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Senofilcon A contact lenses and UV-blocking spectacle lenses provide equal protection against UV-induced expression of p53 and caspase-3 in lens epithelial cells

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ABSTRACT

The importance of safe and effective efforts to prevent cataract incidence, one of which is by providing direct ultraviolet light protection to the eye. The objective of this study was to compare the effectiveness of UV-blocking spectacles and Senofilcon A contact lenses in protecting against UV-B-induced cataract. The associated lens damage was measured through the expression of P53 and caspase-3 in lens epithelial cells. Rats were exposed to direct UV-B irradiation from a UV-B lamp positioned 18 cm anterior to the right eye for 30 minutes, with an average irradiation energy of 6.5 kJ/m². Rats were irradiated without protection (P1), with UV-blocking spectacles protection (P2), and Senofilcon A contact lens protection (P3). Rats were euthanized, and eyes were enucleated on day 3 after UV-B exposure. UV-B exposure to unprotected eyes causes increased expression of P53 as a marker of DNA damage and increased caspase-3 as an executor of apoptosis in the lens epithelial cells. Immunohistochemistry was utilized to assess the expression of p53 and caspase-3 in the lens epithelial cells. The UV-B irradiation group showed the highest mean expression of P53 and caspase-3. The expression of p53 in both protection groups was significantly lower compared to the unprotected radiation groups (p =0.042, p =0.001). Similar results were obtained in the expression of caspase-3 of both protection groups compared to unprotected radiation (p = 0.017; p =0.002). The expression of p53 and caspase-3 was not significantly different when comparing the two protection groups (p =0.386, p =0.158). UV-blocking spectacles and Senofilcon A contact lenses provided equally effective protection in preventing UV-B radiation-induced P53 and caspase-3 expression. Finally, the data suggest that both of these protective measures can be employed as a means of preventing UV-B-induced cataract.

INTRODUCTION

Cataracts are the primary cause of avoidable vision loss globally, and so far, little cataract prevention technique has shown efficacy in decreasing the occurrence of cataracts [1]. The only effective treatment is cataract surgery, which can only be performed by eye surgeons supported with the systems and capacity to perform cataract surgery and manage postoperative complications [2]. Data summarized in VISION 2020: The right to sight, the global burden of disease study states that the target of reducing preventable blindness due to cataracts is not being achieved due to the continuous increase in incidence in the age group above 50 years [1]. This underlies the importance of safe and effective efforts to prevent cataract incidence, one of which is by providing direct ultraviolet light protection to the eye.

The ultraviolet spectrum with a wavelength of 200-400 nm is known to be damaging to human cells. Exposure to ultraviolet-A (UV-A) and ultraviolet-B (UV-B) radiation can trigger cataract formation [3]. Several studies have linked UV-B radiation (wavelength

290-315 nm) found in sunlight to the incidence of lens cortex opacity [4]. UV-B radiation causes alterations in the lens that show a correlation with the depletion of lens epithelial cells. Lens epithelial cells serve as a crucial transport function within the lens and are the primary location for enzymatic reactions that safeguard the lens against oxidative stress. An in vitro study showed that UV-B radiation damages the DNA and changes the way certain proteins are made, which starts the DNA repair process [5]. Homeostasis of lens fibre cells and consequent lens clarity are highly dependent on the function and intercellular communication of the lens epithelium. Lens ageing and the development of cataract formation are linked by a shared mechanism: reduced activity of the oxidative stress repair system, which leads to the buildup of reactive oxygen species (ROS), biomolecular damage to lens cells, and ultimately cellular dysfunction and pathology in the lens [6]. Exposure of ultraviolet radiation to lens epithelial cells that exceeds the maximum tolerated dose can cause direct damage to DNA and indirect damage through oxidative stress mechanisms. Lens epithelial cell damage will eventually cause opacity in the lens [7]. The phosphoprotein P53 is involved in the DNA damage response induced by UV radiation, whereas caspase-3 is a crucial protein involved in the execution of apoptosis in lens epithelial cells [8]. The use of UV protection modalities such as UV-blocking spectacles and UV-blocking contact lenses has been effective in reducing direct UV light transmission [4, 5, 7], but no studies have compared the protective effects of the two modalities.

Therefore, this study aimed to determine the effects of UV-B radiation on rat eye lens epithelial cells and to determine the protective effects of UV-blocking glasses and Senofilcon A against UV-B-induced cataract. The UV-induced damage to the lens epithelial cells was assessed by the expression of P53 and caspase-3. Twenty-eight *Rattus norwegicus* (Wistar rat) were exposed to artificial UV-B light to induce cataract development. Two groups of rats were protected against UV-B light with UV-blocking spectacles and contact lenses. Quantification of caspase-3 and p53 expression of lens epithelial cells was achieved by immunohistochemical analysis.

