International Journal of Scientific Research in Biological Sciences
Research Paper
Vol.9, Issue.1, pp.42-47, February (2022)
E-ISSN: 2347-7520

# Histopathological Changes Caused by Monochromatic Wavelengths in the Retina of Two Species of frogs

**Semaa Ahmed Baker[1*], Ali Ashgar Abd[2]**

[1]Deparment of Biology, College of Education for Pure Science, Mosul University, Mosul, Iraq
[2]Al-Noor University College, Mosul, Iraq

[*]*Corresponding Author: semaa.baker@gmail.com, Tel.: +9647738499144*

**Available online at: www.isroset.org**

Received: 24/Dec/2021, Accepted: 17/Jan/2022, Online: 28/Feb/2022

***Abstract***— The current research deals with the study of the effect of monochromatic visible radiation on the retina of the eye, which are a beam of blue light whose wavelength ranges from 450-500 nanometers and intensity of illumination of 200 lux and under a temperature of 10oC in winter and 20℃ in summer, and a beam of yellow light whose wavelength ranges from From 550-600 nanometers, with an intensity of 225 lux, and under the same temperatures, for three months for each of the two lights. I took two types of frogs, the first is the swamp frog Rana ridibunda ridibunda and the second tree frog Hyla arborea savignyi. The effects in type 1 retina were generally greater than in type 2. On the other hand, the effects of blue light were more than yellow light in general, with close damage to the two lights in some cases, and the damage was at a high temperature of 20℃ more than at the temperature of 10℃ for the two types of light. The damage caused by monochromatic colors of visible light appeared to follow the wavelength, temperature, and intensity of illumination, as the shorter the wavelength, the higher the temperature and the higher the intensity of the illumination, the greater the damage to the components of the eye, and the absence of clots in the tissue of the eye indicates that the light damage occurred by the mechanism photochemical.

***Keywords***— *Retina, Monochromatic Wavelengths, Swamp Frog, Tree Frog*

## I. INTRODUCTION

The diversity of the eyes is astonishing, reflecting the wide range of adaptations resulting from the euphoric pressure to enable the animal to see in different environments. Since the eye collects and concentrates light, its composition depends on the physical properties of the light, which sets restrictions on the optical qualities of the eye. The reason for this is due to the early emergence of the eye that took place in water, as water absorbs or filters light strongly [1], and thus the primary euphoric pressure on the primary organisms was to enable vision in narrow limits of wavelengths. The selection of biochemical mechanisms to sense this narrow range of wavelengths made the eye the ability to sense the wavelength during development. Although many types of animals moved from water to land and were exposed to a wide spectrum of electromagnetic rays coming from the sun, most of the animals' eyes remained restricted to seeing within the narrow band of lights, however, some species of fish and birds later developed additional receptors to sense ultraviolet rays. Suggest that the position of the eye in terms of type, size, and location can be easily developed [2].

In vertebrates, the eye is designed to separate visual tasks into three structural levels: the optical parts that do not sense light, including (the cornea, lens, iris, and vitreous humor), and the second level are the photoreceptor neurons and their associated neurons that complete the transmission of nerve impulses, and these It is represented by the retina, and the third level is the photochemical pigment called the visual pigment, which is concentrated in the outer segments of the photoreceptor cells and represents the first part that light strikes, as well as the accessory layers, the choroid, and sclera, which perform the processes of attachment and nourishment of the previous components [3].

Amphibians are the most primitive terrestrial animals of the vertebrates and are an intermediate state in the phylogenetic site between fish and reptiles. Amphibians are subject to the impossibility of total or partial respiration on land, as they have moist skin [4]. Amphibians have greatly contributed to the knowledge of the visual system of vertebrates, including humans. Vision is the main sense used in detecting the movement of prey, and on the other hand, information about amphibian eyesight was obtained mainly from studies of the retina. Anura (frogs) depend on vision for their nutrition and movement. Frogs have larger and more developed eyes among amphibians [5].

## II. MATERIAL AND METHODS

The current study dealt with two species of frogs, the first is the swamp frog, Rana ridibunda ridibunda, and the second is the tree frog. Hyla arborea savignyi Samples of the first type were collected from the two areas of Hamdaniya, which is about 30 km east of Mosul, and

Ghara village, which is about 80 km north of Mosul, and the second type was collected from Si Kurdak village, about 90 km north of Mosul.

