized during UVB exposure with intraperitoneal ketamine (6 mg/ml) and xyladine (4 mg/ml) anesthesia.

After the mice were anesthetized, the soft contact lenses trephined centrally with a 3mm dermal biopsy punch were held in place with a non-toothed forceps. The eyelids were opened with an additional non toothed forceps, and the soft contact lenses were placed and centered on the corneas. The centration and fit of the soft contact lenses were then confirmed before the animals were exposed to UVB. The mice were positioned on a paper towel in the same manner and the same location directly under the UV light source.

The soft contact lenses were worn only during UVB exposure, which was 10 minutes per day.

After placing a new lens on the opposite eye, the mice were turned on the opposite side and received identical 10 minutes UVB exposure. Each day assessment tests were performed with a hand-held slit lamp microscope. On day 6, all mice were euthanized. Whole eye globes were retrieved for histopathological analyses. All examinations and procedures were performed in accordance with the ARVO.

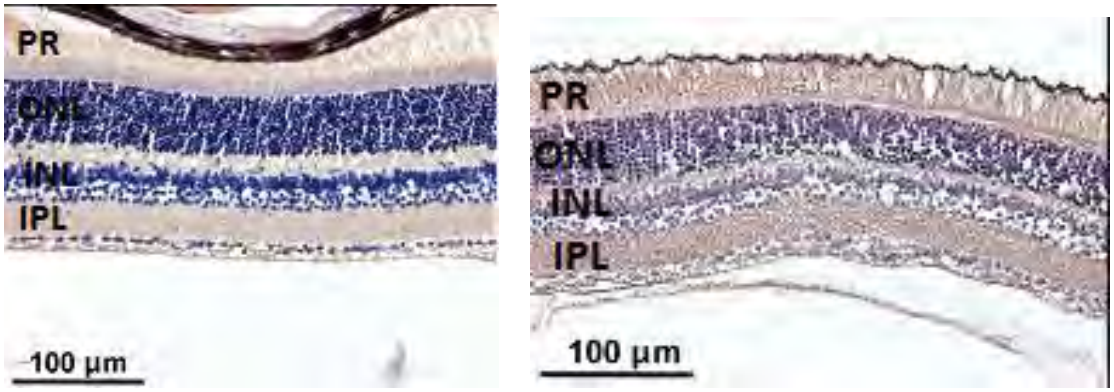
Oxidative stress induced lipid peroxidation was assessed by immunohistochemistry detection of 4-hydroxynonenal (4- HNE) staining. To block nonspecific background staining, sections were treated with normal horse serum for 2 hours at 25°C. The tissues were then treated with mouse anti-4-HNE monoclonal antibodies (25 μg/mL) for two hours at room temperature.

Using image processing software (Adobe Photoshop, San Jose, CA) a subset of color that indicated the stained areas (brown color) was selected from the raw pictures and another image analysis program was used to measure the intensity of staining for each image, and the area of staining was calculated and expressed in square micrometers.

The result demonstrated that more intense staining with anti-4HNE antibodies in the inner and outer photo segments, inner and outer plexiform, and ganglion cell layers in specimens of group III mice (with lotrafilcon A exposed to UVB) and more extensive staining consistently in all retinal specimens of group IV mice (without soft contact lens exposed to UVB) compared with control group (mice without soft contact lens and not exposed to UVB) as well as group II mice (with senofilcon A soft contact lens exposed to UVB) as showen in fig. 11 & 12.

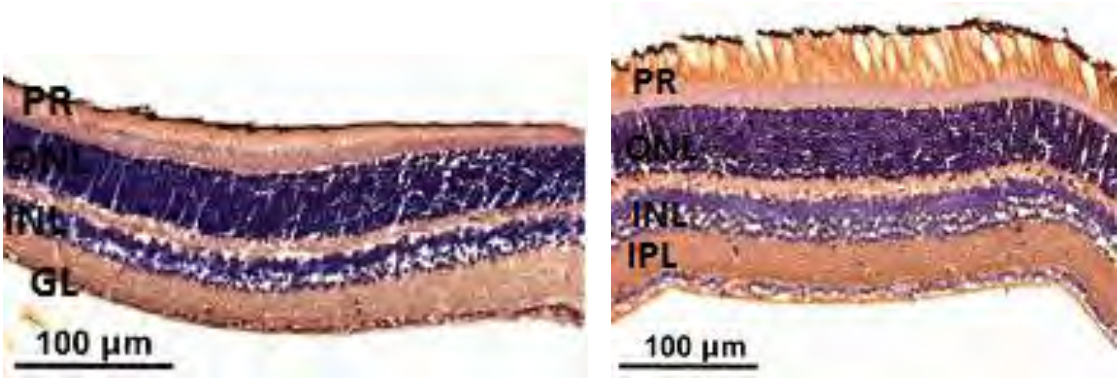
The retinal samples in mice exposed to group III & IV appeared to develop comparatively higher lipid and DNA oxidative stress damage than group I & II. The number of retinal cells positively stained was significantly higher in groups III and IV compared with groups I and II ($P < 0.05$). These results suggest that UVB exposure is associated with increased lipid and DNA oxidative damage in mice retinal tissues with possible protection from such damage with use of silicone hydrogel soft contact lens wear.