MATERIALS AND METHODS

Experimental animals

Twenty-eight robust male Wistar rats, weighing between 250 and 300 g, and aged between 6 and 8 weeks, were acquired from the experimental animal breeder at the Veterinary Faculty, Universitas Airlangga. The research was carried out in the *in vivo* laboratory of the Veterinary Medicine Faculty at Universitas Airlangga. Ethical feasibility was obtained from the Research Animal Ethics Commission of the Faculty of Veterinary Medicine Animal Care and Use Committee (ACUC) Universitas Airlangga, Surabaya, with an ethical feasibility letter number: 2.KEH.018.02.2023.

This work used an experimental methodology that included a post-test-only control group design. There were four groups: one negative control group (K0), radiation group (P1), and two protection groups, each protected by a UV-blocking spectacle lens (P2) and Senofilcon A contact lens (P3) while given UV-B exposure. All rats were euthanized by decapitation on the 3rd day after treatment and enucleated.

UV-B radiation

Each rat in the treatment group was exposed to UV-B light on the right eye under general anesthesia by replicating the method conducted by Giblin *et al.* 2011 [4]. General anaesthesia was administered by injecting 50 mg/kg of ketamine hydrochloride (Ketamine HCl injeksi 100mg/ml, Dexa Medica, Palembang-Indonesia) and 20 mg/kg of xylazine hydrocloride (Showvet Xylazyne 10% injection, ShowvetPharm, Henan Showvet Industrial Co, Henan-China). Tropicamide 1% eye drops (Cendo mydriatil, Cendo Pharmaceutical Industries, Bandung-Indonesia) were administered to the right eye in order to achieve maximum pupil dilation. During radiation, the rat's body was protected with a surgical drape except for the head. The left eye was patched.

The right eye of the rats was exposed to an artificial UV-B source (PL-S 9W/01 narrowband 311 nm, Philips Lighting, Batam-Indonesia) at an 18 cm distance. The lamp produced UV-B with a peak wavelength at 311nm. Radiant energy was measured using a UV-340A LUTRON radiometer (LUTRON UV-340A, Lutron Electronics co., Pennsylvania-USA), and radiation exposure was determined using the radiometric formula as follows: $H \times E_{ë}$

where H is the radiation energy exposure per unit surface area (J/cm^2), t is the duration in seconds, and E_{e^-} is the measured radiation (W/cm^2) obtained from the radiometer measurement. Rat eyes were exposed to UV-B radiation for 30 minutes with radiation generated by a PL-S 9W/01 narrowband UV-B lamp, Philips, at an average of 0.361 mW/cm^2. This value is equivalent to 7.2 times the highest amount of radiation impacted on the cornea by daylight sunlight (0.05 mW/cm^2) [9]. The radiation energy per unit surface area calculated with the radiometric formula was at an average of 0.650 J/cm2, which is 2.95 times greater than the maximum dose of UV radiation that does not cause cataracts [10]. The rat eyes were exposed to UV-B radiation in the presence of either a UV-blocking spectacle lens (Crizal Easy, EssilorLuxottica, Paris-France), a Senofilcon A contact lens (Acuvue OASYS; Johnson and Johnson Vision Inc., New Jersey- USA), or no protection at all.

UV-blocking spectacles

UV-blocking spectacle lenses (Crizal Easy, EssilorLuxottica, Paris-France) were cut in the shape of a 2.5 mm square and attached to a wire support on both the upper and lower sides of the lens. The spectacle lens was positioned 4 mm above the corneal surface of the right eye of the rat.

Senofilcon A UV-blocking contact lenses

Senofilcon A (Acuvue OASYS; Johnson and Johnson Vision Inc., New Jersey- USA) contact lenses were cut with a sterile puncher with a diameter of 5 mm to match the diameter of the rat cornea. The lenses were placed on the corneal surface of anesthetized rats. Prior to the application of the contact lens, a single droplet of normal saline is administered to avoid the entrapment of air between the contact lens and the cornea. The contact lens is subsequently placed on the mouse cornea using straight blunt forceps. Regular moistening of the contact lens with normal saline was performed every 15 minutes throughout the radiation procedure.

Ophthalmic examination

Lens changes caused by UV-B radiation were assessed using a slit lamp immediately after maximal pupil dilatation with 1% tropicamide eye drops for 3 days post-treatment. Documentation of results was conducted using a Fujifilm X-A5 mirrorless digital camera (Fujifilm X-A5, Fujifilm, Tokyo-Japan).

Immunohistochemistry staining

The lens sections underwent standard staining with Hematoxylin Eosin and immunohistochemical staining using the combined indirect approach to quantify the expression of p53 and caspase-3. A caspase-3 monoclonal antibody staining kit (GeneTex Inc., California, USA), and a P53 monoclonal antibody (Biorbyt Ltd., Cambridge, United Kingdom) were utilized with the immunohistochemistry kit (UltraVision Detection System: Anti-Polyvalent, Thermo Fisher Scientific, Chesire-United Kingdom). Data on the expression scores of caspase-3 and P53 were acquired using the modified Remmele method. The Remmele Immuno-Reactive Score (IRS) is a measure of the percentage of multiplication between the score given by positive immunoreactive cells and the score representing colour intensity. Sample data was produced by calculating the average IRS value observed in 5 fields of view at 400x magnification. The immunohistochemically stained samples were documented using a Nikon H600L standard light microscope equipped with a 300-megapixel DS Fi2 digital camera and Nikon Image System software for digital image processing (Nikon H600L, Nikon Instruments Inc., Tokyo, Japan).

