

RESEARCH ARTICLE

Altered retina and cornea of *Clarias gariepinus* (Siluriformes: Clariidae) under the effect of bright and dim lights

Dalia A. Sabry¹ , Dina El-Badry¹

¹Mansoura University, Faculty of Science, Department of Zoology, Mansoura, Dakahlia, Egypt.
Corresponding author: Dalia A. Sabry (drdsabry@hotmail.com)

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ABSTRACT. The purpose of this study was to investigate the influence of constant bright light on the cornea and retina of *Clarias gariepinus* (Burchell, 1822) and to examine whether it can change after constant exposure to dim light. Twenty-one adult individuals of *C. gariepinus* were divided into three groups (n = 7). The first group was maintained under normal light (NL). The second group was exposed to the intense bright light (BL) (3020 Lux) of white light lamps for seven days. The third group was exposed to dim light for seven days (DL) following the previous exposure to intense bright light for seven days. The eyes of each fish group were removed and fixed. The following aspects of the eye were investigated: histopathological, immunohistochemical (GFAP and BAX) staining and biochemical study for lactic dehydrogenase (LDH), superoxide dismutase (SOD), malondialdehyde (MDA) and glucose-6-phosphate-dehydrogenase (G6PDH). Also, isoenzyme electrophoresis of LDH, G6PDH and SOD were performed. The present study found that, seven-days BL exposure caused damage to both cornea and retina. However, after exposure to dim-light after bright light there was partial improvement in corneal and retinal structure and an increase in the assayed SOD and G6PDH levels, along with a reduction in MDA content and activity of LDH. These findings demonstrate a plasticity that may help *C. gariepinus* survive disturbances in the aquatic environment.

KEY WORDS. Catfish, immunohistochemistry; oxidative stress; photoperiods; retina.

INTRODUCTION

Light has a major impact in the life cycle of teleost fish (Vilamizar et al. 2011). There are many anthropogenic and natural disturbances that trigger dramatic changes in the spectrum and intensity of underwater light (Greycay and Targett 1996, Karlsson et al. 2009). For example, visual acuity is decreased in correlation with decreased light and increased turbidity (Caves et al. 2017). Also, the quality of underwater light can be altered by dissolved organic matter (Cronin et al. 2014). Such disturbances can impact the visual acuity of fish (Collin and Hart 2015) and consequently alter their ability to avoid predators, eat and ultimately survive (Schweikert and Grace 2018).

Intense light induces the formation of reactive oxygen species (ROS) within the eye (Rohowetz et al. 2018). ROS are free radicals produced from the normal cell metabolism. They are produced at low levels and are detoxified and metabolized by exogenous and endogenous mechanisms. During cellular oxidative stress, the production of ROS increases and that leads to cellular injury and/or death, and results in tissue and organ dysfunction. Recent studies have examined the role of ROS in disease development and

pathogenesis of the retina. The intensify of the light is correlated with retinal damage and affects mainly the rhodopsin, which is the main component of photoreceptors (Grimm et al. 2000). Bright light activates rhodopsin to produce signals that induce pathological changes in the rod photoreceptor cell. Curcio et al. (1996) and Wenzel et al. (2005) reported that vision impairment results mainly from photoreceptor cell death. Kassen et al. (2007) and Thummel et al. (2008a, b) confirmed that, regular, intense exposure to light induces apoptosis of photoreceptors in the albino trout, *Oncorhynchus mykiss* (Walbaum, 1792), and the albino zebrafish, *Danio rerio* (Hamilton, 1822), although the degenerating cells are replaced by the proliferating neural stem cells.

Teleost fish exhibit changes in their visual perception during cyclic changes between day and night conditions. Furthermore, nocturnal and diurnal fish show varying degrees of accommodations in their retinal structure. For example, the visual cells are larger in nocturnal than in diurnal species (Fishelson et al. 2004, Schmitz and Wainwright 2011). The outer retinas are composed of two types of light-detecting photoreceptor cells: rods, which are extremely sensitive light detectors in low-light conditions, and cones, which are activated when there is more light (Grace and

Taylor 2017). The intensity of light affects fish behavior (Castro and Caballero 2004, Almazan-Rueda et al. 2005) and physiology in various ways. For instance, bright light decreases melatonin secretion and alters the physiological and behavioral activity of fish (Ekstrom and Meissl 1997), while dim light enhances light-collecting power and maximizes light sensitivity (Iglesias et al. 2018).