The experiments in this study support that wearing senofilcon A UV-blocking contact lenses could provide some lipid and DNA oxidative stress protection in the retinas of the UVB exposed mice compared with lotrafilcon A soft contact lens and non-lens wearing (exposed) eyes.



Group I sample Group II sample

Figure 11. Retinal immunohistochemistry staining with anti–4-HNE antibodies showing slight brownish staining in photoreceptor cells (PR), inner plexiform layer (IPL) control (groups I) & II specimens respectively. Group II (senofilcon A soft contact lens with UVB exposure), ONL (outer nuclear layer), INL (inner nuclear layer), (Adopted from Ibrahim *et al.*, 2012).



Group III sample Group IV sample

Figure 12. Retinal immunohistochemistry staining with anti–4-HNE antibodies showing intense brown staining in the photoreceptor cells (PR) segments, inner and outer plexiform (IPL,OPL), and ganglion cell layers (GL) of group-III & IV mice. Group IV (no soft contact lens wear with UVB exposure) shows more extensive staining, group III (mice with lotrafilcon A exposed to UVB). (Adopted from Ibrahim *et al.*, 2012).

Peng *et al*. (2012) demonstrated the irradiation of light emitting diodes (LED) lamps with wavelengths of (300-800) nm were related to photoreceptor loss of retinal tissues in mice.

The study was performed on 40 common black mice of six weeks old (from National laboratory animal center, Taipei, Taiwan). The mice were randomly divided into four groups (each contained 10 mice), including control (no LED exposure), LED white family irradiation for a consecutive 2 week period (2hr/day), 4 week period (2hr/day), and illuminated light of 12hr light/ 12hr dark cycle with white family LED lamps. All experiments were reviewed and approved by the Animal Care and Use Committee in Chung Shan Medical University. Spectrum distribution of LED family lamps was analyzed using an UV-visible spectrophotometer from 300 nm to 800 nm peak at 450 nm which lie within the blue light region.

The entire eyes from light exposed and unexposed (control) mice were perfused with a fixative containing 4% paraformaldehyde in phosphate buffered saline (PBS, pH7.4). Tissues were dehydrated through a graded series of ethanol, and then embedded in paraffin. The retinal sections (70 μm-thick) obtained after light exposure and unexposed controls were collected and stained with hematoxylin. Subsequently, sections were mounted and examined under a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

The retinal sections obtained after light exposure and unexposed controls were stained with hematoxylin. By using image pro plus image analysis system (media cybernetics, silver spring, USA), the outer nuclear layer thickness and area were measured at (0.5, 1, 1.5, 2, 2.5 and 3.0) mm superior and inferior to the optic nerve disc.

The result showed, in control retinas the outer nuclear layer shows 12 to 13 rows of photoreceptor nuclei. However, after 2 to 4 weeks period of light exposure, the thickness of outer nuclear layer was become thinner over time. At 39 weeks after light exposure, photoreceptor cell loss is evident,

with a reduction in the outer nuclear layer thickness in some region to 4 to 5 rows of photoreceptor nuclei. After four weeks exposure the rows of photoreceptor nuclei were significantly decreased as compared with control group (fig.13).

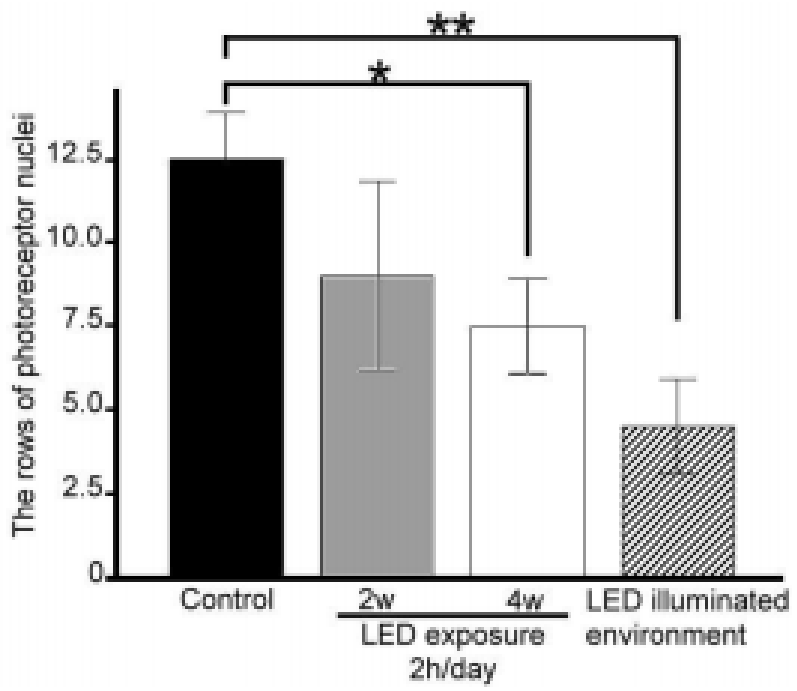


Figure. 13. Quantitative analysis the rows of photoreceptor nuclei in outer nuclear layer after low-powered LED exposure. Data are expressed as means (±S.D.); ** indicate a value statistically different ($p < 0.01$) from the control, and * indicate a value statistically different ($p < 0.05$) from the control. (Adopted from Peng *et al*., 2012).