Statistical analysis

The entire data was analysed using SPSS 25.0 (SPSS Inc, Chicago-USA). The binomial test was employed to analyse all descriptive data. The statistical analysis results were reported as the mean ± standard deviation. The normality and homogeneity of the data were assessed using the Shapiro-Wilk and homogeneity statistical tests. Comparative analysis of P53 and caspase-3 expression was conducted among groups using the one-way ANOVA test, followed by the LSD multiple comparison test. Statistical significance was established when the p was less than 0.05.

RESULTS

Variation of lens opacification

The rat lenses were examined before and after exposure to UV-B radiation using a handheld slit lamp and documented. Variation of lens opacification was observed in the radiation group (P1) (Figure 1).

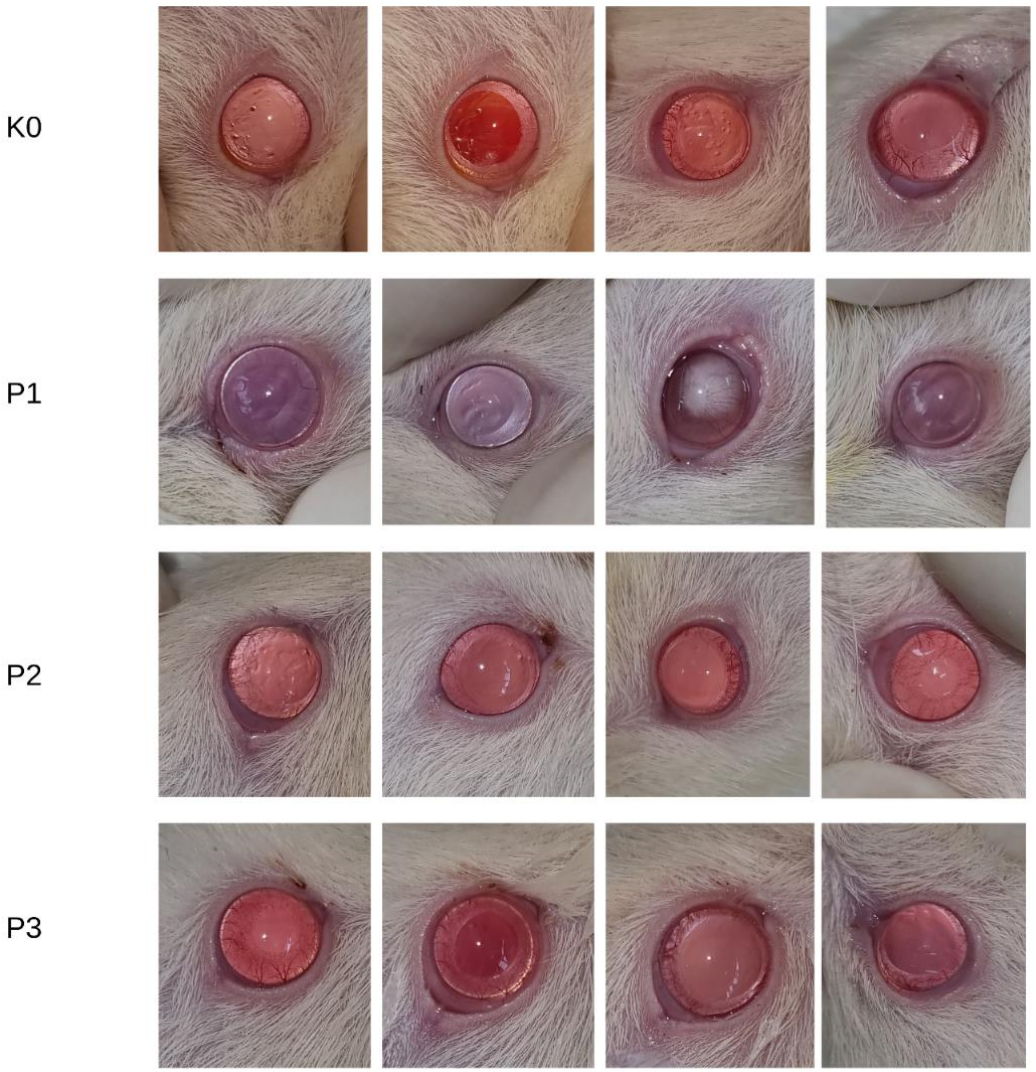

Figure 1. Anterior segment examination of rat eyes three days after UV-B irradiation showed obvious lens opacification in the P1 group compared to the other group. (K0) negative control group, (P1) rats received UV-B irradiation without protection, (P2) rats received UV-B irradiation with spectacle lens protection, and (P3) rats received UV-B irradiation with contact lens protection.

UV-B radiation increases P53 expression in lens epithelial cells

Rat lens epithelial cells in the radiation group (P1) exhibited double the magnitude of P53 expression on day 3 post-exposure, in comparison to the control group (K0), as shown in Table 1. A p of 0.000 ($P<0.05$) indicated that the expression of P53 in the lens epithelium of the P1 group was substantially greater than that of the lens epithelium in the K0 group.