In experiments, forced exposure to light damaged the cone-photoreceptors of diurnal albino fish (Teleostei) and the rod photoreceptors of albino rats, which renders them good models for the study of the damage light can do to the human retina (Bernardos et al. 2007, Fimbel et al. 2007, Kassen et al. 2007, Thummel et al. 2008a, b, Santos et al. 2010). The rod-dominated retina of a nocturnal fish should be liable to light damage and could be a good model to understand photoreceptor degeneration (Bejarano-Escobar et al. 2012).

Clarias gariepinus (Burchell, 1822) is a nocturnal teleost fish native to many countries of Africa and Asia. It has successfully colonized 37 countries (Weyl et al. 2016). It is abundant in the Nile River (Hossain et al. 2002, Martinez-Chavez et al. 2008, Anoop et al. 2009) consumes 70% of its food during the night. During the day, its feeding activity is reduced (Hossain et al. 2002). *Clarias gariepinus* has the ability to reproduce, survive and overcome different environmental barriers (Blackburn et al. 2011).

The purpose of this study was to investigate the accommodation of the cornea and retina of *C. gariepinus* to the disturbances of bright and dim light exposures.

MATERIAL AND METHODS

Experimental design. Twenty-one adult individuals of *C. gariepinus* were captured from the Nile River, Egypt. They were collected in the morning using fishermen nets. Their weight ranged from 1250–1500 g and their body length was about 46–55 cm. The photic injury to the retina and cornea of *C. gariepinus* was carried out according Bejarano-Escobar (2012) with some modifications. The fish were reared in aquaria, fed tad liver and were arranged in three groups (n = 7). The first group served as the control and was maintained under normal light condition (NL) which is 12-hour light and dark cycle (400 Lux in the light – 1 Lux in the dark). The second group was exposed to overhead bright light (BL) for seven days (24 hours per day) using two LED white light lamps (each has electric power 8.8 w and luminous efficiency 100 Lumens/watt). Light-emitting diode (LED) is an artificial sunlight source system that can emit a spectral power distribution approximating ground level sunlight (Fujiwara 2007). LED lamps were suspended 10 cm above the water surface. At this distance the intensity of light was 3020 Lux, measured by using UNI-T UT383S digital light meter. The third group was exposed to dim light (DL) (0.00 Lux). Dim light was obtained by covering all surfaces of the aquarium in black for seven days. This was done after the previous exposure to bright light (24 hours per day). The present experiment was performed according to the local experimental animal ethics committee, code number RZ 19002. At the end of experiment, the

fish were euthanized by 1percent clove oil and sacrificed. Their eyes were dissected and processed for the following investigations.

Histological investigation. The eyes of the studied groups were fixed in phosphate buffered formalin, dehydrated in ascending series of ethyl alcohol, cleared in xylene for five minutes and mounted in melted paraffin wax (58–62 °C). Five µm histological serial sections of the eye tissues were cut by using microtome and stained with hematoxylin and eosin to be examined under bright field Olympus light microscope. The whole thickness of the cornea, retina and the retinal layers were measured using ocular micrometer.

Immunohistochemical staining for GFAP and BAX expression. The paraffin-embedded tissue sections of the retina were cleared and rehydrated using a decreasing series of ethyl alcohol. The specimens were incubated in 2% hydrogen peroxidase for five minutes to block the activity of the peroxidase. Antigen retrieval of the sections was executed by microwaving the sections for 10 min at 95–100 °C in 10 mM citrate buffer (pH 6.0). Then, the slides were incubated overnight with the primary antibodies of GFAP (mouse, Santa Cruz) and BAX (rabbit, Santa Cruz) in a humidified chamber at 4 °C followed by incubation at room temperature in biotinylated secondary antibody for 50 minutes. Then, conjugation with Avidin-Biotin horseradish peroxidase was carried out for 30 minutes. Sections were stained with 0.04% 3, 3-diamino-benzidine tetrahydrochloride and counterstained with hematoxylin. The resulting images of immunohistochemical staining for GFAP and BAX reaction were analyzed on Intel Core I3 based computer using Video Test Morphology software (Russia) with a specific built-in routine for area, area percentage measurement and object counting.

Biochemical analysis. The fresh eye specimens were cleaned with ice-cold isotonic and homogenized with 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose and centrifuged. The supernatant was kept in a deep freezer at (–20 °C) for biochemical assay. The used kits were purchased from Bio Vision incorporated (155 S. Milpitas Boulevard, Milpitas, CA 95035 USA) for the measurement of Superoxide dismutase (SOD) (Catalog number: K335-100), Lactic dehydrogenase (LDH) (Catalog number: K726-500), malondialdehyde (MDA) (Catalog number: K739-100). While, the assay kit of glucose-6-phosphate-dehydrogenase (G6PDH) was purchased from Sigma-Aldrich (St. Louis, MO, USA) (Catalog number: MAK015).