2.4. Histopathological changes of ultraviolet radiation exposure on the conjunctiva and eyelid basal cell carcinoma

Chen *et al*. (2013) investigated the effects of ultraviolet B (UVB) on the conjunctival epithelium and reversal role of dietary alpha lipoic acid against UVB induced conjunctiva in mice models.

Six weeks old mice with average body weight of 26g were purchased from National laboratory animal center, Taipei, Taiwan. The mice were examined with a slit lamp prior to experiments. The mice were randomly split into five groups: Group I control (no UVB exposure, no alpha lipoic acid treatment), group II (exposure to UVB, no alpha lipoic acid treatment), group III, IV and V (exposure to UVB with daily alpha lipoic acid treatment at (1, 10, and 100) mg/kg body weight). To expose the conjunctiva to UVB irradiation, the mice were anesthetized with intraperitoneal sodium pentobarbital injection (45 mg/kg body weight), with both of their eyes exposed to daily UVB light for 10 minutes.

Each day UVB exposure was performed to reach a total amount of 0.72 J/cm^2 with the entire UVB (peak wavelength of 312nm) exposure course completed in a consecutive 10-day period. For the groups with alpha lipoic acid treatments, alpha lipoic acid was supplemented in mouse chow. All mice were killed on day 11 for analysis. The extracted eyes, including the conjunctivas, were processed for hematoxylin–eosin (HE) stain following conventional procedures. All experiment protocols were reviewed and approved by the animal care and use committee of Chung Shan medical university and were performed in accordance with the ARVO statement.

The result showed that in the UVB-exposed group, the conjunctival surface showed more epithelial layers with a marked thickening on the outermost area in some parts of the surface as in (fig.14B) , indicating the occurrence of conjunctival strafied columnar epithelium metaplasia as compared with the control group(fig.14A) .

The number of goblet cells in the conjunctiva was significantly lower in the UVB exposed group than in the control group (fig.15). Dietary alpha lipoic acid at 1 mg/kg body weight did not alleviate the conjunctival goblet cells induced by UVB, but at with given of 100 mg/kg body weight dietary alpha lipoic acid, the number of goblet cells were similar to the normal status (fig. 15).

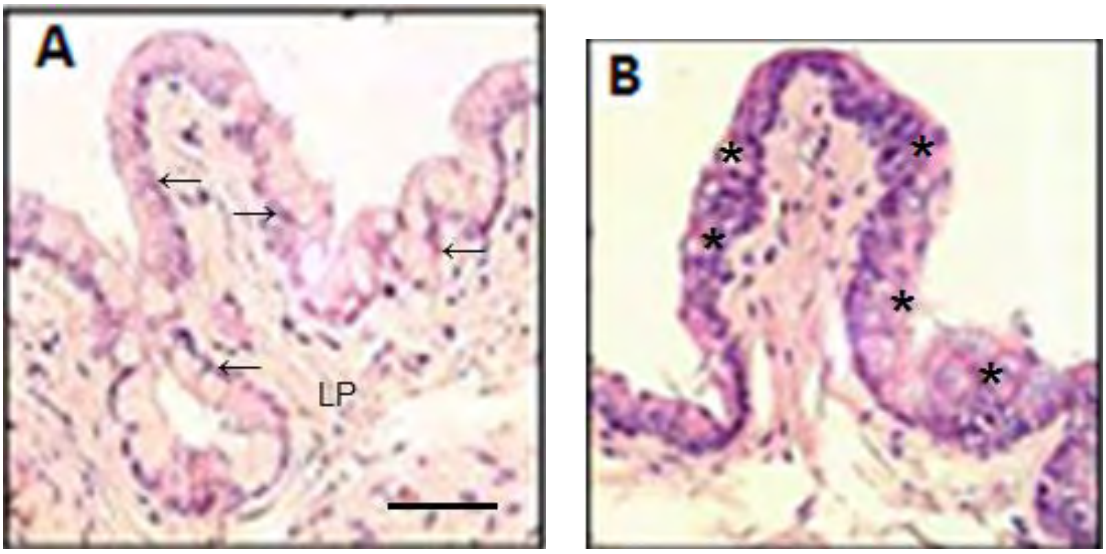


Figure 14. The effects of UVB on conjunctival epithelium. (A) control group showing normal histology of conjunctival stratified columnar epithelium with goblet cells (arrows) and rests on a lamina propria (LP). (B) UVB-exposed group showing typical metaplasia of epithelial layers (asterisks). Scale bar: 20μm, (Adopted from Chen *et al*., 2013).

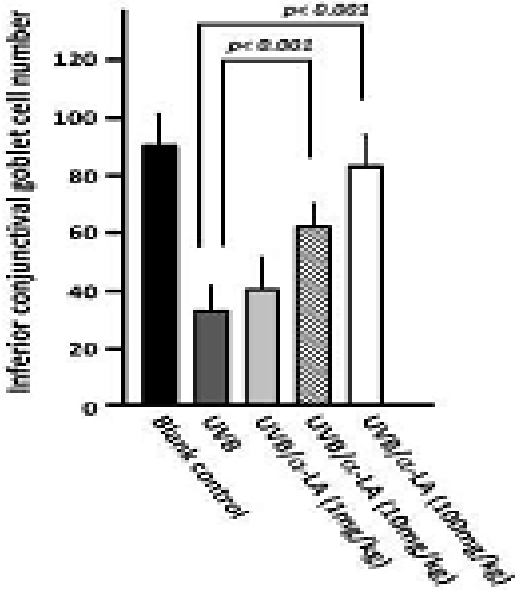


Figure 15. Quantitative analysis showing the number of inferior conjunctiva goblet cells was significantly maintained in the groups given UVB/α-LA (10 mg/kg) and UVB/α-LA (100 mg/kg) ($P < 0.001$), as compared with the UVB exposed group. α-LA (dietary alpha lipoic acid), (Adopted from Chen *et al*., 2013).