The weakest p53 expression in lens epithelial cells was found in the negative control group (K0). Quantitative p53 expression results based on IRS scores obtained the highest mean value of 5.150 with a standard deviation of ± 1.563 in group P1 and the lowest mean value of 2.375 with a standard deviation of ± 1.678 in group P3, while in group P2 the average was 3.500 with a standard deviation of 1.365 and in group K0 it was 2.550 with a standard deviation of 1.577.

Table 1. Levels of P53 and Caspase-3 expression.

	P53 expression		Caspase-3 expression	
Groups	**Mean**	**SD**	**Mean**	**SD**
K0	2.550	± 1.577	0.925	± 0.650
P1	5.150	± 1.563	3.200	± 1.190
P2	3.500	± 1.365	1.825	± 0.971
P3	2.375	± 1.678	1.350	± 1.368

P53 and Caspase-3 expression were the highest in the P1 group. (K0) negative control group, (P1) rats received UV-B irradiation without protection, (P2) rats received UV-B irradiation with spectacle lens protection, (P3) rats received UV-B irradiation with contact lens protection.

UV-B radiation increases caspase-3 expression of lens epithelial cells

The level of caspase-3 expression in lens epithelial cells exposed to UV-B radiation at a dose of 6.5 kJ/m2 for 30 minutes was 3.5 times greater than that of the control group (p 0.000; $p < 0.005$), as shown in Table 1. The expression of caspase-3 in lens epithelial cells was strongest in group P1, which was the group that received UV-B exposure for 30 minutes without protection. The weakest caspase-3 lens epithelial cells were found in group K0. The results of caspase-3 expression obtained the highest mean value of 3.200 with a standard deviation of ± 1.190 in group P1 and the lowest mean value of 0.925 with a standard deviation of ± 0.650 in group K0. The results of caspase-3 expression in group P2 obtained a mean value of 1.825 with a standard deviation of 0.971, and in group P3 obtained a mean value of 1.350 with a standard deviation of 1.368. Figure 2 presents representative IHC slides from each group.

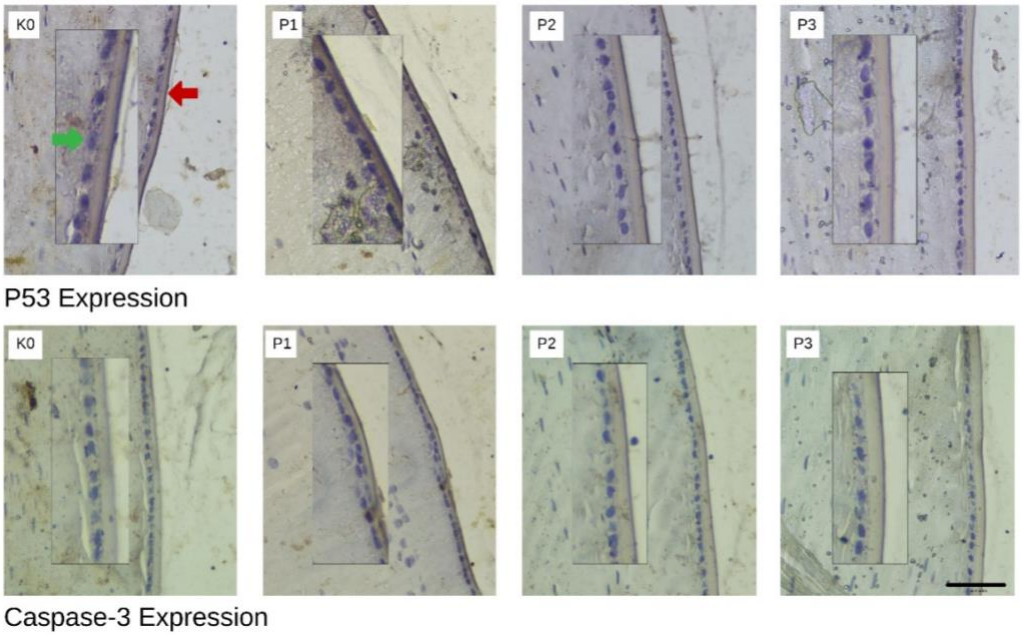

Figure 2. Immunohistochemistry staining of anterior lens tissue shows P53 and caspase-3 expression variables in epithelial cells (inlet) of the lens among groups. The strongest expression of P53 and caspase-3 was observed in the P1 group (immunohistochemical staining, objective lens 40x; bar = 50 microns; Eclipse E-i microscope; DS Fi2 300 megapixel camera). (K0) negative control group, (P1) rats received UV-B irradiation without protection, (P2) rats received UV-B irradiation with UV blocking spectacle lens protection, (P3) rats received UV-B irradiation with Senofilcon A contact lens protection. Green arrow: lens epithelial cells; Red arrow: anterior lens capsule.

