RESEARCH ARTICLE

The histological comparison of the effect of 50mW green and blue laser on the human eye at variable time intervals: an animal model

Qamar Aqeel Shams, Ameen Mohammad Alsudani, Mustafa Salih Almusawi

Abstract

Objective: To compare the effects of 50mW green and blue laser pointers on retinal tissue with different exposure durations.

Method: The experimental, histopathological study was conducted from November 2021 to April 2022 at the Postgraduate Laboratory of the Department of Physiology and Medical Physics, College of Medicine, Mustansiriyah University, Iraq, and comprised adult male mice. They were divided into green group G and blue group B. Group G was exposed to a 50mW green laser pointer with wavelength 532nm, while group B was exposed to a blue laser pointer with wavelength 415nm. The groups were subdivided with respect to duration of exposure. Subgroups G1 and B1 had exposure time 1min, G2 and B2 had exposure time 2min and G3 and B3 had exposure time 3min. Across the groups, the overall supplied power density was 130mW/cm^2. Conventional Haemotoxylin and Eosin stain was used to assess the damage on the retinal tissues of the mice post-exposure. Data was analysed using SPSS 24.

Results: Of the 24 groups, 12(50%) were in each of the 2 groups, while each subgroup had 4(16.66%) mice. There was more retinal tissue damage in group G compared to group B ($p<0.05$). The damage increased significantly with increase in exposure duration in both groups ($p<0.05$).

Conclusion: The traditional green laser pointers caused more damage to the retina of mice than the blue laser.

Key Words: Eosine, Retina, Lasers

(JPMA 74: S447 (Supple-8); 2024) **DOI: https://doi.org/10.47391/JPMA-BAGH-16-100**

Introduction

Blue light is more likely to be absorbed in ~450nm, while green light is more resistant to absorption. Additionally, the longer is the wavelength, the deeper is the penetration, since the wavelength is directly proportional to the penetration.[1]Blue light and other shorter wavelengths of visible white light scatter more intensely than longer wavelengths[2]. This is caused by a scattering cross-section that is inverse of λ^4.3

The high-powered green lasers could be directly emitted using green laser diodes (515/520nm). There are several different power outputs available for green laser pointers. The best laser pointers to use and point with are 5mW green laser pointers Classes II and IIIa[4]. The green laser beam can be clearly seen in the dark night sky, where it can point to a single star [5]. Blue lasers are gallium nitride (GaN) laser diodes having a peak wavelength in the blue light spectrum (400-480nm). The blue laser system can be utilised for laser cladding, heat treatment and other applications because of its high absorption rate, which makes it attractive for energy conservation[6].

Laser pointers and laser equipment of different power and colours ranging from a small number of milliwatts (mW) to several watts are now widely accessible. As a result, laser pointers can be potentially in the hands of people who are unaware of the risks associated with their use[7], and that is why there are not many laser pointers readily available to the general public in various countries[8]. The United States Food and Drug Administration (FDA) advises consumers not to buy a laser pointer with a 5mW power without reading the labels or instructions first, and not to give them to kids since the risk of misuse is too great, and there is a risk of eye injury if they are exposed to class III lasers with a power of up to 5mW[9].

The back of the eye is covered by a delicate neural membrane called the retina[10]. It is similar to how a camera works with film. It receives the light from the image we see, and converts it into electrical impulses that are transmitted to the brain through the optic nerve fibres[11].

The retina is made up of two layers: the sensory retina, and the retinal pigment epithelium (RPE)[12]. The neural retina consists of several layers of neurons, including the inside out retinal ganglionic cell (RGC), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segment (IS) and outer segment (OS), which are the layers that make up the retina[4]. The photoreceptor cells (rods and cones) are

College of Medicine, Mustansiriyah University, Baghdad, Iraq.

Correspondence: Qamar Aqeel Shams

Email: qamaraqeel90@gmail.com

made up of ONL, which are the retina's central light-sensitive cells[13].

Daytime colour vision and night-time black-and-white vision are both controlled by cones and rods[14]. It enables the neural network to detect photons and generate a neurophysiological signal through a process called phototransduction[13].