Isoenzyme electrophoresis. The collected eye samples were cleaned and homogenized using 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose. Electrophoresis was carried out according to Laemmli (1970) for enzymes Lactic dehydrogenase, glucose-6-phosphate-dehydrogenase and Superoxide dismutase. The protein bands were stained using Coomassie blue R-250 (60 mg/l) (Andrews 1986). For visualization of the tested enzymes, electrophoresis process was carried out for each kind of the enzymes in the selected incubated medium.

Lactic dehydrogenase isoenzyme was examined depending on the method of Sarkar et al. (1978). After electrophoresis,

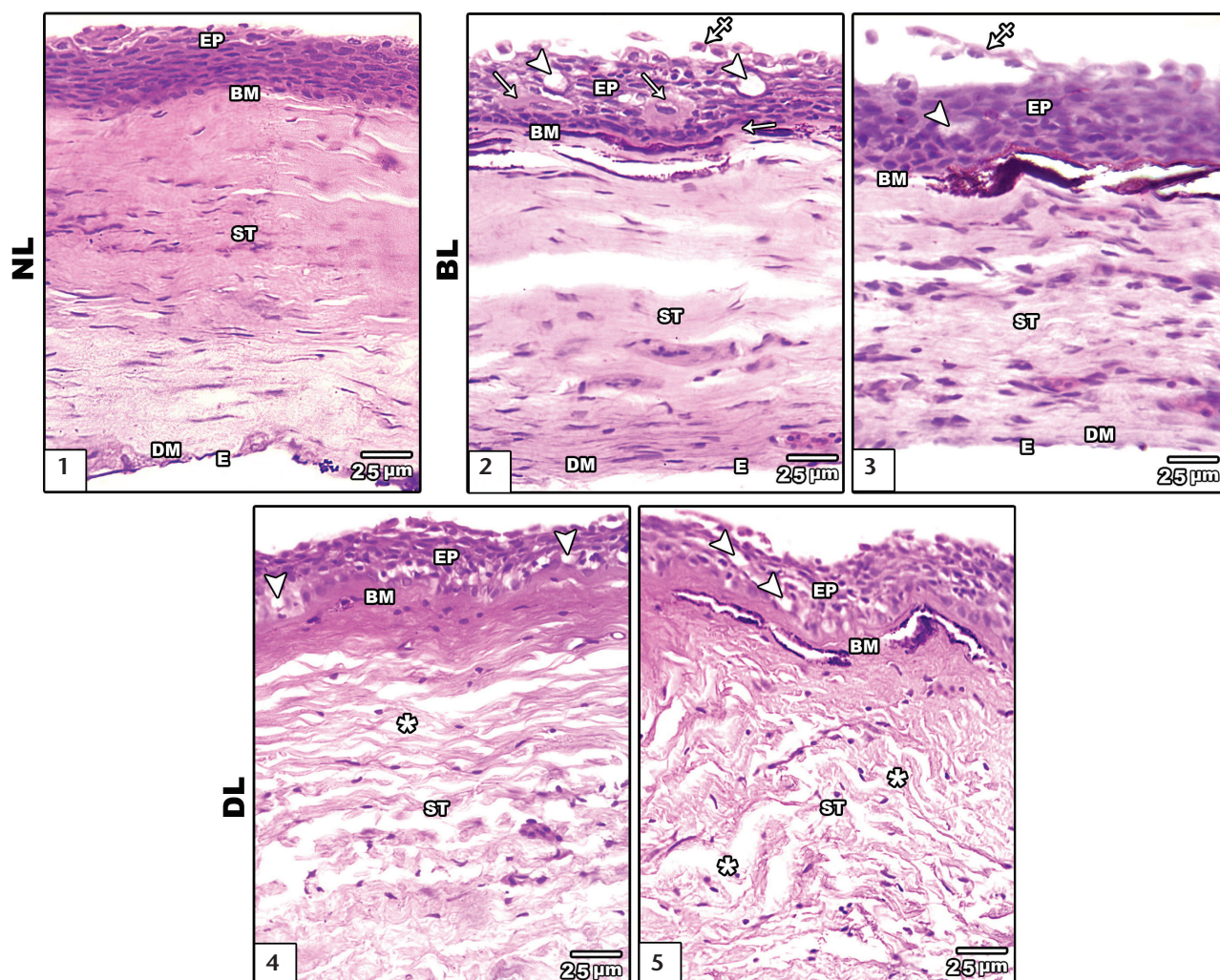
the gel was incubated for 20 min with H₂O 18.4 ml, Tris 1 M, phenazine methosulphate 1.6 mg/ml tetrazolium-blue 1 mg/ml, NAD 10 mM and Na-lactate 10 mM to develop color reaction. In this reaction, phenazine-methosulphate is the primary electron acceptor, NAD and lactate serve as substrates, and tetrazolium-blue is the electron acceptor.