Lindgren *et al*. (1998) compared the distribution of eyelid basal cell carcinoma (BCC) with the relative ultraviolet radiation exposure (UVR) to different sites on the eyelids.

The locations of the BCCs were studied in an outpatient clinic for evaluation and treatment of eyelid tumours in the Sahlgrenska university hospital, Gothenborg, Sweden. The exposure was recorded with a polymer film attached to the eyelids at seven sites in a manikin and in human subjects. The histologically verified location of BCC on the eyelids was allocated to one of seven regions (upper medial, upper middle, upper lateral, lower medial, lower middle, lower lateral & medial canthal area).

In all, 329 tumours in 319 patients were investigated from June 1981 to October 1997. The age (median 73, range 22–98) at treatment and sex distribution (140 men, 189 women). The measurements of UVR exposure to the eyelids were performed from May to August 1997. The dosimeters were constructed using a skin adhesive tape on each eyelid.

In 18 of the exposure measurements the heads of manikins used with the dosimeters attached to the eyelids and the apertures close to the eyelid margins. In 12 of the 18 measurements the manikins were sitting upright and six the manikins were in recumbent positions. Each manikin was kept in the same position during the exposure time of 6–8 hours.

In eight exposures the dosimeters were attached to the skin of the eyelids. Seven exposures were made on person during different conditions (two while sunbathing and walking at the seaside, two while sunbathing and walking in the garden, two while gardening in the sun, and one while sitting in shade). The rest one was exposed in a recumbent position sunbathing in the garden. The subjects wore the dosimeters for 3–4 hours in the middle of the day. The optical absorbance at 330 nm through each aperture was measured.

The result indicated the tumours were mainly on the lower eyelids (68%; 225 tumours) and the medial canthal area (26%; 87 tumours). There was no significant difference between the right and the left eye ($p=0.44$).

The results from the manikins showed the upper eyelids of the manikins received slightly more UVR than the lower in both sitting and recumbent position. The lateral areas of both the upper and lower eyelids showed low

values as well as the medial canthal area. In the human subjects the medial part of both the upper and lower eyelids and the medial canthal areas received the lowest UVR.

The calculated UVR doses of the eyelids and the location of the 329 BCCs located on the eyelids the different areas were regarded to be approximately of the same size. Figure 17 shows the tumour distribution and the UVR exposure at the seven sites expressed as a proportion of the total weighted from all the measurements. BCCs were much more common on the lower eyelid while the UVR exposure did not show preponderance for the lower eyelid. Further, the medial parts of the eyelids received relatively less UV exposure although the incidence of BCCs at this site is high and the lateral part of the eyelids received the highest doses of UVR in the humans.

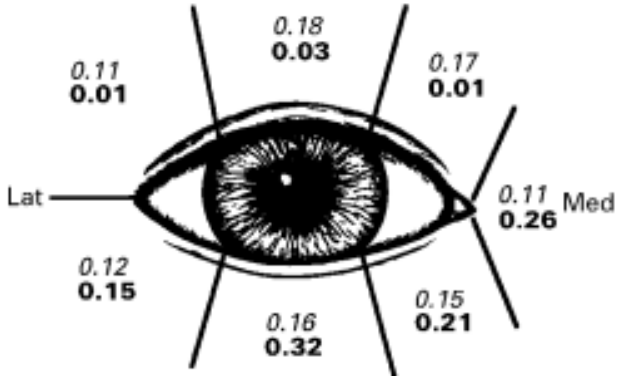


Figure 16. Fraction of the total number of the basal cell carcinomas (bold) and fraction of the total UVR (italics) at the seven different sites of the eyelids (assuming that the areas are approximately of the same size). Right and left eye combined. (Lindgren *et al*., 1998).

3. Discussion

3.1. Histopathological effect of ultraviolet radiation on crystalline lens and related ocular disorders

Lens epithelial cells are a likely target for UVB damage because they are the first cells in the lens to be exposed to UV radiation. Risa *et al*. (2004) studied dose response relationship between UVB irradiation and subsequent lens opacities by inducing apoptosis in the lens epithelium which increased with increasing UV radiation dose from (2.5 to 7.5) kJ/m^2. On the other hand, oxidative damage to the lens epithelium (Choudhary *et al*., 2005) and fibro-protein (Staniszewska & Nagaraj, 2005) play significant roles in cataract formation. Cataract (clouding of the crystalline lens of the eye), is the sunlight related eye disease with the most serious public health implications (De Gruijl *et al*., 2003) and is the leading cause of avoidable blindness worldwide (Lui *et al*., 2009).