Before light reaches the retina, it must move through many mediums, including the tear, cornea, aqueous fluid, lens and vitreous humor[15]. Along with visible (that is, 'light') wavelengths, some near-infrared wavelengths also reach the retina. The iris and pupil are both protected by the transparent cornea. This lens, together with the crystalline lens, is the earliest and strongest in the eye's optical system, allowing sharp images to be produced at the level of photoreceptors in the retina[16].

Furthermore, because photopigments only absorb a small portion of the input energy, most of the radiation that reaches the fundus of the eye is absorbed by the pigmented epithelium and choroid[17]. The melanin, a dark pigment that absorbs any light that the retina is unable to detect, is found inside the pigment epithelial cells. By doing this, light is stopped from reflecting off the retina and back onto the back of the eye. Consequently, it is believed that the RPE will sustain the most damage[18]. Then starts a sequence of electrical and chemical reactions that finally activate nerve impulses that are sent through the optic nerve's fibres to various vision centres in the brain[4].

Retinal burns caused by a portable laser pointer represent a serious and increasing community health concern. Such gadgets are getting more powerful, less expensive, and more freely available online[19]. The retina may suffer irreversible damage from this hail of photons when exposed to excessive levels of light. An instant thermal damage might result from a brief exposure to excessively intense lights. The quantity of thermal damage severity and degree in tissues subjected to laser beams is determined by irradiance, spot size, pulse length and wavelength[20].

The current study was planned to compare the effects of 50mW green and blue laser pointers on retinal tissue with different exposure durations.

Materials and Methods

The experimental, histopathological study was conducted from November 2021 to April 2022 at the Postgraduate Laboratory of the Department of Physiology and Medical Physics, College of Medicine, Mustansiriyah University, Iraq.

After approval from the institutional ethics review committee, the sample was raised, comprising adult male mice. They were divided into green group G and blue group B. Group G was exposed to a 50mW green laser pointer with wavelength 532nm, while group B was exposed to a blue laser pointer with wavelength 415nm.

The groups were subdivided with respect to duration of exposure. Subgroups G1 and B1 had exposure time 1min, G2 and B2 had exposure time 2min, and G3 and B3 had exposure time 3min. Across the groups, the overall supplied power density was 130mW/cm^2.

All of the mice had free access to food and drink. Diode laser pointer emitting gallium-aluminum-arsenide (Shenzhen Huianqi Technology Co., Ltd. China). The emission was through a small handpiece that made it possible to focus the beam. Using an optical power meter (Model 2936-C, Gentec-E, Mestro, Canada), energy was measured and modulated by the size of the irradiation point, the distance travelled, and the time. The laser beam was focused on the mice's right eyes after they had had anaesthesia with sodium pentobarbital, sparing the mice's left eyes, which were utilised as the control group. Laser irradiations were carried out in the dark and appropriate safety goggles were donned. The temperature used to irradiate the eyes was 23±2°C.

After being sacrificed by cervical dislocation, the mice had their right (exposed) and left (non-exposed) eyes quickly inoculated and perfused with 10% neutral-buffered formalin solution for at least 24h. The eyeball samples were divided into two sections longitudinally. They were embedded in paraffin blocks, sectioned into 4µm sections, and mounted on slides, to be stained with the standard Haemotoxylin and Eosin (H&E) stain. A double-blind histopathologist examined each section under a light microscope to determine the extent of any destruction of the retinal tissue. The severity of the destruction was graded as <5% = no lesion, 5-25% = mild, 26-50% = moderate and >50% = severe. The damage was assessed with respect to reversible and permanent cellular alterations, cellular layer misalignment, thinning, and the development of interstitial oedema within various retinal layers.

Data was analysed using SPSS 24. Data was presented as mean ± standard deviation, and statistical tests were run with 95% confidence level. $P<0.05$ was considered statistically significant.