Two groups of each type of frogs were placed, each group in a separate basin containing water 15 cm high interspersed with groups of stones consisting of 12 frogs in transparent plastic basins and in the darkroom. Shine blue light on two basins, one for each type, and yellow light also on two basins, one for each type. Colored lamps were used, the capacity of each lamp is 5 watts, four for each basin, and they were installed at a height of 50 cm from the bottom of the basin. The basins were covered with lamps with black cloth to prevent the light from spreading to the neighboring basins. The wavelengths, intensity of lighting, exposure period and temperature of the basin were as in the table below for each From my winter and summer experiences, the temperature was set by the HAAKE K F3 digital refrigeration unit.

Table 1

| Visible light color spectrum | Wavelength used | Intensity of illumination | Time period | temperature | |
|---|---|---|---|---|---|
| | | | | Summer experience | Winter experience |
| Blue | 450-500nm | 200lux | Three months | 20°C | 10°C |
| Yellow | 550-600nm | 225lux | Three months | 20°C | 10°C |

The wavelengths were determined in the physics laboratory at the College of Education and the intensity of light for each color was measured with a Luxmeter Model Lx 101 Sampo/china 2007.

Both types of frogs were dissected after being anesthetized with chloroform at ten o'clock in the morning and then the eyes were taken out using curved fine tweezers after cutting the surrounding bones with fine scissors. Immediately after extraction, the eye was transferred to a Petri dish containing a piece of gauze or filter paper to clean the eye and immersed in a physiological solution [6]. The duration of the autopsy was as fast as possible to minimize postmortem changes in tissues that may occur after death. The subsequent operations of fixation, washing, dehydration, clearing, infiltration, blocking , trimming, cutting,mounting, and coloring were carried out to prepare the tissue sections. The tissue sections fixed on the slides were colored with general textile and other histological chemical dyes, including Hematoxylin-Delafield′s Hematoxylin and Eosin Stain. (HE) was prepared and stained with Hematoxylin Delafield according to [6], eosin was prepared and stained according to [7], The Periodic Acid Schiff Technique (PAS) was prepared and stained As stated in [8], Toluidin Blue Technique was prepared and colored as stated in [8]. The selected sections were imaged using a Reichert Neovar Type 300422 Compound Microscope equipped with a Japanese-made MDCE-5A camera with an ASH 100 sensitivity.

## III. Results

The results of the current study showed the occurrence of various histopathological changes in the histological structure of some components of the eye when exposed to visible blue and yellow light at different temperatures for three consecutive months. A discrepancy appeared in the effect between the two types of frogs on the one hand and the two lights used in each type on the other. In general, the results showed that the effect of blue light was more than the yellow light on the one hand, and the effect of both lights was more in summer than in winter, and on the other hand, it became clear that the effect on the components of the eye of the frog Rana was more than that of the frog Hyla. The following is a summary of the effects that occurred in the two types.

**Blue light**

After three months of exposure in the winter, the effects of blue light appeared in the retina. In the first type of frog Rana, various damages occurred, as the degeneration of the epithelial pigment cells and necrosis of some of them appeared with the occurrence of edema and hemorrhage. Extensive damage to the photoreceptor cells was also shown in this part (Fig. 1). Damage to some bacilli was demonstrated with fascicle (Fig. 2).

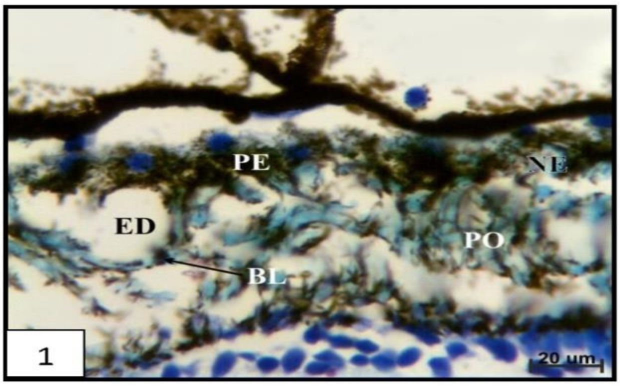


Figure (1): Cross-section of the eyeball of the first type exposed to blue light for three months in winter. Note the degeneration of epithelial cells PE , and some necrosis NE, edema ED of photoreceptors, bleeding BL, and extensive damage of photoreceptors PO, TB stained.

