

RESEARCH ARTICLE

Histological assessment of the effect of 50mW red laser on retina at variable time intervals: animal model

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Abstract

Objective: To assess the histopathological effect of 50milliWatt red laser pointer on mice retinal tissue at variable exposure times.

Method: The experimental study was conducted in the Postgraduate Laboratory of the Unit of Medical Physics, Department of Physiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, from November 2020 to April 2021, and comprised albino mice. Histopathological effects on the retinal tissues were evaluated microscopically using conventional haematoxylin and eosin stain just after being exposed to commercially available 50 milliWatt red laser pointers with 630nm wavelength at 1, 2 and 3 minutes, grouped as R1, R2 and R3, respectively. The laser beam was focussed on the right eye of the mice sedated with sodium pentobarbital, reserving the left eye for use as a control group. Data was analysed using SPSS 24.

Results: Of the 12 mice, 4(33.3%) were in each of the 3 groups. There was significantly destructive effect of red laser on retinal outer cellular layers in all the groups compared to the controls ($p<0.05$). The effect was significant between R1 and R2 ($p=0.0001$), and between R2 and R3 ($p=0.02$).

Conclusion: The handheld commercial red laser pointers could harmfully affect a wide range of mice retina and choroid.

Key Words: Eosine, Pentobarbital, Retina, Lasers, Choroid, Sodium.

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Introduction

Laser energy in the ultraviolet (UV) and far-infrared parts of the spectrum does not reach the eye, and, hence, it is not concentrated in the retina that may cause corneal damage¹. Laser pointers can transfer more light energy into the eye than staring directly into the sun, and that could be harmful. This is owing to the refractive characteristics of cornea and crystalline lens that enhance irradiance (W/cm^2) reaching the retina by 105 times². Because of its consistent qualities and inexpensive cost, the red laser pointer is one of the most commonly used light sources³. By adding Indium gallium nitride (InGaN) or gallium nitride (GaN) self-organised quantum dots as the gain media, red lasers emit at 630nm, the longest wavelength obtained with the nitride system⁴. Red laser pointers can generate light at a variety of wavelengths, including infrared radiation, which is invisible to the naked eye⁵. The red laser's beam is difficult to perceive in a normal night environment⁶. During visual presentations, laser pointers have been used for years to highlight crucial regions on charts and screens. Laser pointers are not considered dangerous when they are

used responsibly. While brief ocular exposure to the light emitted by these devices can be frightening and cause transient vision impairment, such exposures are too brief to cause permanent harm to the eye². The majority of laser pointers on the market today have wavelengths of 670nm to 632nm with a power of 5mW. Class 3R lasers, which have a maximum strength of 5mW, are potentially harmful to the eyes, and direct eye contact should be avoided⁷. The manufacturing of laser pointers for the general public is generally limited, but varies from country to country. The United Kingdom's Health Protection Agency suggested that laser pointers for the general public be limited to 1 milliWatt (mW) because no injuries have been documented at this level. Regulatory authorities in the United States allow lasers with a maximum power of 5mW. Studies have revealed that when observed for several seconds, laser beams with a low strength of <5mW induce irreversible retinal damage, although this is difficult due to the blink reflex of the eye⁸.

The retina is the eye's light-sensitive innermost layer. The eye optics produce concentrated two-dimensional (2D) images on the retina, which are then translated into electrical impulses by the brain to produce visual impressions¹. The sensory retina and the retinal pigment epithelium are the two layers that make up the retina⁹. The layers of neurons that make up the neural retina are retinal ganglionic cell (RGC), inner plexiform layer (IPL),