Results

Of the 24 groups, 12(50%) were in each of the 2 groups, while each subgroup had 4(16.66%) mice. There was

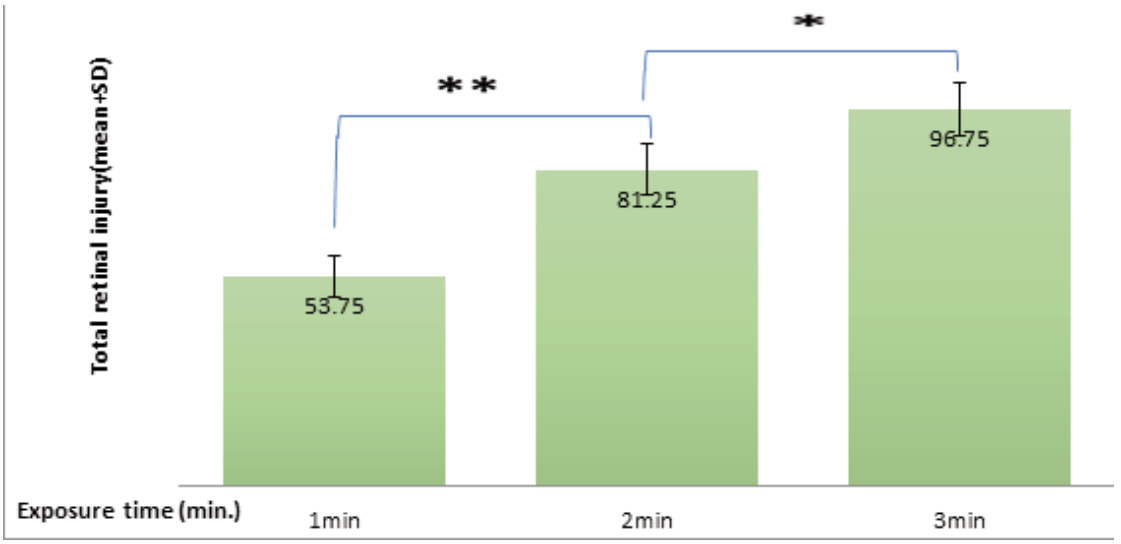

Figure-1: Total retinal damage following exposure to a green laser 50mW power at 1min, 2min and 3min*p<0.05, **p<0.001.

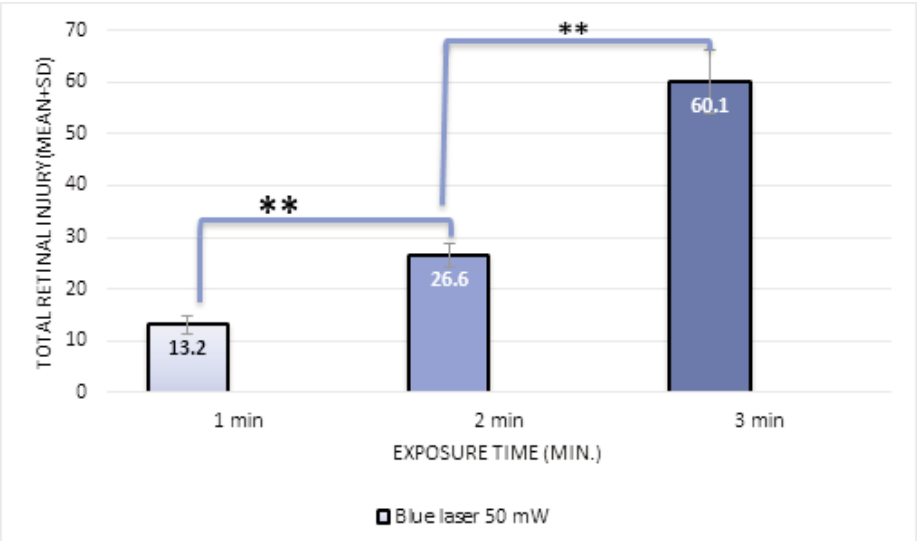

Figure-2: Total retinal damage following exposure to a blue laser 50mW power at 1min, 2min and 3min.
**p<0.001.

more retinal tissue damage in group G compared to group B (p<0.05), and the damage increased significantly with increase in exposure duration in both groups (Figures 1-2).