Epidemiological studies have verified certain types of cataract result from exposure to UV radiation (Katoh *et al*., 2001). Zhang *et al*. (2012) demonstrated mice subjected to *in vivo* UV irradiation (15 minutes at 20.6 kJ/m^2) can form subcapsular cataract within 2 days. There are three primary types of age related cataracts: nuclear sclerosis, cortical, and posterior subcapsular. As a person ages, any one type or a combination of the three types are likely to develop. Ultraviolet radiation and increased exposure to sunlight has been shown in many studies to be one of the major risk factors for cortical cataract (Taylor *et al.,* 2005).

Katoh *et al*. (2001) conducted a study on 1045 people who were older than 50 years in Iceland showed a significant increase in the risks for two different grades of cortical opacification (the process of becoming cloudy or opaque) in people who spend more than 4 hours per day in the sun on weekdays. Schein *et al*. (1994) also showed a positive relationship between UVB exposure and cortical cataract. UV radiation may have been a causative factor

as watermen in the highest quartile had a three-fold increased risk for cortical cataract (Taylor *et al.,* 2005).

West *et al*. (2005) conducted research in Maryland re-affirmed the relationship between cortical cataract and sun exposure indicating 10% risk. Additional reported risks from sun UV exposure in populations living at latitudes with differences in ambient UV exposure can be found in an Australian study where good correlations have been established between cataracts and ambient UV exposure in populations in Australia (West *et al*., 1998; Schein *et al*., 1994). This study also showed that cortical cataract is relatively more prevalent than other types of cataract in populations living in temperate climates and the incidence increases toward lower latitudes.

The risk of presbyopia may also be increased with greater exposure to UV radiation and higher climate temperatures. Populations that live in close proximity to the equator, in areas that experience hot climate conditions and are exposed to high levels of sun are at a greater risk of developing presbyopia in their earlier years (Stevens & Bergmanson, 1989).

The radiation that hits the lens is first filtered by the lens capsule. Söderberg *et al*. (1996) have shown that at 300 nm, approximately 60% of the radiation is transmitted by the anterior capsule. The transmitted radiation impacts and damages the lens epithelial cells and thereafter the cortical fibers. Because lens epithelial cells are responsible for maintaining much of the homeostasis of the underlying fibers, damage to lens epithelial cells may also result in abnormalities in lens fibers.

Pajer *et al*. (2013) showed UV absorbance differences at various anteroposterior sections of the human lens and that of the anterior and posterior lens capsules in the range of 240–400 nm and it was observed that the posterior layers of the lens reveal higher absorption coefficients than the anterior layers at wavelengths shorter than 310 nm. In addition, it was observed that the posterior lens capsules show higher absorption in the UVB and UVC range than the anterior capsules. Another study by Gaillard *et al*.

(2000) showed the posterior layers of the lens show higher UV absorbance than the anterior ones in the 300 –440 nm range.

Studying protein expression differences between the anterior and posterior lenticular pools revealed that levels of human Beta-crystallin B2 and that of glyceraldehyde 3-phosphate dehydrogenase were significantly higher in the anterior cortex of the lens whereas levels of Alpha-crystallin A chain and Beta-crystallin B1 were higher in the posterior segments. The individual crystallins may play distinct roles under UV irradiation: Alpha-crystallin, a major constituent of the lens inhibits UV-light induced aggregation of other lens proteins (Lee *et al*., 1997) and is thought to be a major target for UV irradiation due to its higher degree of unfolding (Weinreb *et al*., 2000).

Aquilina & Truscott, (2002) identified sites of attachment of UV filters could be linked to beta B1-crystallin. Gamma-crystallin D is an essential lens protein and exposure to UVC is thought to perturb protein structure and probably leading to aggregation (Wang & Wen, 2010) and a gamma-crystallin fold may have been evolved to protect tryptophan from UV radiation damage (Chen *et al.*, 2009).

These crystallins may present the molecular basis for differences in the anterior and posterior UV absorbance because the higher degree of unfolding of Alpha-crystalline A and Beta-crystalline B1 chains may render the posterior part of the lens a more UV absorbing structure. The manifold increase of glyceraldehyde-3-phosphate dehydrogenase in the anterior section pool may be due to differences in carbohydrate metabolism within the lens. Interestingly, this enzyme was described as a target of UV-B (Schmidt *et al*., 1992). Metabolic differences were also detected among the compartments in the lens (anterior, nuclear, posterior and equatorial), from lenses exposed to *in vivo* UVB (Tessem *et al*., 2006).

The role of different wavelengths of near-UV radiation on the etiology of cortical cataracts varies widely among species (Dillon, 1999). Mouse lenses mainly absorb UVB, whereas guinea pig and rabbit lenses also contain

chromophores that absorb UVA. It is interesting that the age of an animal appears to play a very important role in the susceptibility of UV-induced cataract. Aged lens is very vulnerable to UV exposure, resulting in extensive metabolic and structural damage. In contrast, young lenses were resistant to UV stress, and capable of repairing damage. Since young animals likely have a high metabolic activity for synthesis and repair.

3.2. Histopathological effects of ultraviolet radiation on cornea and related ocular disorders

The injuries caused by UV irradiation in human's cornea are named photokeratitis (also known as ultraviolet keratitis). It is characterized by exfoliation of the corneal epithelium, diminished visual perception, inflammation, edema, eye redness, and burning-like pain from the ocular surface (Tenkate, 1999; Lassen *et al*., 2008).