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inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segment (IS), and outer segment (OS). The ONL's photoreceptor cells (rods and cones) are the retina's principal light-sensitive cells and the image-forming visual system's sensors^{1,10}. Black-and-white vision is provided by using rods in low light. A brightly lit environment is best for cones because they are responsible for colour perception and high-acuity vision¹¹. The neural retina of the eye is meant to provide a visual image of the environment. The only transparent structures in the human body are the clear ocular media; cornea, aqueous humour, crystalline lens and vitreous humour. This is owing to the clarity of the eye media and the eye's inherent focussing characteristics in the visible and near-infrared regions of the spectrum¹². As a result, if light reaches the retina, the visual pigments in the rods and cones absorb a tiny quantity of light to trigger the visual response, while the remaining energy is absorbed in the retinal pigment epithelium (RPE) and choroid¹³. So, a large portion of the beam passes through the pigment epithelium, which is the fundus' first absorbing layer. Within this layer, the maximum absorption per unit volume occurs because it includes melanin granules¹. It serves as a light absorber and is also the largest energy absorber in laser exposure⁵. It then starts a chain of chemical and electrical reactions that finally activate nerve impulses sent through the optic nerve fibres to different vision centres in the brain¹⁴. Laser pointer-induced retinopathy is known to occur when certain laser pointers are focussed into a person's eye¹⁵. Misuse of a laser pointer can result in irreversible retinal damage, depending on wavelength, radiation output power, period of exposure, laser spot size, and damage localisation⁵.

The current study was planned to assess the histopathological effect of 50mw red laser pointer on mice retinal tissue at variable exposure times.

Materials and Methods

The experimental study was conducted in the Postgraduate Laboratory of the Unit of Medical Physics, Department of Physiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, from November 2020 to April 2021.

After approval from the institutional ethics committee, the sample was raised with albino male mice who were divided into three equal groups in relation to the duration of exposure to red laser. The mice in all the groups had free access to food and fluids.

Diode laser pointers (Shenzhen Huianqi Technology Co., Ltd, China) were chosen with a gallium-aluminium-

arsenide emitter having an output power of 50mW and a wavelength of 630nm for red laser. Using a small handpiece, it was possible to produce a focussed beam. The energy was measured using an optical power meter (Gentec-E, Mestro, Canada).

The laser beam was focussed on the right eye of mice sedated with sodium pentobarbital, reserving the left eye for use as a control group. Histopathological effects on the retinal tissues were evaluated microscopically using conventional haematoxylin and eosin (H&E) stain just after exposure to 130mW/cm² of total power density for 1, 2 and 3 minutes, grouped as R1, R2 and R3, respectively. The laser irradiations were carried out in the dark with proper protective eyewear on. The irradiation was carried out at a mean temperature of 23±2°C.

The exposed and non-exposed eyes of the experimental animals were quickly infected and perfused with 10% neutral-buffered formalin solution for at least 24 hours after they were sacrificed by cervical dislocation.

The eyeball samples were sliced into two pieces, embedded in paraffin blocks, sectioned into 4µm sections, and then placed on adhesive slides to be stained with H&E. Using a light microscope, each section was examined by a double-blind histopathologist to determine the extent to which the retinal tissue had been destroyed, if any had been, and classified as no lesion, mild, moderate or severe, which corresponded to <5%, 5-25 %, 25-50% and 50-100% of normal retinal tissue in each section¹.

Data was analysed using SPSS 24. Data was expressed as mean + standard deviation. Data was analysed at 95% confidence interval (CI), and p<0.05 was considered statistically significant.

Results

Of the 12 mice, 4(33.3%) were in each of the 3 groups. There was significantly destructive effect of red laser on retinal outer cellular layers in all the groups compared to the controls (p<0.05). Group R1 showed retinal tissue injury at the site of exposure with mean destruction score 15.25±0.957%, which was mild and mainly reversible injury. The changes included mild thinning in OPL, mild loss in the cellular stratification of ONL with few cytoplasmic vacuolation, disrupted IS and OS due to mild interstitial oedema, a few vesicular hydropic swellings in RPE with mild choroid vascular congestion (Figure 1A).