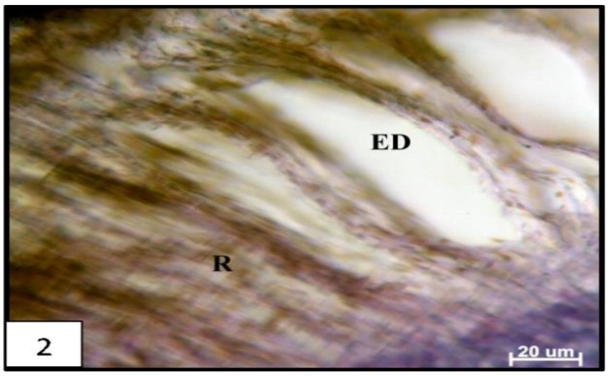


Figure (2): Cross-section of the eyeball of the first type exposed to blue light for three months in winter. Note damage to some rods R with edema ED, PAS stained .

 

In the second type of frog Hyla, the degeneration of the epithelial pigment cells appeared with the appearance of phagocytic melanocytes in the layer of the photoreceptor cells, and necrosis appeared in some of the outer and inner segments of the photoreceptor cells with the disintegration of their nuclei and the appearance of a tumor in the inner nuclear layer (Fig. 3). It also appeared in addition to the degeneration of the epithelial pigment cells, damage to some rods, and disintegration in the inner and outer nuclear layers with local necrosis in the inner plexiform layer (Fig. 4).

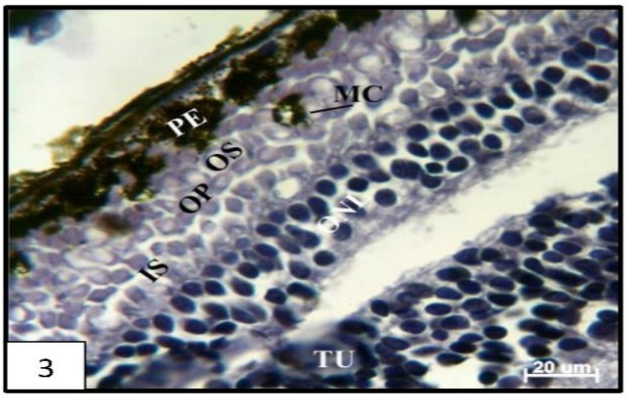


Figure (3): Cross-section of the eyeball of the second type exposed to blue light for three months of winter. Note the degeneration of pigment epithelial cells PE, phagocytic melanocytes MC appearing in the photoreceptor layer, necrosis of some outer segments OS of photoreceptors, and some inner segments IS, the disintegration of their nuclei (outer nuclear layer) ONL, tumor TU in the inner nuclear layer, TB stained .

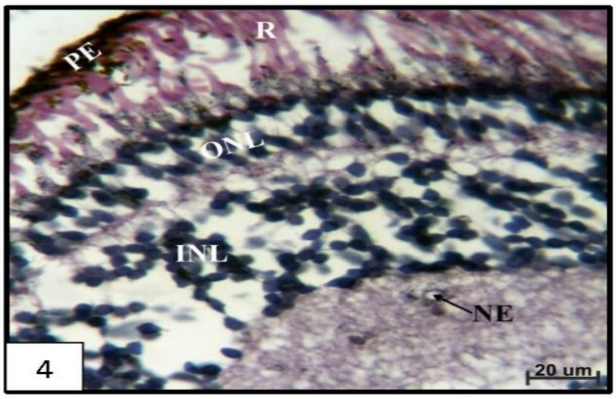


Figure (4): Cross-section of the eyeball of the second type exposed to blue light for three months in winter. Note the degeneration of pigment epithelial cells PE, damage to some rods R, the disintegration of the outer nuclear layer ONL, and the inner INL, local necrosis NE of the inner plexiform layer, PAS stained.

As for the blue light in summer, it also caused histopathological effects, but more than in winter in the two species of frogs. In the first type of frog, retinal damage appeared, including extensive and almost complete damage in some parts of the epithelial pigment cells, photoreceptor cells, and their nuclei (Fig. 5). Also, fibrosis and tumors occurred in the inner plexiform layer (Fig. 6).