Group G1 displayed severe and mainly irreversible retinal tissue damage with mean destruction of 53.751+5.469%. These changes included: moderate interstitial oedema within IS and OS, moderate interstitial thinning in OPL, loss of cellular stratification and cytoplasmic vacuolation in ONL, moderately vesicular hydropic swelling in RPE, and moderate choroidal vascular congestion. Group B1 showed mild and mainly reversible injury with mean destruction of 13.2±1.76%. The changes included minor interstitial oedema within IS and OS, few vesicular hydropic swelling in RPE, slight choroid vascular congestion, mild loss of cellular stratification, and few cytoplasmic vacuolations. Group G2 showed severe and mainly irreversible retinal tissue damage with mean destruction of 81.25+6.7%. These changes included slight loss of cellular stratification at INL, considerable thinning of OPL, severe disruption of IS and OS, moderate vesicular hydropic swelling of RPE, predominant cellular necrosis, moderate choroid vascular congestion, localised bleeding, and vascular occlusions. Group B2 showed moderate and mainly reversible damage with mean destruction of 26.6%+2.32%. The changes included slight

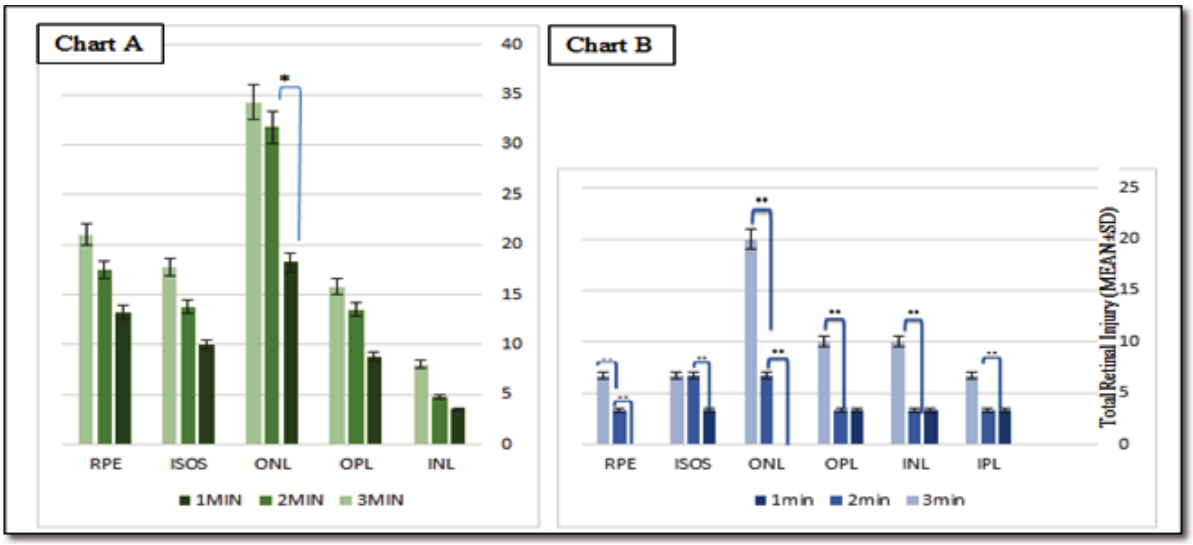

Figure-3: (A) Comparison among retinal layer injury following exposure to 50mW green laser for 1min, 2min and 3min, (B) following exposure to 50mW blue laser for 1min, 2min and 3min.

RPE: Retinal pigment epithelium, ISOS: Inner segment and outer segment, ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: Inner plexiform layer.

loss of stratification in INL, moderate thinning in OPL, moderate disruption of IS and OS, moderate choroid vascular congestion, localised bleeding at the site of exposure, and moderate loss of cellular stratification in ONL with pyknosis and interstitial oedema. Group G3 showed severe injury with mean destruction of 96.75+6.62%. The changes included severe loss of cellular stratification at INL, extended predominate cellular necrosis in RPE, extensive disruption of OPL, extended pyknosis and complete loss of cellular stratification in ONL, complete loss of IS and OS, severe vascular congestion, localised haemorrhage, and choriocapillaris occlusions in the choroid. Group B3 showed severe damage with mean destruction of 60.1+6.04%. The changes included complete loss of IS and OS, total loss of INL cellular stratification, extensive OPL disruption, extensive pyknosis, complete loss of ONL cellular stratification, significant vascular congestion, localised bleeding, choriocapillaris occlusions in the choroid, moderate vesicular hydropic oedema and extended necrosis in RPE.