Group R2 showed retinal tissue injury with mean destruction score 30 ±3.366%, which was moderate and irreversible injury. The changes included mild loss of

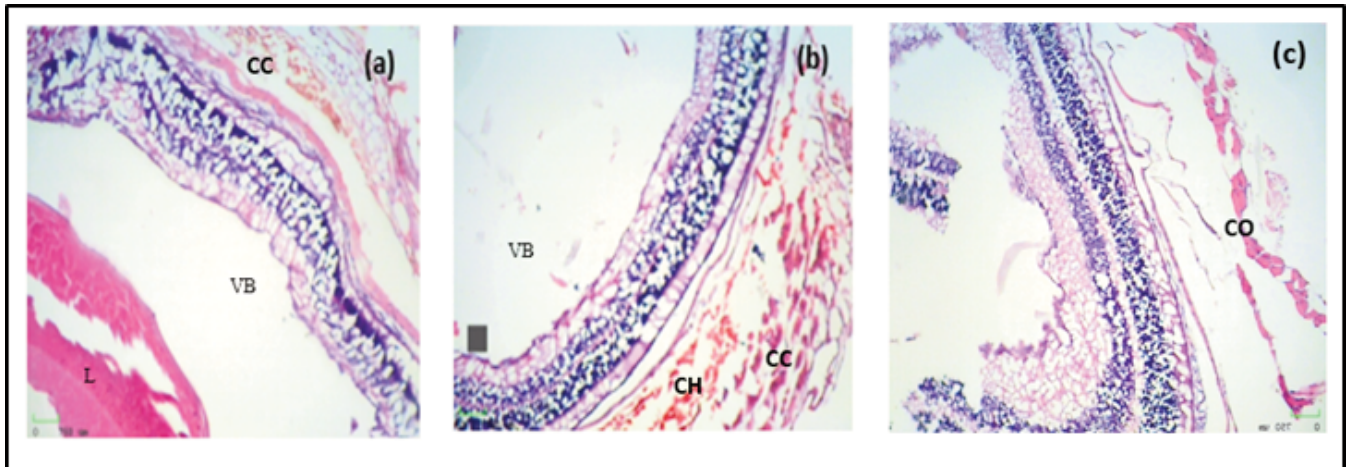


Figure-1: Microscopic changes seen in formalin-fixed-paraffin-embedded haematoxylin and eosin (H&E) stained mice retinal tissue section of (a) group R1 that was exposed to 50mW red laser for 1min showing mild reversible retinal tissue injury $15.25 \pm 0.957\%$ compared to (b) group R2 that was exposed to 50mW red laser for 2min and showed moderate mainly reversible retinal tissue injury $30 \pm 3.366\%$ and (c) group R3 that was exposed to 50mW red laser for 3min showing moderate irreversible retinal tissue injury $49.25 \pm 6.55\%$. X100. L: Lens, VB: Vitreous body, CC: Choroidal vascular congestion, CH: Choroidal haemorrhage, CO: Chorio-capillary occlusions.

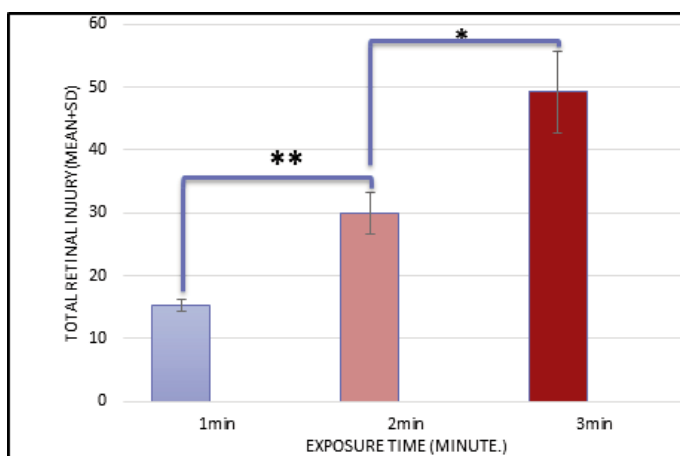


Figure-2: Total retinal damage following exposure to red laser of 50mW power at different 1min, 2min and 3min.