Giblin *et al*. (2011) reported UVB of 310 nm cause nearly complete exfoliation of rabbit corneal epithelium after exposure of 3 J/cm^2 dose for 30 minutes. Golu *et al*. (2013) also observed epithelial lesions and erosion of the Bowman's membrane by UV dose of 6.5 J/cm^2 in the wavelength range of 290–400 nm. On the other hand corneal exfoliation alone is sufficient to cause loss of keratocytes in the anterior stroma (Ivarsen *et al*., 2004). Moreover, exposing the de-epithelialized rabbit cornea to UVR results in loss of keratocytes in the whole corneal thickness (Podskochy, 2004).

Some other histological and ultrastructural details have been reported by Mahmoud *et al*. (2010) rats' cornea exposed to a single UVB irradiation at a dose of 1.2 J/cm^2 revealed epithelial thinning with cellular degeneration, exfoliation and Bowman's membrane was also discontinuous or absent. Similar findings have been noticed by Chen *et al*. (2011) in rats' cornea exposed to UVB light 0.72 J/cm^2 daily were considerably thinner corneal epithelial layers with UVB exposure than those of the control group. Absence of Bowman's membrane could be referred to loss of keratocytes, as stromal

keratocytes are responsible for synthesis and secretion of collagen fibers of Bowman's membrane.

Moreover, the damages induced by photokeratitis might not be limited only to the corneal epithelium because UV irradiation can go deeper through the epithelial layer and provoke inflammatory responses that involve the full corneal thickness (Newkirk *et al*., 2007). In Mureşan *et al*. (2013) experiment on rat, there were significant inflammatory responses (infiltration of polymorphonuclear leukocytes), hemorrhage following the increase of UVB radiation intensity from 45 mJ UVB/cm^2 to 360 mJ UVB/cm^2 and increased exposure period from 47seconds to five minutes and 26 seconds. Giblin *et al*. (2011) also observed the formation of interstitial edema with widely separated collagen fibers and few keratocytes within corneal stroma. In deed normal cornea contains no blood vessels or pigments. During an inflammatory response involving the cornea, large numbers of neutrophilic leukocytes and lymphocytes migrate from blood vessels of the corneoscleral limbus and penetrate the stromal lamellae.

Besides, the corneas with UVB exposure frequently contained invasive polymorphonuclear leukocytes in the stroma. Some polymorphonuclear leukocytes appeared to fasten to the endothelial layer, suggesting a potential threat of attack to the corneal endothelial cells (Chen *et al*., 2011). However, Kolozsvari *et al*. (2002) reported Descemet's membrane and endothelium were minimally affected in some animals only as they were less important for absorption of UVB rays than corneal epithelium and stroma.

Loss of collagen fibers might be explained by either decreased collagen synthesis due to loss of keratocytes or increased its degeneration due to increased production of matrix metalloproteinases (MMPs). MMPs are collagen degradation enzymes leading to increased collagen turn over (Kozak *et al*., 2003). Chandler *et al*. (2010) observed a significant increase of MMP-2 and MMP-9 in the lotrafilcon A group compared with the senofilcon A group at the dose of 1.667 J/cm^2 UVB exposure, showing corneal stromal degradation

and induction of apoptosis in rabbit. Human corneal fibroblasts have been shown to produce MMP-2 after UV radiation (Kozak *et al*., 2003). Both MMP-9 and MMP-2 have been shown to degrade the basement membranes resulting in ulceration in human cornea (Woessner, 1991). It is likely that UV induction of matrix metalloproteinases production may have played a role in initiating a proteolysis in the corneas of UV-exposed mice (Newkirk *et al*., 2007).

Degeneration and loss of epithelial cells and keratocytes might be due to oxidative stress caused by UVB (Cullen, 2002). UVR lead to generation of free radicals (such as superoxide anion and hydroxyl radicals) and related reactive oxygen species (such as hydrogen peroxide and singlet oxygen). These substances are dangerous for biological systems as they react with deoxyribonuclcic acid (DNA), proteins and lipids leading to cellular damage (Mehlhase & Grune, 2002). UVB rays could also decrease antioxidant enzymes (mainly catalase and glutathione peroxidase) in the corneal epithelium thus increasing the oxidative damage (Lofgren & Soderberg, 2001).

Epithelial thinning might be due to cellular degeneration with loss of dead cells as well as slow rate of cellular proliferation and regeneration. Since the continued UVR exposure to cornea may prevent replacement keratocytes and as the functions of keratocytes are to synthesize and maintain stromal collagen, loss of keratocytes would result in decreased collagen production resulting in loss of stromal collagen and subsequent corneal thinning. Phagocytes may remove degenerate collagen and may also contribute to the stromal thinning.

3.3. Ultraviolet radiation exposure effects on retina

UV radiations which are capable of reaching the retina have shown alterations which affect the functions of the retina. Patton *et al.* (1999) indicated that relatively low doses of UVA (0.9 J/cm^2) and UVB (0.09 J/cm^2) can induce the formation of DNA strand breaks in cultured retinal pigment epithelium (RPE) and apoptosis in the exposed cells. The young retina is at particular risk for damage from UV exposure because the young lens has not as yet synthesized the yellow chromophore that prevents UV transmission to the retina (Dillon, 1991). UV damage to the eye may increase the possibility of developing eye disorders (ocular melanoma and macular degeneration).