Protective effect of UV-blocking spectacles and Senofilcon A contact lenses on p53 expression of lens epithelial cells

The present investigation demonstrated that the expression of p53 in lens epithelial cells exposed to UV-B radiation with UV-blocking spectacle lens protection was 1.4 times higher than that of the control group (Figure 3). However, this difference was not statistically significant when compared to the control group ($p = 0.230$). The group that received UV-blocking spectacle lens protection (P2) had 1.4 times less p53 expression compared to the lens epithelial cells of rats exposed to UV-B without protection (P1). This comparison yielded statistically significant results with a p of 0.001 ($p < 0.05$). The relative expression of p53 in lens epithelial cells exposed to UV-blocking contact lens protection was not statistically different from the negative control group, as indicated by a p of 0.158 ($p < 0.05$).

The p53 expression in lens epithelial cells subjected to UV-B radiation while protected by Senofilcon A contact lenses was determined to be 2.2 times lower than that of the control group ($p = 0.01$) (Figure 3). The p53 expression in lens epithelial cells exposed to UV-B while protected by Senofilcon A contact lenses did not significantly differ from that in control lens epithelial cells ($p = 0.823$).

The mean expression of p53 in the radiation group was 2 times greater than the control (5.150 ± 1.563; 2.550 ± 1.577). Notably lower p53 expression was detected in the P2 and P3 groups ($p = 0.042$, $p = 0.001$), while the p53 expression was not statistically different between groups P2 and P3 ($p = 0.158$).

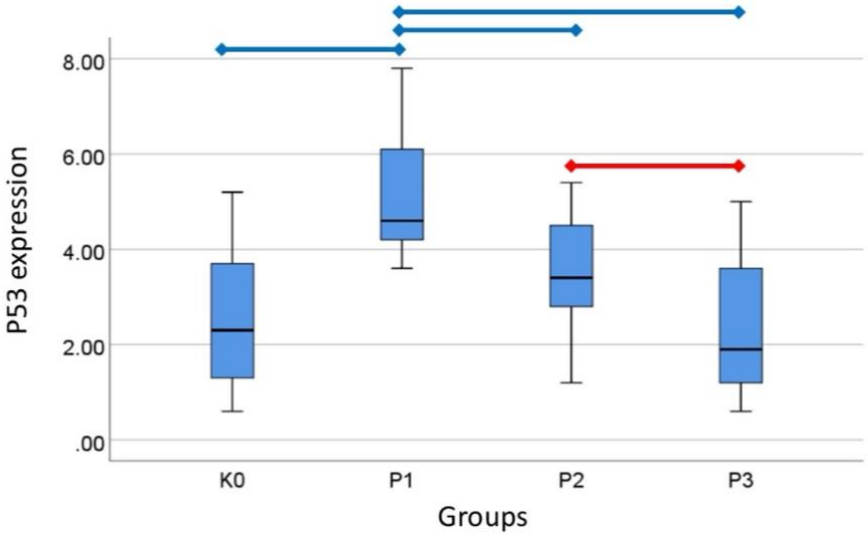

Figure 3. Statistical analysis of P53 expression of lens epithelial cells in UV-B exposure without protection groups (P1) was significantly higher compared to the negative control group (K0), and those of UV-B protection groups (P2 and P3). P53 expression of lens epithelial cells in groups with UV blocking spectacle protection was insignificantly different from P53 expression in Senofilcon A contact lens protection group. Data are presented as mean ± SD. Blue arrows indicate statistical significance in the comparison between groups ($p<0.05$), meanwhile, red arrow indicates statistical insignificance.

Protective effect of UV-blocking spectacles and Senofilcon A contact lenses on caspase-3 expression of lens epithelial cells

Lens epithelial cells subjected to UV-B exposure with UV-blocking spectacles protection exhibited a 1.8-fold lower caspase-3 expression compared to those exposed to UV-B without such protection (Figure 4). The difference yielded significant results in the post-hoc analysis, with a p of 0.017 ($p>0.05$). The expression of caspase-3 in the epithelial

cells of the rat eye lens with UV-blocking eyewear protection was not statistically different from the negative control group (p =0.106). UV-blocking spectacle successfully inhibits apoptosis of lens epithelial cells, as indicated by the expression of caspase-3, which is significantly lower in the spectacle lens protection group (P2) compared to the non-protection group (P1) after UV-B exposure of 6.5 kJ/m2 for 30 minutes in this study.

The mean expression of caspase-3 in the radiation group was 3.5 times greater than that of K (3.200 ± 1.190; 0.925 ± 0.650). The levels of caspase-3 expression were significantly lower in groups P2 and P3 compared to group P1 (p =0.017; p = 0.002). Statistically insignificant differences were seen in the expression of caspase-3 between the P2 and P3 groups (p =0.386). Statistical analysis of Caspase-3 expression is presented in Figure 4.