* $p < 0.05$, ** $p < 0.001$, SD: Standard deviation.

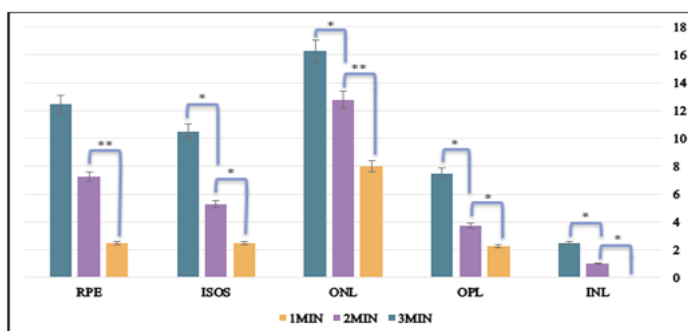


Figure-3: Comparison among retinal layer injury following exposure to 50mW red laser for 1min, 2min and 3min. * $p < 0.05$, ** $p < 0.001$. RPE: Retinal pigment epithelium, ISOS: Inner segment and outer segment, ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer.

stratification in INL, moderate thinning in OPL, moderate loss of cellular stratification in ONL with pyknosis and interstitial oedema, moderate disruption of IS and OS, moderate choroid vascular congestion and focal haemorrhage at the site of exposure (Figure 1B).

Group R3 showed retinal tissue injury with mean destruction score at site of exposure $49.25 \pm 6.55\%$, which was moderate to severe injury. The changes included loss of cellular stratification of INL, extensive disruption of OPL, extensive pyknosis and complete loss of cellular stratification in ONL, complete loss of IS and OS, moderate vesicular hydropic swelling and extended necrosis in RPE, severe vascular congestion, focal haemorrhage and chorio-capillary occlusions in the choroid (Figure 1C).

The effect was significant between groups R1 and R2 ($p = 0.0001$), and between groups R2 and R3 ($p = 0.02$) (Figure 2).

Duration of exposure increased injury score directly in INL, OPL and IS/OS ($p < 0.05$). The difference in the effect in ONL and RPE between groups R1 and R2 was significant ($p < 0.001$), but there was no significant difference related to RPE between groups R2 and R3 ($p > 0.05$) (Figure 3).

Discussion

The misuse of commercially available low-power laser pointers has witnessed increasing public concern, because it not only can be distracting and annoying, but carry the potential risk of producing permanent retinal damage from transient, inadvertent laser pointer exposure¹⁶. The factors

that determine the initial tissue effect include the laser wavelength, laser power, laser waveform, tissue optical properties and tissue thermal properties. Light interacts in many ways with biological tissues that are influenced by the recipient's optical properties so that when laser light strikes the tissue, it can reflect, scatter, be absorbed or get transmitted to the surrounding tissue¹⁷. The biological action in the tissue can only be realised if it is absorbed and converted into heat energy in the process. This indicates that the amount of tissue damage caused by the laser is dependent on the energy density, the pulse width/duration, and the heat conduction rate¹⁸. Laser can damage the retina by photochemical or/and thermal mechanisms¹⁹ depending on the laser wavelength, spot size, power, exposure time and other factors¹. The light wavelengths, in the visible light spectrum, have been shown to be more readily absorbed than those on the infrared spectrum. Visible laser light, of short wavelength between 400nm and 700nm is highly absorbed by melanin, haemoglobin (Hb) and myoglobin, while infrared wavelengths have little pigment-specificity and the prime absorbing media are proteins and water²⁰. Furthermore, longer wavelengths are able to reach and target deeper structures within the tissue, and, therefore, light transmission is critical¹⁸.