In addition to UV damage to the young retina, Roberts, (2001) reported the short blue visible light (430 nm) damages the retina in persons over 50 years of age through photo-oxidation reactions. Marc *et al*. (2008) performed studies on primates (monkeys) have shown that short-term light exposures (less than 12 hours) at relatively high intensity can induce damage in photoreceptors and the RPE cells. Glickman, (2002) also reported human retinal pigment epithelium (RPE) and anterior layers of the retina in adults constantly receive intense visible light of wavelength greater than 400 nm.

According to Ueda *et al*. (2009), the most phototoxic effect was produced by light at wavelengths around 430 nm, then referred as the ''blue light hazard''. Agarwal *et al*. (2002) observed that thickness of the retinal outer nuclear layer, photoreceptor segment length and RPE thickness were 60–70% reduced when rat retinas were evaluated 4 weeks after blue light exposure. Light damage observed in rats resulted in a retinal degeneration exhibiting features of atrophic age related macular degeneration (AMD), including photoreceptor and RPE degeneration and chorio-capillaris atrophy (Marc *et al*., 2008). Wielgus *et al*. (2010) demonstrated that six hours blue light (450 nm) exposure of rats caused significant shortening of the photoreceptor inner and outer segment lengths, when retinas were evaluated immediately after irradiation. In addition, 40–50% of the photoreceptor nuclei were pyknotic

with condensation of chromatin and dark staining nuclei. Peng *et al*. (2012) study also showed significant atrophy of the outer nuclear layer thickness after 4 week period of light emitting diode exposure 2 hr/day, and more serious atrophy over time analyzed from the UV-wavelengths of 300 nm to 800 nm.

Several molecules in the retinal cell are implicated in mediating the phototoxicity of light in retina. The retinal pigment epithelium(RPE) is especially susceptible to oxidative stress because of its high light, oxygen, fluorophore (e.g. lipofuscin) and membrane lipid (e.g. polyunsaturated fatty acids) levels (Radu *et al.*, 2011). The photodamage observed in the RPE cells is oxygen dependent and is diminished by antioxidants (Organisciak & Vaughan, 2010), which suggests that the blue light effect is associated with oxidative stress.

Moreover, Ibrahim *et al*. (2012) investigated the lipid and DNA oxidative stress in retinal effects after UVB exposure in mice by using with or without UV-absorbing silicon hydrogel soft contact lenses. Retinal samples in mice exposed to UVB but without soft contact lenses appeared to develop comparatively higher lipid and DNA oxidative stress damage which shown to promote inflammation compared to control group (non-lens wear and no UVB exposed). Oxidative stress may be mediator of apoptosis and onset retinal degeneration. In addition the RPE and choroid contains melanin, which absorbs UV and short wavelength visible light and protects the retina against photo damage. However, with age ocular melanin and naturally protective antioxidant systems may decrease protective effectiveness against UV damage.

3.4. The histopathology and carcinogenic effects of UV radiation on conjunctiva and eyelid

UVB that reaches the eye is primarily absorbed by the epithelium and Bowman layer of the cornea (Bdour & Latayfeh, 2004) however, acute and chronic exposure to UV radiation can cause acute and chronic pain and inflammatory changes in the exposed conjunctiva (thin membrane covering the inner surface of the eyelid and the white part of the eyeball) (Oliva & Taylor, 2005).

Chen *et al*. (2013) studied mice's conjunctiva following UVB (peak wavelength of 312nm) exposure for a consecutive ten days period. The mice were anaesthetized with their ocular surfaces exposed to UVB light (0.72 J/cm^2 per day). Histologically, the conjunctival epithelial layers with UVB exposure were considerably showed marked thickening on the outermost area in some parts of the surface indicating the occurrence of conjunctival epithelium metaplasia as compared with the control group. The number of goblet cells in the conjunctiva were also significantly lower in the UVB exposed group than in the control group (Chen *et al*., 2013).

Acute and chronic exposure of the eyelid to UVA and UVB solar radiation causes UV radiation related damage to the skin of the eyelids as the skin becomes more susceptible to changes in melanin pigmentation, erythema (redness of the skin), and histopathological (dead tissue) production (Oliva & Taylor, 2005). According to Neff & Carter, (1999), approximately 5% to 10% of all skin cancers occur on the eyelid and the area around the eyes which include the lid margins, canthi, eyebrows or adjacent area of the face. These areas may become susceptible to benign malignant lesions or tumours as a result of increased exposure to solar UV radiation.

Lindgren *et al.* (1998) compared the distribution of eyelid basal cell carcinoma (BCC) with the relative ultraviolet radiation exposure to different sites on the eyelids. The result indicated the tumours were mainly on the lower eyelids (68%; 225 tumours) and the medial canthal area (26%; 87

tumours) of 329 BCCs with no significant difference between the right and the left eye (p=0.44). Eventhough BCCs were much more common on the lower eyelid, the UVR exposure did not show preponderance for the lower eyelid. Further, the medial parts of the eyelids received relatively less UV exposure although the incidence of BCCs at this site is high and the lateral part of the eyelids received the highest doses of UVR in the humans.