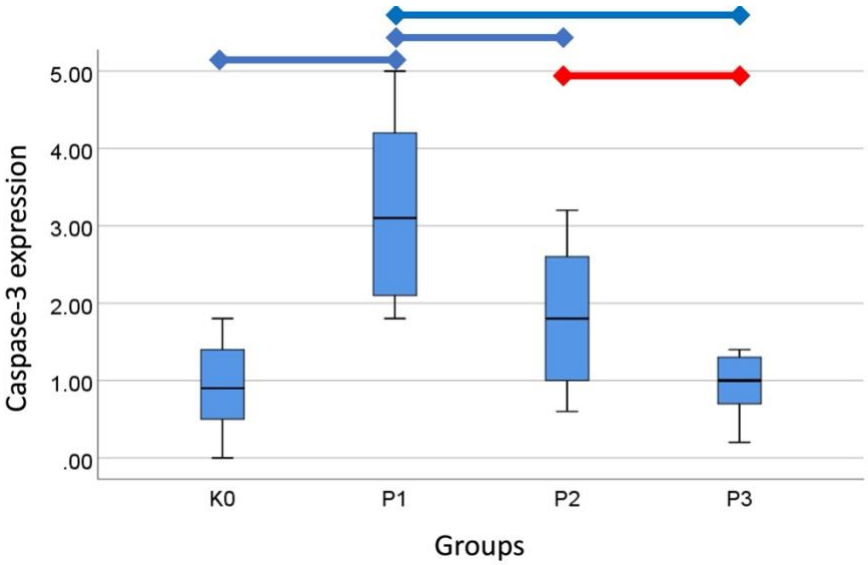

Figure 4. Statistical analysis of Caspase-3 expression of lens epithelial cells in UV-B exposure without protection groups (P1) was significantly higher compared to the negative control group (K0), and those of UV-B protection groups (P2 and P3). Caspase-3 expression of lens epithelial cells in groups with UV blocking spectacle protection (P2) was insignificantly different from caspase-3 expression in Senofilcon A contact lens protection group (P3). Data are presented as mean ± SD. Blue arrows indicate statistical significance in the comparison between groups ($p<0.05$), meanwhile, red arrow indicates statistical insignificance.

Comparison of the protective effect of UV-blocking spectacle lenses with Senofilcon A contact lenses on p53 and caspase-3 expression of lens epithelial cells

The p53 expression in the Senofilcon A contact lenses group showed p53 expression of 2.375 ± 1.678, while the UV-blocking spectacle lenses group showed p53 expression of 3.500 ± 1.365. The p53 expression in both protection groups yielded significantly different results compared to the UV-B exposure group without protection. The difference in p53 expression of the Senofilcon A contact lens group was not significant from the UV-blocking spectacles group (Figure 3), indicating that both UV-blocking spectacles and Senofilcon A contact lenses are equally effective in preventing DNA damage due to UV-B exposure as assessed by p53 expression in lens epithelial cells. The caspase-3 expression in the Senofilcon A contact lens group showed a score of 1.350 ± 1.368, while the UV-blocking spectacles group showed a caspase-3 expression of 1.825 ± 0.971. The caspase-3 expression in both protection groups showed significantly lower results compared to the UV-B exposure group without protection (p =0.017 and p =0.002) (Figure 4). The caspase-3 expression in the UV-B exposure group protected by Senofilcon A contact lenses was not substantially different from that in the UV-B exposure group protected by UV-blocking spectacles (Figure 4).

DISCUSSION

The study demonstrated that the expression levels of p53 and caspase-3 in lens epithelial cells were markedly elevated in the group of rats subjected to 6.5 kJ/m2 of UV-B exposure for 30 minutes, in comparison to the control group. The cohort of rats equipped with UV-blocking spectacles and Class I UV-blocking contact lenses (Senofilcon A) exhibited p53 expression that was not statistically distinct from the control group. The expression levels of p53 and caspase-3 in the UV-blocking spectacle protection group were not substantially different from those in the class I UV-blocking contact lens (Senofilcon A) protection group. The research indicated that UV-B exposure resulted in elevated p53 expression, signifying DNA damage, and an increase in caspase-3, indicating apoptosis. The study demonstrated that UV-blocking glasses and class I UV-blocking contact lenses (Senofilcon A) offered comparable efficacy in protection against UV-B induced damage characterized by the elevation of p53 and caspase-3 expression.

Exposure of ultraviolet radiation, especially UV-B, to lens epithelial cells is proposed as one of the initiating mechanisms for senile cataracts [6]. UV-B radiation can have direct or indirect biological consequences, specifically induced damage to nucleic acids, proteins, and lipids. UV-B rays indirectly activate free radicals, and directly damage DNA by the mechanism of excitation of nucleic bases that produce dimer products. Besides that, radiation energy that transfers to oxygen molecules can emulate the generation of reactive oxygen species and other compounds resulting from photo-oxidation [11]. DNA damage to lens epithelial cells activates p53 and initiates the intrinsic apoptotic pathway. Cell death through the p53 pathway is further executed by caspase proteinases. Activated caspase (cleaved caspase) gives rise to a phenotype typical of apoptosis. In response to caspase activation, mitochondria secrete apoptogenic substances, including Cytochrome C. The liberation of Cytochrome C thereafter stimulates the activity of Apaf-1 and caspase-9. Caspase-9, which has been activated and joins the apoptosome, will activate caspase-3 and caspase-7 that execute cell death [12]. Exposure to UV radiation with a wavelength of 300 nm in the eye lens of rats showed damage to lens epithelial cells, induced apoptosis, and increased caspase-3 expression in the lens epithelium [13-15].