Glucose-6-phosphate-dehydrogenase was determined according to method of Gaal et al. (1980). The separating gel (5% acrylamide) was intended in 0.75 M Tris-Pi (pH 8) and the stacking gel (2.8% acrylamide) in 20% sucrose and 50 mm Tris-Pi (pH 6.3). The electrophoretic buffer contained 80 mm aspartate, five mM Tris and 20 μ M NADP⁺ at pH 7.4. For determining G6PDH activity, Gels were stained at 30 °C in a solution of 20

ml volume containing 25% (v/v) glycerol, 1.2 mmol Tris-Pi (pH 8.5), 30 μ mol glucose-6-P, 6 mg p-nitro blue tetrazolium, 4 g mol NADP⁺, and 0.5 mg phenazine methosulfate.

Superoxide dismutase was determined according to method of Jevremovic et al. (2010). After electrophoresis, the gels were incubated with 30% H₂O₂ and 1 mM KCN, followed by incubation for 30 min in the dark with a reaction mixture (0.098 mM NBT, 0.1 M EDTA, 2 mM N, N, N, N tetramethyl ethylene diamine in K phosphate buffer, pH 7.8 and 0.030 mM riboflavin).

Statistical analysis. Data were presented as mean \pm standard deviation. The statistical analysis was performed with analysis of variance (ANOVA) and post hoc analysis using SPSS (version 15) software package for windows.



Figures 1–5. Photomicrograph of sagittal histological sections of cornea of *Clarias gariepinus*: (1) control showing normal stratified epithelium, Bowman's layer, heavily nucleated stroma, Descemet's membrane and endothelium; (2–3) bright light exposed group showing damaged epithelium and fragility of stroma; (4–5) dim light exposed group showing less improved stroma. Arrow head refer to vacuolization. Arrow refers to pyknosis. Crossed arrow refers to epithelial cell loss. Star refers to stromal edema. (Ep) Epithelium, (BM) Bowman's membrane, (St) stroma, (DM) Descemet's membrane, (E) endothelium, (NL) normal light, (BL) bright light, (DL) dim light.

RESULTS

Histopathological observations

Cornea

In fish exposed to normal light, the cornea is composed of four layers. They are, from front to back: an outer stratified squamous epithelium, Bowman's layer, the stroma, which is an composed of organized parallel collagenous fibers infiltrated with keratocytes, and complemented with a Descemet membrane containing endothelial cells (Fig. 1).

In BL group, cornea revealed vacuolization in the epithelial cell layer and loss of epithelial cells. Pyknotic cells and disorganized basal lamina of epithelial layer were observed (Figs 2, 3).

In the DL group, the cornea appeared partially recovered. It showed widening of the median collagenous fibrils of the stroma. The size of the vacuolar degenerated epithelium appeared comparatively smaller. There is no detected pyknotic epithelium (Figs 4, 5). The thickness of the cornea in both bright and dim light groups was significantly increased from the group exposed to normal light (Fig. 6).

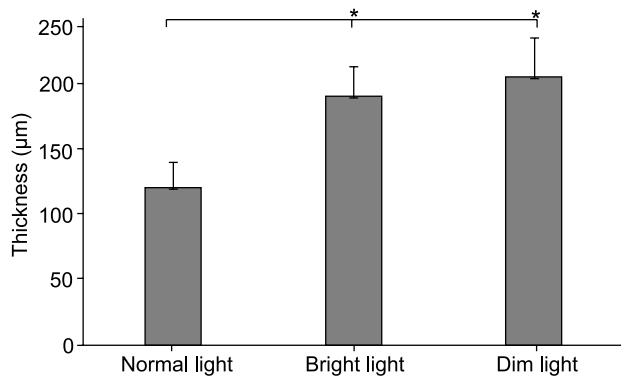


Figure 6. Mean thickness of the cornea in *Clarias gariepinus*. Each column represents the mean value \pm SD ($n = 7$); * significant at $p < 0.05$.

Retina

In fish exposed to normal light, the retina is composed of six layers: ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer nuclear and photoreceptor layer, which is in contact with the pigmented epithelium. Both outer plexiform and outer nuclear layers were thicker than the corresponding inner layers. The ganglion cells were distributed among bundles of the nerve fibers (Fig. 7).