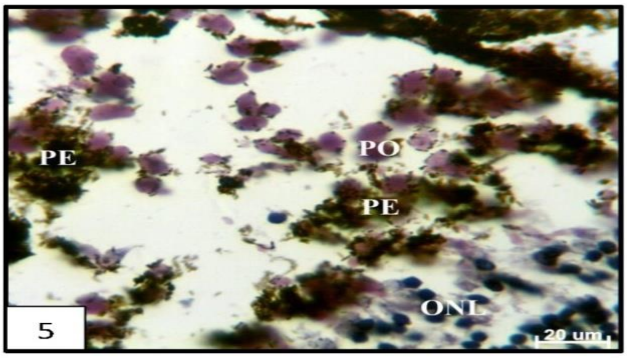


Figure (5): Cross-section of the eyeball of the first type exposed to blue light for three months in summer. Note extensive and almost complete damage in some parts in pigment epithelial cells PE, photoreceptors PO, and their nuclei (outer nuclear layer) ONL. The remains of the removal cells took high famine with , PAS stained , PAS stained.

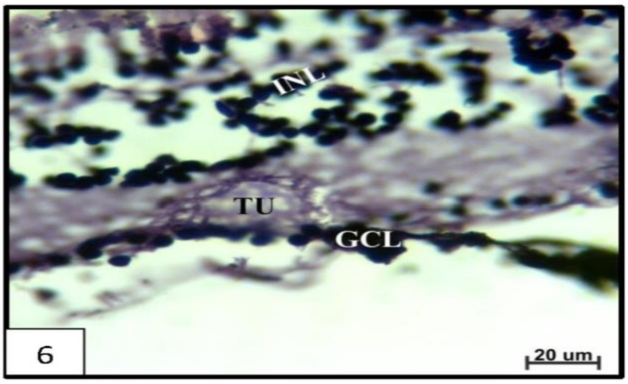


Figure (6): Cross-section of the eyeball of the first type exposed to blue light for three months in summer. Note fibrosis and tumor TU in the inner plexiform layer, the inner nuclear layer INL, the ganglion cell layer GCL, HE stained.

In the second type of frog, some rods and cones were damaged, epithelial cells degenerated, granulomas appeared, and hyperdegeneration occurred in a part of them (Fig. 7). In addition to the degeneration of the pigmented epithelial cells, edema appeared between them and the photoreceptor cells, some photoreceptor cells were damaged and some were swollen with the disintegration of their nuclei, and the photoreceptor cells took a positive dye with PAS color (Fig. 8).

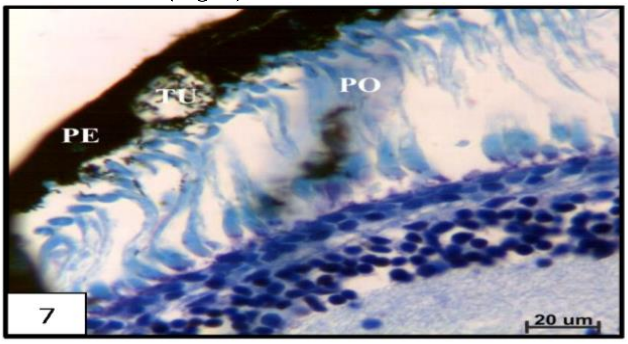


Figure (7): Cross-section of the eyeball of the second type exposed to blue light for three months in summer. Note damage to some rods and cones (photoreceptor cells) PO, degeneration of pigment epithelial cells PE, the appearance of granulomas TU in which , hyperplasia in part, TB stained .

 

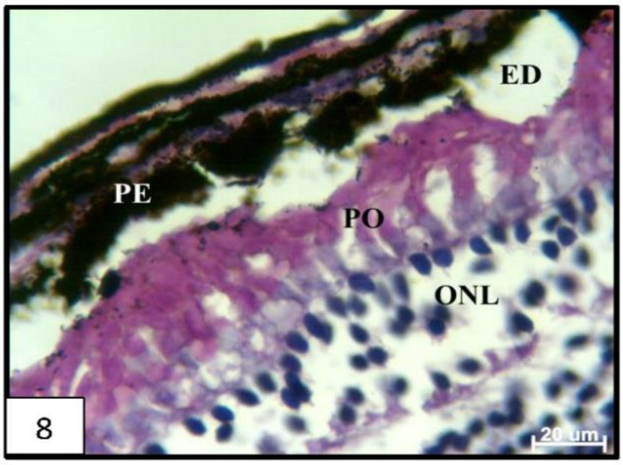


Figure (8): Cross-section of the eyeball of the second type exposed to blue light for three months in summer. Note the degeneration of pigment epithelial cells PE, edema ED between pigment epithelial cells and between photoreceptors, damage to some photoreceptors PO, swelling of some, the disintegration of their nuclei (outer nuclear layer) ONL, photoreceptors received positive dye with PAS stain, PAS stained.