Rat lens epithelial cells in the radiation group (P1) exhibited double the magnitude of P53 expression on day 3 post-exposure, in comparison to the control group (K0). A p =0.000 ($P<0.05$) indicated that the expression of P53 in the lens epithelium of the P1 group was substantially greater than that of the lens epithelium in the K0 group. The present finding corroborates a prior investigation conducted by Sun et al. (2001), which demonstrated a substantial upregulation of p53 expression in lens epithelial cells 24 hours post-exposure to UV light [14]. P53 expression was positively correlated with time after UV-B exposure, it was explained by the experimental research by Lv and Xing (2018) that showed the p53 expression increased after 15 minutes of UV-B exposure (UV light with a wavelength of 300 to 350 nm, 1.0x103μW/cm2), where P53 expression on day 7 was higher than expression on day 5, and expression on day 5 was higher than expression on day 3 [15]. This shows that lens epithelial cell apoptosis increases over time, even though UV-B exposure is only done once. Both studies link increased p53 expression with lens epithelial cell apoptosis and cataracts. An in vitro study concluded that microRNA directly targets p53 and induces lens epithelial cell apoptosis through a mechanism involving p53 activation [13]

The level of caspase-3 expression in lens epithelial cells exposed to UV-B radiation at a dose of 6.5 kJ/m2 for 30 minutes was 3.5 times greater than that of the control group ($p = 0.000$; $p < 0.005$). These findings are consistent with prior investigations, including a

study conducted by Talebizadeh (2016), which revealed a 12% increase in caspase-3 expression in lenses exposed to UV-B radiation. This study identified that apoptotic cells are more prevalent in the central region of the lens epithelium compared to the nuclear bow region. This is in line with the investigation conducted on albino rats exposed to UV-B radiation for 15 minutes, with a total dose of 8 kJ/m2 and a wavelength of 300 nm, which revealed that activated caspase-3 was more prevalent in the central region of the lens epithelium than in the nuclear bow [16].

Caspase-3 functions as a primary executor protein in the majority of apoptosis models. The normal lens also expresses classical endogenous caspases, including caspase-3, which undergo proteolysis during the process of lens fibre cell development [16]. Evidence from experimental model studies has demonstrated that caspase-3 is a major indicator of apoptosis, exhibiting superior selectivity compared to the TUNEL assay [17]. A prior investigation revealed that the presence of activated caspase-3 in lens epithelial cells exposed to UV-B (300nm) resulted in a higher rate of apoptosis at 6-16 hours after exposure [17]. The expression of caspase-3 reached its highest point 16 hours after exposure to UV-B radiation and then started to decrease 24 hours after exposure [18]. The reduction in caspase-3 expression 24 hours after exposure may be attributed to the spontaneous termination of caspase-3 expression in lens epithelial cells as a result of cell death [18]. This experimental study provides evidence of the association of acute UV-B exposure with lens epithelial cell apoptosis and the risk of cataract formation.

The protection of UV-blocking spectacles against UV-B exposure has not been widely studied in vivo, but several publications describe the UV transmission ability of spectacle lenses, both clear and tinted or sunglass lenses. Polycarbonate materials are calculated to absorb 100% of UV radiation up to 375 nm wavelength, transmit 1% of UV with 380 nm wavelength, and 10% of 385 nm wavelength [19]. Standard clear spectacle lenses can reduce exposure to UV radiation by 31% [20]. UV-blocking spectacles that have a UV-absorbing base material efficiently reduce UV radiation by 93%, and only 7% of UV radiation directly reaches the surface of the eye [21].

The p53 expression in lens epithelial cells subjected to UV-B radiation while protected by Senofilcon A contact lenses was determined to be 2.2 times lower than that of the control group ($p = 0.01$). The p53 expression in lens epithelial cells exposed to UV-B while protected by Senofilcon A contact lenses did not significantly differ from that in control lens epithelial cells (p of 0.823). The findings of this study align with prior research on UV-blocking contact lenses, including an in vivo experimental investigation conducted by Giblin et al. (2011). This study revealed that rabbit eyes exposed to UV-B radiation for 30 minutes (wavelength 270-360 nm, dose 1.7mW/cm2) exhibited lens epithelial cell loss, edema, vacuole formation, DNA single-strand breaks, and anterior subcapsular opacification. The results differed from those of rabbit eyes treated with Senofilcon A contact lens protection, where the adverse UV-B effects were negligible [4]. In other studies, rabbit eyes protected by Senofilcon A contact lenses exhibited almost no DNA damage, whereas DNA single-strand breaks occurred in rat eyes lacking contact lens protection [22]. The expression of pro-inflammatory factors, including nuclear factor-kappa B (p65), cyclooxygenase-2, Fas L, and Fas, was significantly lower in corneas protected by Etafilcon A, which belongs to the same UV-blocking contact lens classification as Senofilcon A [23]. Senofilcon A (ACUVUE Oasys) has the lowest UV transmittance of 0.24% UV-B, while the UV-blocking contact lens class 2, Etafilcon A, has a UV transmittance of 1.46% [24]. This data indicates that Senofilcon A's UV-B transmittance aligns with the manufacturer's assertion of being below 10%. In this study, Senofilcon A markedly inhibited the elevation of p53 expression following UV-B

exposure in mouse lens epithelial cells, indicating its efficacy in preventing DNA damage to these cells caused by UV-B radiation.