In the group exposed to BL, there was a relative increase of dark-brown deposits of retinal pigment among the damaged photoreceptors and dispersed through the outer molecular layer. Also, the thickness of the photoreceptor layer was significantly reduced ($p < 0.05$) compared to the group exposed to NL. The photoreceptor of the DL group regenerated and became considerably thick. The thickness of the outer nuclear layer in the BL

retina increased significantly when compared to both NL and DL groups (Figs 7–11).

In the group exposed to DL, there was no evidence of dispersed pigments in the outer nuclear layer. The density of the nerve fibers in the outer plexiform layer was decreased (Figs 7–10). In addition, the thickness of the outer nuclear layer, outer plexiform layer and inner plexiform layer were significantly reduced ($p < 0.05$) with respect to the BL and NL groups (Fig. 11). The whole DL retina showed a significantly decreased thickness if compared to the whole retina in both NL and BL groups (Fig. 12).

Immunohistochemical observations

The dark brown immunohistochemical reaction of GFAP is over expressed, particularly in the inner nuclear, outer nuclear and photoreceptors layers of the BL group compared to the DL group. The group exposed to normal light showed the weakest immune reaction (Figs 13–15). Concerning the BAX immunohistochemistry, there was a significant increase of the immune reaction in BL more than in DL. The immunohistochemical reaction was located at the inner nuclear, outer nuclear and photoreceptors. The normal light group showed the least immunohistochemical reaction (Figs 16–18). Image analysis revealed a significant increase ($p < 0.05$) in the immunohistochemical reaction of GFAP and BAX in the bright light more than that of dim light group. The normal light group showed a significant decrease ($p < 0.05$) of the immune reaction with respect to the other ones (Fig. 19).

Biochemical analysis

Table 1 shows that there was a significant increase in LDH, MDA ($p < 0.05$), coinciding with a significant decrease in SOD ($p < 0.05$) in both BL and DL groups compared to the NL group. Also, G6PDH was markedly decreased ($p < 0.05$) in the BL group in comparison with the NL and DL groups. In the DL group, the level of both LDH and MDA decreased significantly if compared to the BL group, while the level of SOD in the DL retina increased significantly.

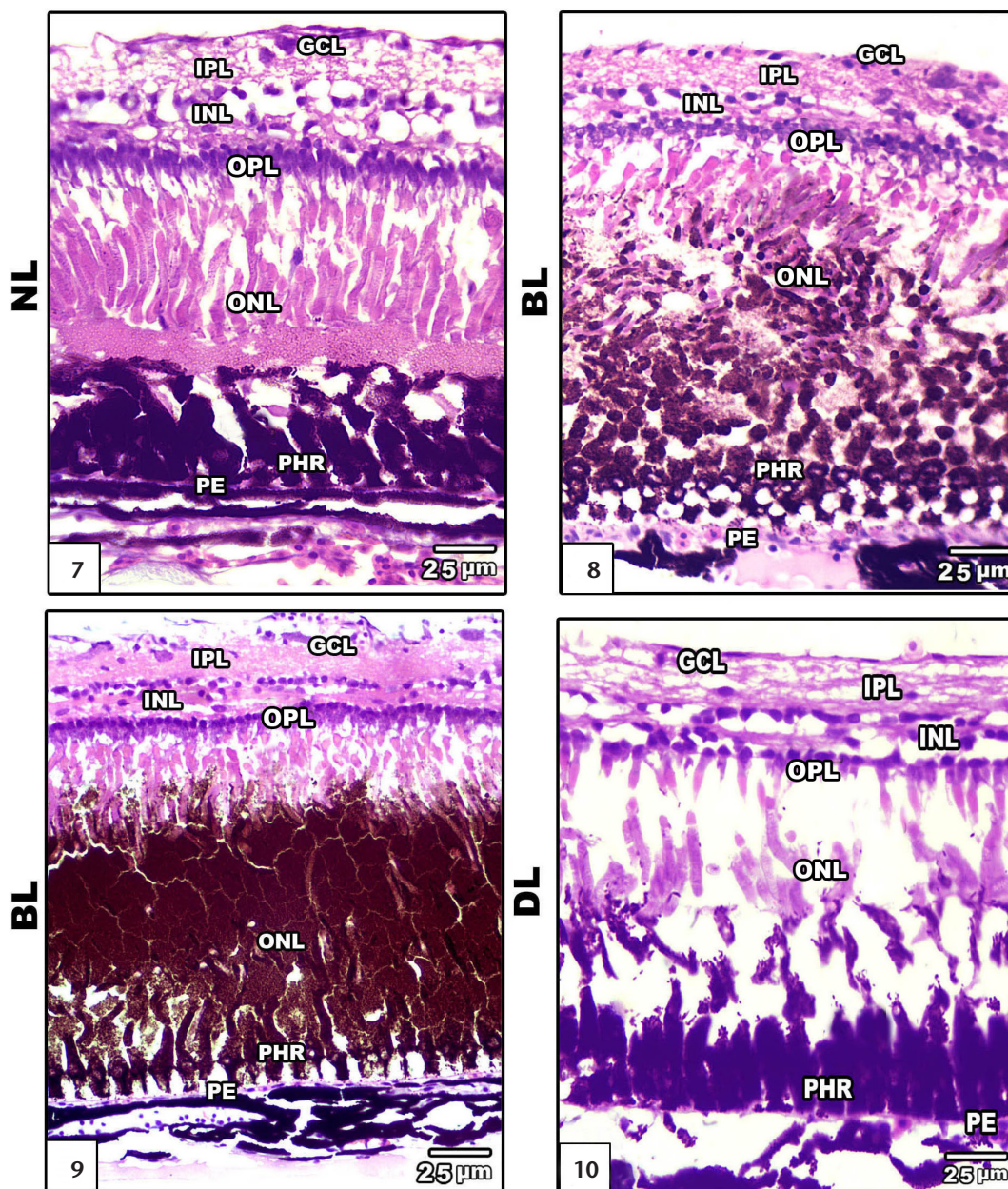
Table 1. Lactic dehydrogenase (LDH), glucose-6-phosphate-dehydrogenase (G6PDH) superoxide dismutase (SOD) activities and malondialdehyde (MDA) content in samples of the exposed eye to NL, BL and DL.