In the current study, it was obvious that the red laser reached the retina, but its effect was mild-moderate, because red lasers have a long wavelength and so they generate lower energy levels and cause less damage when they pass through tissues, which make the energy of red laser pointer insufficient to be absorbed by RPE. Therefore, the red laser pointer requires longer durations of exposure and high power to cause permanent damage to the retina, but the harmful effect can spread to a wider extent on the retina. Thus, red laser can damage the outer retina due to the presence of photo-acceptor melanin granules where the highest absorption per unit volume usually occurs, and choroidal layers as well due to its high penetration⁷.

Conclusion

The handheld commercial red laser pointers could harmfully affect a wide range of mice retina and choroid.

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Conflict of Interest: None.

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References

1. Yahia MJ, Hasan JA, AL 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.
2. Nakagawara VB, Montgomery RW. Laser pointers: Their potential affects on vision and aviation safety. [Online] 2001 [Cited 2024 August 22]. Available from URL: https://www.faa.gov/sites/faa.gov/files/data_research/research/med_humanfacs/oamtechreports/0107.pdf
3. 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.
4. Frost T, Banerjee A, Sun K, Chuang SL, Bhattacharya P. InGaN/GaN Quantum Dot Red ($\lambda=630$ nm) Laser. *IEEE J Quantum Electron* 2013;49:923-31. doi: 10.1109/JQE.2013.2281062
5. 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.
6. Robertson DM, McLaren JW, Salomao DR, Link TP. Retinopathy from a green laser pointer: a clinicopathologic study. *Arch Ophthalmol* 2005;123:629-33. doi: 10.1001/archophth.123.5.629.
7. Kaya M, Yagci BA. Bilateral macular injury following red laser pointer exposure: A case report. *Eur Eye Res* 2021;1:170-3.
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. doi: 10.1016/j.ophtha.2013.09.006.
9. Evangelou N, Alrawashdeh OSM. Anatomy of the Retina and the Optic Nerve. In: Petzold A, eds. *Optical Coherence Tomography in Multiple Sclerosis*, 1st ed. Cham, Switzerland: Springer Cham, 2015: pp 3-19.
10. Hildebrand GD, Fielder AR. Anatomy and Physiology of the Retina. In: Reynolds JD, Olitsky SE, eds. *Pediatric Retina*, 1st ed. Berlin, Germany: Springer Berlin Heidelberg, 2010: pp 39-65.
11. Bommel WV. Interior Lighting: Fundamentals, Technology and Application, 1st ed. Cham, Switzerland: Springer Cham; 2019.
12. Sliney DH. How light reaches the eye and its components. *Int J Toxicol* 2002;21:501-9. doi: 10.1080/10915810290169927.
13. Marmor MF, Wolfensberger TJ. The retinal pigment epithelium: function and disease, 1st ed. Oxford, England: Oxford University Press; 1998.
14. Buehler A, Sitaras N, Favret S, Bucher F, Berger S, Pielan A, et al. Semaphorin 3F forms an anti-angiogenic barrier in outer retina. *FEBS Lett* 2013;587:1650-5. doi: 10.1016/j.febslet.2013.04.008.
15. Petrou P, Patwary S, Banerjee PJ, Kirkby GR. Bilateral macular hole from a handheld laser pointer. *Lancet* 2014;383:1780. doi: 10.1016/S0140-6736(14)60757-1.
16. 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/archophth.118.12.1686.
17. Dederich DN. Laser/tissue interaction: what happens to laser light when it strikes tissue? *J Am Dent Assoc* 1993;124:57-61. doi: 10.14219/jada.archive.1993.0036.
18. In: Allemann IB, Goldberg DJ, eds. *Basics in Dermatological Laser Applications*, 42 vol. Basel, Switzerland: S. Karger AG; 2011.
19. Mainster MA. Retinal laser accidents: mechanisms and management. *J Laser Appl* 2000;12:3-9. doi: 10.2351/1.521906.
20. 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.