**Yellow light:**
After three months of exposure to yellow light in both winter and summer, different effects appeared in the eye components of the two types of frogs. In the first type of frog, and in winter, degeneration of the epithelial pigment cells appeared, with damage to some bacilli and cones, and the appearance of the outer nuclear layer in two rows. The upper is compact and the lower is loose, and the inner nuclear layer is separated from it with necrosis in the outer plexiform layer (Fig. 9). As for the second type of frog, the degeneration of the epithelial pigment cells and swelling in some of them and necrosis in some photoreceptor cells, and disintegration of the inner nuclear layer in its superficial part appeared ( Figure 10).

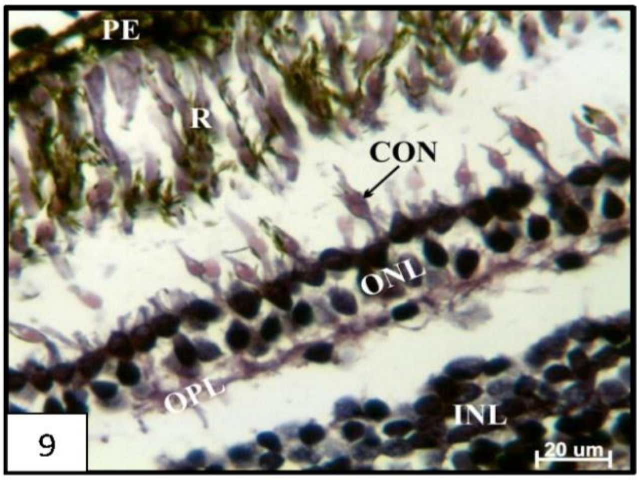


Figure (9): Cross-section of the eyeball of the first type exposed to a yellow light for three months in winter. Note the degeneration of pigment epithelial cells PE, damage to some rods R and cones CON , the appearance of the outer nuclear layer ONL in two rows, the upper one is compact and the lower one is loose, the separation of the inner nuclear layer INL from it, necrosis of the outer plexiform layer OPL, HE stained.

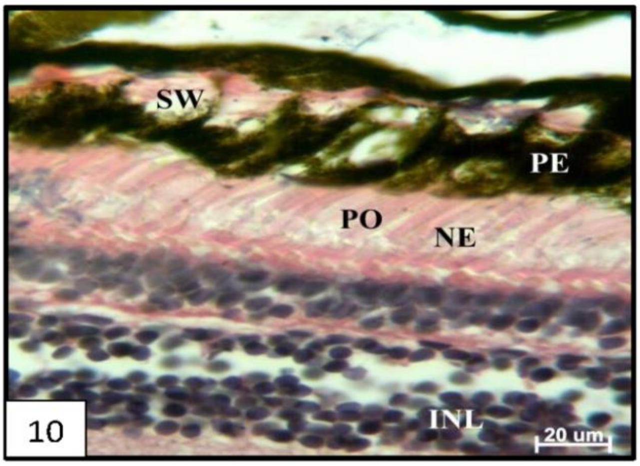


Figure 10: Cross-section of the eyeball of the second type exposed to a yellow light for three months in winter. Note the degeneration of epithelial cells PE, swelling SW of some, necrosis NE of some photoreceptor cells, disintegration of the inner nuclear layer INL in its superficial part, HE stained.

During exposure to yellow light in summer, the first type of frog showed damage to photoreceptor cells, epithelial pigment cells, and their nuclei, with disruption of the inner nuclear layer (Fig. 11). In the second type of frog, the degeneration of the epithelial pigment cells appeared, with bleeding and their separation from the photoreceptor cell layer, with damage to the photoreceptor cells in some parts with their nuclei, disintegration in the inner nuclear layer, reduction in the number of nuclei of the inner nuclear layer, and some nuclei undergoing apoptosis. (Fig. 12). used along with appropriate statistical methods used clearly along with the year of experimentation (field and laboratory).