Animal groups	LDH (mµ/gm)	G6PDH (mµ/gm)	SOD (mµ/gm)	MDA (nmol/gm)
Normal light	6.9500 \pm 1.00 ^{ab}	13.26 \pm 0.58 ^a	42.49 \pm 1.00 ^{ab}	6.05 \pm 1.00 ^{ab}
Bright light	10.93 \pm 0.81 ^{ac}	10.13 \pm 0.51 ^{ab}	30.17 \pm 1.00 ^{bc}	9.81 \pm 0.58 ^{ac}
Dim light	8.92 \pm 0.62 ^{bc}	12.31 \pm 1.00 ^b	36.10 \pm 1.00 ^{bc}	7.71 \pm 0.86 ^{bc}

Values are given as mean \pm SD ($n = 7$). The same superscript letters of each column mean significant difference at $p < 0.05$.

Isoenzymes electrophoresis

From Fig. 20, LDH expressed four faint isoenzyme fractions in the NL group compared to more dense bands in both BL and DL



Figures 7–10. Photomicrograph of sagittal histological sections of retina of *Clarias gariepinus*, showing ganglion cell layer, inner and outer plexiform layer, inner and outer nuclear layer, photoreceptor layer: (7) normal light showing ordinary retinal structure; (8–9) exposure to bright light showing damaged photoreceptor and increased infiltration of dark-brown pigments; (10) dim light exposure showing regenerated photoreceptors layer and less dense nerve fibers in the outer plexiform layer and outer nuclear layers. (GCL) Ganglion cell layer, (IPL) inner plexiform layer, (INL) inner nuclear layer, (OPL) outer plexiform layer, (ONL) outer nuclear layer, (PHR) photoreceptors, (PE) pigmented epithelium, (NL) normal light, (BL) bright light, (DL) dim light.

groups. In the DL group, the third fraction of LDH appeared with less intensity compared to the BL group. G6PDH showed two faint isoenzyme fractions in the BL and DL groups in comparison with three dense isoenzyme fractions in the NL group. SOD appeared

with three faint isoenzyme fractions in the BL and DL groups in comparison with three dense isoenzyme fractions in the NL group. In the BL group, the density of the isoenzyme fraction of G6PDH and SOD was comparatively decreased compared to the DL group.

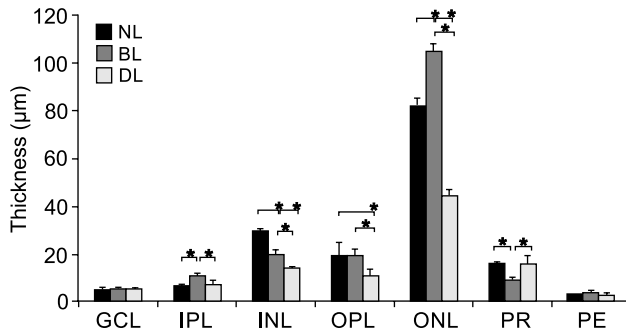


Figure 11. Mean thickness of the different retinal layers in *Clarias gariepinus*. Each column represents the mean value \pm SD ($n = 7$); * significant at $p < 0.05$. (NL) Normal light, (BL) bright light, (DL) dim light, (GCL) ganglion cell layer, (IPL) inner plexiform layer, (INL) inner nuclear layer, (OPL) outer plexiform layer, (ONL) outer nuclear layer, (PR) photoreceptors, (PE) pigmented epithelium.