Across group G, microscopic changes shows that exposure to 50mW green laser continuously for 1min had a significant effect on the tissue of the retina ($p=0.0002$) between G1 and G2, and between G2 and G3 ($p=0.01$).

Across group B, exposure to 50mW blue laser for 1min had a significant difference in the effect on retinal tissue ($p=0.00007$) between B1 and B2, and between B2 and B3 ($p=0.0000006$).

Continuous exposure to green laser for 1min, 2min and 3min had significant difference ($p<0.05$) in ONL, and in ONL, IS/OS and RPE between G1 and G2 ($p<0.001$). No significant difference existed in RPE between G2 and G3 ($p>0.05$).

Continuous exposure to blue laser for 1min, 2min and 3min had significant difference in OPL, ONL, IS/OS and RPE ($p<0.05$), while the difference was highly significant in IS/OS between B1 and B2 ($p<0.001$). There was no significant difference in RPE between B2 and B3 ($p=0.591$) (Figure 3).

Discussion

The commercially available low-power laser pointers can cause retinal injury from unintentional short-term exposure[21]. The laser wavelength, laser power, tissue optical properties, and tissue thermal parameters are the variables that define the first tissue effect. When laser light comes into contact with the biological tissue, it can do a variety of things depending on the recipient's optical characteristics, like reflect, transmit to nearby tissue, scatter, and absorb[22].

The laser must be absorbed and transformed into heat energy for such a chemical biological action to take place in the tissue. This demonstrates that the density of energy, pulse width/duration, and rate of heat conduction all affect how much tissue damage the laser may cause[23]. Lasers can harm the retina through photochemical, thermal, or other means[24].

The key characteristics in this regard are the laser wavelength, spot size, power, exposure duration and

others[4]. It has been demonstrated that light wavelengths in the visible light spectrum are more easily absorbed than those in the infrared range. Melanin, haemoglobin and myoglobin strongly absorb short-wavelength visible laser light (between 400nm and 700nm), whereas infrared wavelengths have limited pigment-specificity, and proteins and water are the main absorbers[25]. Light transmission is essential because longer wavelengths must be able to penetrate the tissue and target deeper regions[23].

The statistical analysis of microscopic changes of all experimental groups showed that continuous 50mW green and blue laser exposure had significantly high differences in their effect on the tissue of the retina ($p<0.001$) between groups G and B at the same exposure time in the current study.

It was found that exposure to both types of lasers used could cause irreversible retinal layers injury, especially the outer nuclear cells and the retinal pigmented cells, so that the exposure to 50mW blue laser for >3min caused irreversible retinal tissue injury at the site of exposure with mean destruction of 60.1+6.04%, and exposure to green laser 50mW for only 1min caused severe irreversible retinal tissue injury at the site of exposure with mean destruction of 53.751+5.469%.

Therefore, the green laser pointer had more effect on the outer layers of the retina than the blue laser pointers, and it is possible that the green lasers had more absorption by RPE than other wavelengths. While in the case of blue laser, it was obvious that it had less effect on the outer nuclear layers than green laser because it underwent scattering and absorption by the anterior parts of the eye (cornea, lens, vitreous humour, aqueous humour) before reaching the retina, and this corresponded with the finding of an earlier study[26]. One study[27] mentioned that the kind of tissue, laser wavelength, and laser power affect how deeply the signal is transmitted to the tissue.

In the case of blue laser, a study[28] found that eye injury was intimately related to shortwave blue light with wavelengths between 415nm and 455nm. This intense blue light reached the retina after passing through the cornea and lens, which could cause different degrees of retinal, crystal-clear and corneal damage. In the current study, the effect of a blue laser pointer on the RPE was mild-moderate, and affected the anterior parts of the eye (cornea, lens, vitreous humour, aqueous humour) where it was absorbed in the cornea, unlike other lasers. This was due to the scattering effect, where the scattering of tissue is always a combination of Rayleigh and Mie scattering depending on what structures are dominant[3]. So, violet and blue light of shorter wavelength will scatter more than longer wavelength light because Rayleigh scattering is inversely proportional to the 4^{th} power of wavelength (yellow and especially red light)[29]. Therefore, light with shorter wavelengths, like the blue light, is absorbed by the cornea more readily than light with a longer wavelength[30].