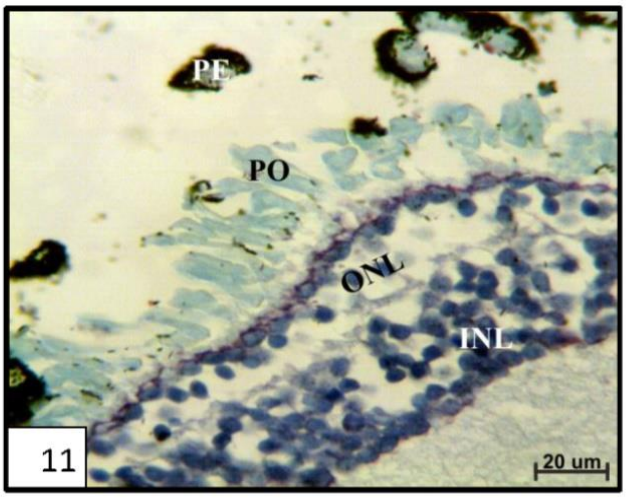


Figure (11): Cross-section of the eyeball of the first type exposed to a yellow light for three months in summer. Note damage to pigment epithelial cells PE and photoreceptors PO, damage to their nuclei (outer nuclear layer) ONL, disruption of the inner nuclear layer INL, TB stained.

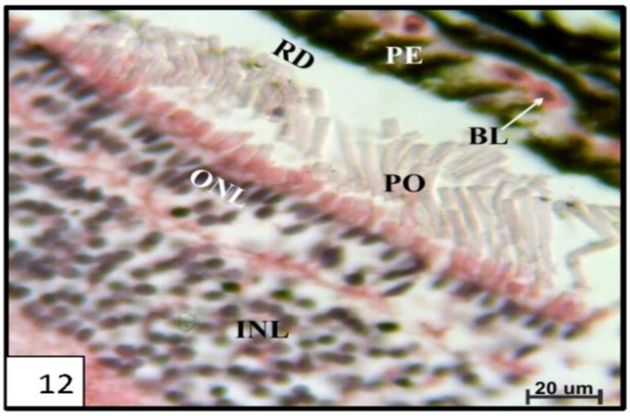


Figure (12): Cross-section of the eyeball of the second type exposed to a yellow light for three months in summer. Note the degeneration of pigment epithelial cells PE, bleeding BL towards, detachment from the photoreceptor layer RD , damage to photoreceptors PO in some parts with their nuclei (outer nuclear layer) ONL, dissociation in the inner nuclear layer INL, reduction in the number of nuclei in the inner nuclear layer, Some nuclei undergo apoptosis, HE stained.

## IV. DISCUSSION

The extent of damage inflicted by light on the tissue structure of the eye depends on approximately twelve biological and physical factors, and among these factors that pertain to our current study is the intensity of illumination, wavelength, animal type, temperature, pigmentation, and period time [9][10]. Accordingly, the damages that occurred in our current study were caused by the effect of different light intensity, different wavelengths, different types of frogs used, temperature, and eye pigmentation, as all are considered variable factors in this study.

Our study showed that after three months of continuous exposure to blue and yellow light to two different species of frogs, different histopathological changes appeared in the retina of the two species of frogs. The effects that appeared differ between the two types, as they were in the first type more than the second type in general. On the other hand, the effect of blue light was more than that of yellow light in general, with close damage to the two lights in some cases, especially at high temperature, and the effects were more at a temperature of 20 °C (in summer) than at a temperature of 10 °C (in winter ).

Regarding the blue light, histopathological changes occurred in the retina of the two types of frogs at low temperatures. As for the high temperature, the damages were more and more severe than at the low temperature in the first type of frog. As for the second type, the damage was generally less, with damage occurring close to the first type in some cases.