The expression of caspase-3 in lens epithelial cells subjected to UV-B exposure with Senofilcon A was 2.4 times lower than that in lens epithelial cells exposed to UV-B without protection, demonstrating a significant difference with a p of 0.002 ($p<0.05$), as illustrated in Figure 4. The caspase-3 expression in lens epithelial cells subjected to UV-B exposure with Senofilcon A did not significantly differ from that in the control group (p 0.437; $p > 0.005$). The findings of this study corroborate earlier research about the protective efficacy of Senofilcon A, which found no significant difference in caspase-3 activity or the number of positive cells in the TUNEL assay for lens epithelial cells after UV-B exposure with Senofilcon A contact lens protection compared to control lenses [11]. This study is also in line with research by Giblin et al. (2011), where rabbit eyes protected with Senofilcon A contact lenses showed no significant lens epithelial cell apoptosis compared to unexposed control eyes [4]. UV-blocking Senofilcon A contact lens protection proved effective in preventing lens epithelial cell apoptosis, in which was reflected through expression of caspase-3.

UV-blocking contact lenses and UV-blocking spectacles can be proposed as one of the easy and inexpensive modalities of protection against UV exposure. Contact lenses theoretically protect the lens from all UV light entering the eye, but UV-blocking spectacles produce a reflection of UV rays originating from the anti-reflective coating on the back of the spectacle lens onto the surface of the eyeball. The UV radiation enters through an angle of 135°-150° behind the spectacle wearer and is reflected by the back of the lens into the eye [25, 26]. Things to consider in choosing between the UV-blocking contact lens modality or UV-blocking spectacles include the use of contact lenses that must be prescribed by an optometrist or ophthalmologist, involving a professional fitting process to ensure that contact lens wear will not cause corneal damage. Contact lenses only protect the surface of the cornea and limbus, whereas UV-blocking spectacles may have the advantage of providing protection to other eye structures, including the conjunctiva and eyelids. The choice of UV protection modality can be tailored to each individual's needs. The combination of UV-blocking contact lenses and UV-blocking spectacles can be advised for patients with outdoor activities and high UV exposure [24, 27]. This study is the first to compare the protective effect of UV-blocking spectacle lenses with UV-blocking contact lenses. Both protection modalities can be promoted as a method of preventing damage to eye structures due to UV-B exposure, especially DNA damage and apoptosis of lens epithelial cells, as shown in this study.

This study fills an information gap in the field of cataract prevention studies due to ultraviolet radiation by comparing the protective effect of UV-blocking contact lenses with UV-blocking spectacles. This study focuses on the DNA damage response of lens epithelial cells reflected by p53 expression and the execution of lens epithelial cell apoptosis reflected by caspase-3 expression. However, this study has several limitations. Assessment of p53 and caspase-3 expression was done in a cross-sectional manner at 3 days after exposure, so it does not describe the time course of p53 and caspase-3 expression induced by UV-B radiation. This research cannot explain the processes that occur between UV-B radiation and increased expression of caspase-3 and p53, as this process might also involve cellular damage response mechanisms such as oxidative stress, inflammation, and DNA repair mechanisms. The UV-B protection devices studied in this study must be modified to fit the size of the rat eyes. This may alter the effectiveness of the product used, especially the contact lenses. Further studies with experimental animals that have larger corneal diameters, such as rabbits, may reflect

the protective effect on human eyes more accurately. Further research can be done to study other markers involved in the process of apoptosis of lens epithelial cells to get a broader picture of the effect of UV-B radiation. Research on reactive oxygen species and DNA repair mechanisms after UV-B exposure can complement and show the relationship between UV-B exposure, DNA damage, lens epithelial cell apoptosis, and cataract formation. Finally, further research can use rabbits that have a corneal diameter that is not much different from the human eye, so that a model that better reflects the actual protective effect on the human eye.

CONCLUSIONS

This study demonstrated that direct UV-B exposure results in elevated levels of P53, a hallmark of DNA damage, and increased expression of caspase-3, an important executor marker in the process of apoptosis. The study further revealed that UV-blocking spectacles and Senofilcon A contact lenses offer comparable protection against UV-B-induced P53 and caspase-3 expression. This research suggests that these protective measures can serve as effective and readily available strategies to prevent UV-B-induced cataracts.

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AUTHOR CONTRIBUTIONS

Study conception and design - (NS, NPA, DH), Data collection - (NS, DH, NPA, RF, MDG), Data analysis and interpretation - (NPA, RF, MDG), Drafting of the article - (NPA, NS), Critical revision of the article – (NPA, NS, DH). All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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