Conclusion

The green laser pointer of 50 mW caused more intensive and irreversible damage to retinal layers than the blue laser pointers at the same exposure time. Besides, 1min of continuous exposure to a 50mW green laser was enough to elicit irreversible retinal tissue injury, while continuous exposure to 50mW blue laser for >3 minutes elicited the same irreversible retinal tissue injury.

Acknowledgement: We are grateful to the College of Medicine and Mustansiriyah University for facilitating the study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Al Musawi MS, Al-Gailani BT. In Vitro Biostimulation of Low-Power Diode Pumping Solid State Laser Irradiation on Human Serum Proteins. Photobiomodul Photomed Laser Surg 2020;38:667-72. doi: 10.1089/photob.2020.4873.
2. Al-Musawi MS, Jaafar MS, Al-Gailani TB, Ahmed NM, Suhaimi FM. In vitro effects of low level yellow laser irradiation on human red blood cells. In: IEEE 6th International Conference on Photonics (ICP). Kuching, Malaysia, 2016, pp 1-3. doi: 10.1109/ICP.2016.7510024.
3. Ansari MA, Erfanzadeh M, Mohajerani E. Mechanisms of Laser-Tissue Interaction: II. Tissue Thermal Properties. J Lasers Med Sci 2013;4:99-106.
4. Al-Kaabi AAK, Al-Musawi MS, Hasan AA. In Vitro Effect of Low-Level Lasers on Total Bilirubin Concentration in Human Blood Plasma Using 375 and 650 nm Lasers. Photobiomodul Photomed Laser Surg 2024;42:49-53. doi: 10.1089/photob.2023.0141.
5. Ristova M, Kuo Y, Lee HH. Study of hydrogenated amorphous silicon thin films as a potential sensor for He–Ne laser light detection. Appl sur sci 2003;218:44-53. doi: 10.1016/S0169-4332(03)00698-6.
6. Yahia MJ, Hasan JA, Musawi MS. Biostimulation effect of DPSS laser irradiation with different power densities and radiation times on blood viscosity in vitro. AIP Conf Proc 2020;2213,020118. doi: 10.1063/5.0000438.
7. Nontapot K, Rujirat N. High Accuracy Laser Power Measurement for Laser Pointers Safety Assessment. In: Ribeiro P, Raposo M, eds. Optics, Photonics and Laser Technology. Cham, Switzerland; Springer, 2018; pp 67-82.
8. Alsulaiman SM, Alrushood AA, Almasaud J, Alzaaidi S, Alzahrani Y, Arevalo JF, et al. High-power handheld blue laser-induced maculopathy: the results of the King Khaled Eye Specialist Hospital Collaborative Retina Study Group. Ophthalmology 2014;121:566-72.e1. doi: 10.1016/j.ophtha.2013.09.006.