Both environmental and hereditary factors are known to increase the risk of developing BCC. Compared with squamous cell cancer (SCC) the evidence that exposure to sunlight is the predominant cause of BCC is less compelling. The lack of association between relative UVR exposure on the eyelids and BCC location indicates that UVR exposure only partially explains the etiology of periorbital BCC, and there are probably other factors like an increasing risk with age and a fair skin colour, that contribute to the development of these tumours. Solar UV radiation exposure is known to be an important etiologic factor in countries with intense sunlight exposure, such as Australia in the development of basal cell carcinoma (Marks, 1995), particularly in fair skinned individuals of middle age (Fong & Malhotra, 2005).

SCC occurs almost exclusively on sun exposed skin such as the face, neck, and arms, and the incidence is clearly correlated with geographical latitude, being higher in the more sunny areas of the world. SCC of the eyelid is the second most common, invasive epithelial malignancy that occurs on the squamous layer of the epidermis (outermost layer of cells that make up the skin), and accounts for 5% - 10% of periocular malignancies (Fong & Malhotra, 2005). SCC is more deadly than BCC because BCC rarely metastasizes, while SCC may metastasize and increased immunosuppressed populations.

4. Conclusion

The histopathological effects of UV radiation exposure in anterior ocular structures revealed dose, exposure duration and cellular susceptibility in human and animal models. With decreasing wavelengths, spectral energy increases, and higher spectral energy raises the potential for ocular damage. Much of the biological damage caused by solar irradiation is due to absorption of light at or above 300 nm.

The cornea absorbs most of the UVB and all of the UVC radiation reaching the eye. This energy is primarily absorbed in the epithelium and Bowman layer. The high absorption rate appears to be due to high tryptophan content in stromal proteins, a high ascorbic acid concentration of the epithelium, and the absorbance of UV radiation directly by nucleic acids. Ultraviolet rays can induce production of reactive oxygen species, inactivate corneal antioxidant enzymes, and induce cell death in corneal cells. Photokeratitis associated with UVB exposure in humans and experimental animal models has been proposed to be related to increased expression of matrix metalloproteinases (MMP) with corneal stromal degradation and induction of apoptosis.

UVB cause the nearly complete exfoliation of rabbit corneal epithelium accelerating the loss of corneal epithelial cells observed 15 hours after the UVB dose of wavelength 312 nm that the cornea absorbs 75% to 85% of incident UVB light and much of this is absorbed by the corneal epithelium.

The lens anterior capsule is a poor absorber of UVB, transmitting approximately 60% of UVB radiation at 300 nm. The most harmful wavelengths for the lens are between 300 to 305 nm.

Only a small proportion of light reaching the retina is required for the visual signal, and the excess light energy is thought to be absorbed mainly by the retinal pigment epithelium. Below 510nm appears to be a direct relationship between retinal damage and the wavelength of light, with lower wavelengths

causing greater damage especially RPE. Agents that damage the RPE may have adverse effects on the photoreceptors and ultimately, on vision.

Exposure to UVB at (peak wavelength of 312nm) can cause histopathological and morphometric alterations in the conjunctival surface which lead to conjunctival squamous metaplasia and loss of goblet cells in the conjunctival epithelium, which may subsequently lead to deterioration of tear quality and quantity.

Overall, the results suggest that the UVB had damaging effects on the eye structure after 45 minutes of exposure.

Increased exposure to UV radiation with increasing ozone depletion and global climate changes has broad public health implications for ophthalmologists as an increased burden of UV-related ocular diseases is to be expected.

5. Recommendations

In this review of the histopathological effects of ultraviolet radiation on ocular tissues in human and animal models, the following important concerns are recommended:

Although being intensively investigated in the last decade, cellular and molecular mechanisms underlying UV-induced lesions are still far from being completely discovered.

Limited research studies have been conducted on the impacts of increased UV radiation on the retina; thus chronic exposure to solar radiation and the effects on the retina need to be further explored. Most of the damage to the retina, resulting from UV rays, may be due to acute exposure from viewing the sun directly, however, limited knowledge exists regarding chronic exposure.

Known carcinogenic effects of ultraviolet radiation need to be conducted in human as many people directly or indirectly exposed to its in work place.

Further research should be conducted to further elucidate the mechanisms through which ultraviolet light alters ocular function as well as genetic and genomic markers that could identify individual's susceptibility to ultraviolet radiation toxicity.

There is sufficient evidence that cortical and posterior subcapsular cataracts can be caused by UVB in laboratory animals, but the link between the cataracts in humans to chronic ocular exposure to UVB needs further study.

With increasing levels of solar UV resulting from depletion of the ozone layer, and the continuing rise in the level of melanoma worldwide, people should become more aware of their UV exposure and take appropriate precautions.

These precautions include staying out of the sun during the period around noon (the period when the UV levels are highest), or wearing UV protective clothing, wide brimmed hats and UV absorbing eye glasses.

Health promotion/ prevention strategies are critically needed to create awareness on human eye health and climate related impacts. In addition the eye health community should adopt an approach to ocular morbidity which necessitates active campaigning for the protection of the environment.

Information should be freely and widely available to warn of any increases of ultraviolet radiation exposure related to the thinning of the stratospheric ozone layer.

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