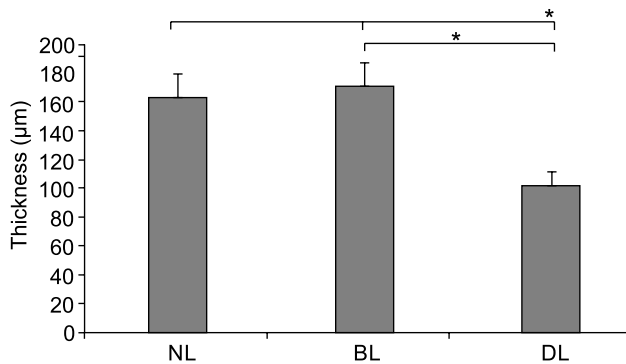


Figure 12. Mean thickness of the whole retina in *Clarias gariepinus*. Each column represents the mean value \pm SD ($n = 7$); * significant at $p < 0.05$. (NL) normal light, (BL) bright light, (DL) dim light.

DISCUSSION

The data obtained in this work suggests that bright light caused intense corneal damage assessed by either vacuolar degeneration or pyknotic epithelium. After an exposure of seven days to DL following BL, the cornea was not completely recovered from the oxidative stress involved in increasing the thickness of the cornea compared to the cornea exposed to NL. The cornea of the adults of *C. gariepinus* exposed to DL following exposure to BL showed insufficient improvement due to a failure to completely regenerate the corneal epithelium, stroma and endothelium. It is known that the structure of the cornea is unique and allows for both mechanical strength and transparency. It is rich in collagen lamellae oriented in criss-cross directions. Although the limbus of the cornea is a border line between the corneal and conjunctival epithelium, it is rich in limbal stem cells for repair (Mobaraki et al. 2019). However, exposure to BL may degenerate the limbal stem cells and consequently delay regeneration.

Jaadane et al. (2015) reported that the ocular tissues of the albino rat, *Rattus norvegicus* (Berkenhout, 1769), exposed to white LED developed intense edema due to abnormal permeable capillaries and vasodilation. Also, exposing rats to an ultraviolet radiation (type B) lamp led to necrosis and corneal epithelium exfoliation (Muresan et al. 2013).

Fish exposed to BL also displayed damaged retinal photoreceptors associated with a substantial reduction in the thickness of the photoreceptor layer compared to both the NL and DL groups. These findings are consistent with the work of Vera and Migaud (2009) and Schweikert and Grace (2018), who exposed the Atlantic salmon, *Salmo salar* (Linnaeus, 1758), and tarpon, *Megalops atlanticus* (Valenciennes, 1847), respectively, to LED light.