As for the yellow light, the effect was less than the blue light, and in the first type of frogs, more than the second type in general, except in some cases, the damage was close, and the damage included the components of the eye, and the damage in high temperature was stronger. Accordingly, the most severe effect caused by blue light is due to the difference in wavelength and the type of animal.
The damage that occurred in the retina has also occurred in many vertebrates, as the frog of the type Rana pipiens was exposed to continuous white light for fourteen months and damage to the epithelial pigment cells [11]. The damage that occurs in epithelial cells, caused by exposure to light, is caused by a deficit in the cells' ability to degrade the molecules within them. In the process of molecular renewal, epithelial cells break down their molecules, as well as the large numbers of membranous discs that shed from the outer pieces of rods and cones. If one of these molecules breaks down, it may not be analyzed, because abnormal shapes of this molecule will lead to its inability to harmonize or match with the active sites of the degrading enzymes. This results in a continuous accumulation of unhelpful and undigested waste within the cell that interferes with the normal function of the cell. This eventually leads to abnormal secretions that pollute the surroundings of the cell and lead to its destruction. In view of the importance of epithelial pigment cells in feeding the photoreceptor cells, the damage it causes to the epithelial cells leads to the destruction of photoreceptors, and this is what may happen in our current study [9].

In a study on two types of bony fish, it was exposed to three different wavelengths of blue, green, and yellow with different light intensities for eight months. It was found that extensive damage occurred to most of the components of the eye, and the effect of blue light was more severe than the green and yellow lights, and the damage was more in one of the species. What is in the second type, which proves that the difference in wavelength and the type of animal leads to the difference in the damage resulting from exposure to the colors of the visible spectrum, and this applies to our current study [12]. On the other hand, a study confirmed that the damage to the photoreceptor cells results from the direct action of light on the photoactive molecules or results from secondary events that cause damage to the retina and that the appearance of free radicals in the cells leads to damage to the components of the retina [13]. On the other hand, one of the studies explained that the capacitance of light photons by the eye tissue increases when exposed to blue light, and this may explain the higher sensitivity of the retina to blue light than to yellow light. Accordingly, blue light affects several enzymes that absorb this light, and this leads to damage to The retina, and this is what may happen in our current study [14].

In another study, it was shown that the damage that occurs to the components of the eye when exposed to optical radiation depends primarily on the depth of penetration of the radiation into the tissue, which in turn depends on the wavelength, intensity of illumination, exposure duration, and frequency of radiation. These factors are complex in their relationships, and it is known that the blue light is shorter than the yellow light, and thus it causes more damage [15].

 

In a study on rats, it was shown that exposure to light during high temperature in the surroundings and inside the body accelerates damage in some eye tissues, and this depends on the value of temperature, exposure time, and intensity of illumination. It appeared that 50% of visual cells are destroyed and lost when exposed to light at a degree of 34.5 degrees. Celsius for an hour and a half, and the same damage occurred after exposure for one hour at a temperature of 37 °C at the same intensity of illumination [16].

In another study, it was shown that the temperature affects the natural properties of the visual pigment, as it appeared that the sensitivity varies according to the temperature. The higher the sensitivity, the greater the sensitivity, causing greater damage to the components of the retina [17]. This is what may happen in our current study.

In another study, it was shown that absorption of different wavelengths of visible light by mitochondria may result in cell breakdown in all layers of the retina. This is what may happen in our current study [18]. Another study also showed that even after long exposure to different wavelengths of the visible light spectrum, damages appear in certain areas and the cells remain healthy in other areas, some of which showed slight changes. all cells. This is what may happen in our current study [19].

The damage caused to the eye tissues in our current study appears to be damage done in a photochemical manner, as there is no clotting in the components of the eye, which is caused by damage to the thermal coagulation mechanism, and this was confirmed by previous studies in this direction [18] [20].

## V. CONCLUSION

It was concluded from the current research that the damage caused by monochromatic colors of visible light appeared to follow the wavelength, temperature, and intensity of illumination, as the shorter the wavelength, the higher the temperature and the higher the intensity of illumination, the greater the damage to the components of the eye, and the absence of clots in the tissue of the eye indicates that Photochemical damage occurred by the photochemical mechanism. On the other hand, the occurrence of damage in certain areas of the eye indicates that the different parts of the eye receive the different intensities of light, and this was confirmed by most of the previous studies.

## ACKNOWLEDGMENT

The authors are very grateful to the University of Mosul, and the Deanship of College of Education for Pure Sciences for their provided facilities, which helped to improve the quality of this work.

## REFERENCES

[1] E. J. Warrant and N. A. Locket, Vision in the deep sea. Biol. Rev.,79, 671–712, 2004.