9. Turaka K, Bryan JS, Gordon AJ, Reddy R, Kwong HM Jr, Sell CH. Laser pointer induced macular damage: case report and mini review. Int Ophthalmol 2012;32:293-7. doi: 10.1007/s10792-012-9555-z.
10. Kolb H. Simple anatomy of the retina by helga kolb. [Online] 2011 [Cited 2024 august 24]. Available from URL: https://webvision.med.utah.edu/book/part-i-foundations/simple-anatomy-of-the-retina/
11. Nguyen KH, Patel BC, Tadi P. Anatomy, Head and Neck: Eye Retina. Fl, US: Statpearls; 2023.
12. Al Musawi MS, Jafaar MS, Ahmed NM, Al-Gailani BT, Suhaimi FM. Effects of Low Power Violet Laser Irradiation on Red Blood Cells Volume and Erythrocyte Sedimentation Rate in Human Blood. AIP Conf Proc 2017;1875:020005. doi: 10.1063/1.4998359.
13. Hildebrand GD, Fielder AR. Anatomy and Physiology of the Retina. In: Reynolds J, Olitsky S, eds. Pediatric Retina. Berlin, Germany: Springer, 2010; pp 39-65.
14. Guido ME, Marchese NA, Rios MN, Morera LP, Diaz NM, Garbarino-Pico E, et al. Non-visual Opsins and Novel Photo-Detectors in the Vertebrate Inner Retina Mediate Light Responses Within the Blue Spectrum Region. Cell Mol Neurobiol 2022;42:59-83. doi: 10.1007/s10571-020-00997-x.
15. Crooke A, Guzmán-Aranguez A, Peral A, Abdurrahman MK, Pintor J. Nucleotides in ocular secretions: their role in ocular physiology. Pharmacol Ther 2008;119:55-73. doi: 10.1016/j.pharmthera.2008.04.002.
16. Disatham J, Brennan L, Cvekl A, Kantorow M. Multiomics Analysis Reveals Novel Genetic Determinants for Lens Differentiation, Structure, and Transparency. Biomolecules 2023;13:693. doi: 10.3390/biom13040693.
17. Yahia MJ, Hasan JA, Musawi MS. Influence of DPSS laser radiation with different power densities and exposure times on erythrocyte sedimentation rate–an in-vitro investigation. AIP Conf Proc 2020;2213,020028. doi: 10.1063/5.0000436.
18. Birtel J, Harmening WM, Krohne TU, Holz FG, Charbel Issa P, Herrmann P. Retinal Injury Following Laser Pointer Exposure. Dtsch Arztebl Int 2017;114:831-7. doi: 10.3238/arztebl.2017.0831.
19. Linton E, Walkden A, Steeples LR, Bhargava A, Williams C, Bailey C, et al. Retinal burns from laser pointers: a risk in children with behavioural problems. Eye (Lond) 2019;33:492-504. doi: 10.1038/s41433-018-0276-z.
20. Hunter JJ, Morgan JI, Merigan WH, Sliney DH, Sparrow JR, Williams DR. The susceptibility of the retina to photochemical damage from visible light. Prog Retin Eye Res 2012;31:28-42. doi: 10.1016/j.preteyeres.2011.11.001.
21. Robertson DM, Lim TH, Salomao DR, Link TP, Rowe RL, McLaren JW. Laser pointers and the human eye: a clinicopathologic study. Arch Ophthalmol 2000;118:1686-91. doi: 10.1001/archopht.118.12.1686.
22. Elgendy H, Maia RR, Skiff F, Denehy G, Qian F. Comparison of light propagation in dental tissues and nano-filled resin-based composite. Clin Oral Investig 2019;23:423-33. doi: 10.1007/s00784-018-2451-9.
23. Allemann IB, Kaufman J. Laser principles. In: Alleman IB, Goldberg DJ, eds. Basics in Dermatological Laser Applications. Basel, Switzerland; Karger, 2011; pp 7-23.
24. Mainster MA. Retinal laser accidents: mechanisms and management. J. Laser Appl 2000;12:3-9. doi: 10.2351/1.521906.
25. Bordin-Aykroyd S, Dias RB, Lynch E. Laser-tissue interaction. EC Dent Sci 2019;18:2303-8.
26. Lee HS, Cui L, Li Y, Choi JS, Choi JH, Li Z et al. Influence of Light Emitting Diode-Derived Blue Light Overexposure on Mouse Ocular Surface. PLoS One 2016;11:e0161041. doi: 10.1371/journal.pone.0161041.
27. Brown MS, Arnold CB. Fundamentals of Laser-Material Interaction and Application to Multiscale Surface Modification. In: Sugioka K, Meunier M, Piqué A, eds. Laser Precision Microfabrication. 1st ed. Berlin, Germany; Springer, 2010; pp 337-44.
28. Zhao ZC, Zhou Y, Tan G, Li J. Research progress about the effect and prevention of blue light on eyes. Int J Ophthalmol 2018;11:1999-2003. doi: 10.18240/ijo.2018.12.20.
29. Al Musawi MS, Jaafar MS, Al-Gailani B, Ahmed NM, Suhaimi FM. Laser-induced changes of in vitro erythrocyte sedimentation rate. Lasers Med Sci 2017;32:2089-95. doi: 10.1007/s10103-017-2340-5.
30. Lee HS, Cui L, Li Y, Choi JS, Choi JH, Li Z, et al. Influence of Light Emitting Diode-Derived Blue Light Overexposure on Mouse Ocular Surface. PLoS One 2016;11:e0161041. doi: 10.1371/journal.pone.0161041.