[2] J. Viltala, E. Korpimaki, P. Palokangas, andM. Koivula, Attraction of kestrels to vole sent marks visible in ultraviolet detection. Nature 373, 425- 427, 1995.

[3] F.T. Ollivier, D.A. Samuelson, D.E. Brooks, D.A. Lewis, M.E. Kallberg,. and A.M. Komaromy, Comparative morophology of the tapetum lucidum (among selected species) J. Comp.Anat., 7, 1, 11-22, 2004.

[4] J.Y. Lettvin, H.R. Maturana, W.S. Mcculloch, and W.H. Pitts, What the Frog's eye tells the frogs brain, Martin Balaban, pp. 233- 258, 1968.

[5] C. Morandini, Zoologia. S.A, fourth ed., Livraria Nobel,

[6] pp. 137-218,1976.

[7] H,A. Al-Hajj, Optical Microscopic Preparations (Microscopic Technologies) Theoretical Foundations and Applications, Jordan Book Center, Amman - Jordan, p. 331, 1998.

[8] E. Gurr, Staining animal tissue, practical and theoretical. London, Leonard, Hill, p. 552, 1962.

[9] A.G.E. Pears, Histochemistry theoretical and applied, 4th . ed., Analytical Technology, Churchill Living Stone, Edinburgh, p. 898,1985.

[10] R.W. Young, Solar radiation and age-related macular degeneration. Surv. Ophthalmol., 32, 232-266, 1988.

[11] D.T. Organisciak and B.S. Winkler, Retinal light damage: Practical and theoretical considerations. In progress in retinal and ocular research. (Eds. N. Osborne and G. Chader), pp.1-29,1994.

[12] M. I. Glad Mohesh , Effect of high-intensity light on the retinal pigment epithelium of zebrafish – An induced retinal damage study model. National Journal of Physiology, Pharmacy and Pharmacology, 10,4,1-3, 2020.

[13] T.S.H. Al-Fakji, A comparative microscopic study and the effect of some electromagnetic rays on the histological structure of the retina of two species of bony fish Calcalburnus mossulensis and Noemacheilus angora, Master Thesis, College of Education, University of Mosul-Iraq, 2003.

[14] X. Ouyang, J. Yang, Z. Hong, Y. Wu, Y. Xie, G. Wang, Mechanisms of blue light-induced eye hazard and protective measures: a review. Biomedicine & Pharmacotherapy, 130,1-8, 2020.

[15] C. Grimm, C.E. Reme and P.O. Rol, Blue light 146 effects on rhodopsin: Photoreversal of bleading in living rat eyes. Invest. Opthalmol, Vis. Sci., 41: 390-398,2000.

[16] H. Siekman, Revision of guidelines on limits for laser radiation of wavelength between 400nm and 1400nm. International commission on non-ionizing radiation protection. Helathpysics., 45,431-440,2000.

[17] P.N. Youssef, N. Sheibani and D.M. Albert, Retinal light toxicity. Eye , 25, 1–14, 2011.

[18] P. Ala- Laurila, R.J.Albert, P.Saarinen andA. Koskelainen, The thermal contribution to photoactivation in A2 Visual pigments studied by temperature effects on spectral properties. Vis. Neurosci., 20, 4 , 411-419, 2003.

[19] D. Norren and J.J. Vos, Light damage to the retina: an historical approach. Eye , 30, 169–172, 2016.

[20] I. Jaadane, G.V. Rodriguez1, P. Boulenguez, S. Carré2, I. Dassieni , C. Lebon, S. Chahory, F. Behar-Cohen1, C. Martinsons and A.Torriglia, Retinal phototoxicity and the evaluation of the blue light hazard of a new solid-state lighting technology. Scientific Reports , 10, , 6733 ,2020.

[21] R.W. Young, The family of sun light related eye diseases, Review. Ophtomet. Vis. Scien., 71, 125- 144,1994.

## AUTHORS PROFILE

Ms. Semaa A. Baker pursed B. Bio., M.Sc., and Ph.D. in Zoology from Mosul University, in 2005, 2010 & 2019.

He is currently working as Lectuer in Histology and Comparative Anatomy at Department of Biology Department, Education college for Pure sciences, Mosul University, Mosul, Iraq since 2010

 