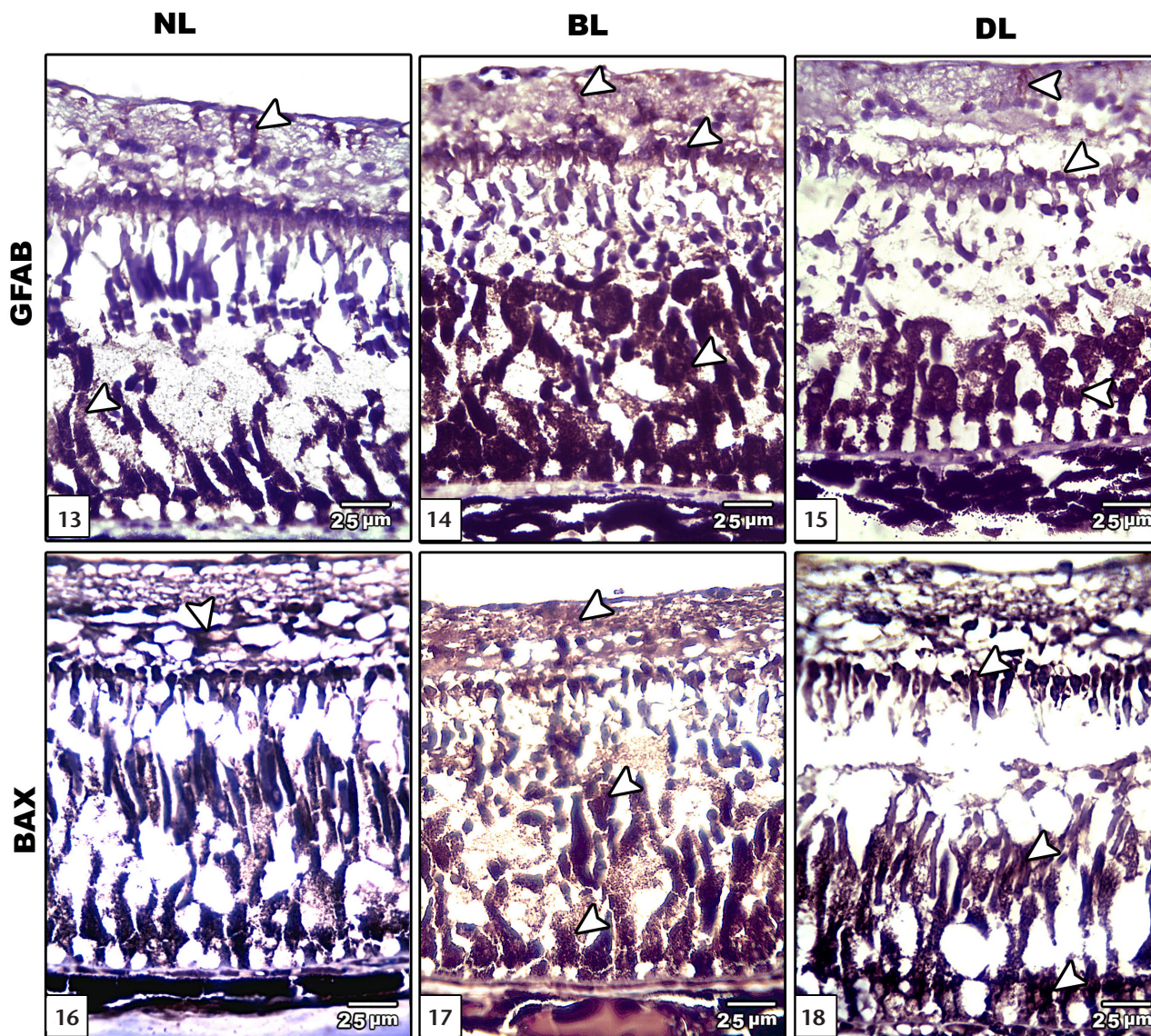
Also, exposure to BL increased the dispersion of pigments through the outer nuclear layer. Organisciak and Vaughan (2010) mentioned that the damage effect of intense light on the photoreceptors/retinal pigment epithelium (RPE) was caused by the release of reactive oxygen species by bleaching the rhodopsin or other compounds from the damaged retinal pigment epithelium (RPE).

Furthermore, exposure to DL following BL showed modest regenerated activity, evidenced by increasing density and thickness of photoreceptors and no pigments infiltrating the outer nuclear layer. These may be due to the induction of Müller cells by the damaged photoreceptors to proliferate and differentiate into new photoreceptor progenies to replace the lost cells (Fimbel et al. 2007, Bailey et al. 2010, Bejarano-Escobar et al. 2012). The absence of the dispersed pigments and damaged photoreceptors can be explained by the fact that microglial cells have a main role in clearing the retina from retinal dead neurons and cellular debris through phagocytosis (Rashid et al. 2019). Also, exposing juvenile teleost fish, *Tinca tinca* (Linnaeus, 1758), to a constant fluorescent light for 96 hours led to an increase of microglia cells (Bejarano-Escobar et al. 2012). Futter et al. (2004) reported that hyper pigmentation helps in scavenging free radicals and toxins.

At the same time, exposure to BL significantly increased the thickness of ONL, together with a reduction in the thickness of the photoreceptor layer. This may be attributed to edematous lesions resulting from extracellular fluid accumulation enhanced by the modulation of the blood retinal barrier (Bandellico et al. 2015).

Also, DL exposure followed by BL decreased the thickness of the ONL, while the photoreceptor layer restored its normal thickness. The present findings are consistent with Vera and Migaud (2009) who reported recovering the retina of *S. salar* exposed to continuous intense light for 3, 7, 15 or 25 days followed by 30 days of 12 hour light:12 hour dark photoperiods.

It is known that BAX is a protein (Kondo et al. 2001) located in the outer membrane of the mitochondria and is associated with the release of cytochrome c into the cytosol, followed by caspase activation and apoptosis (Westphal et al. 2011, Son et al. 2013, Xu et al. 2017). From the present findings, exposure to BL increased retinal damage assessed by overexpression of BAX



Figures 13–18. Photomicrograph of sagittal histological sections of retina of *Clarias gariepinus*: (13–15) showing GFAP immunostaining: (13) control showing decreased GFAP immunohistochemistry; (14) exposure to bright light showing increased immunohistochemical reaction; (15) dim light exposure showing comparatively decreased immune reaction compared to bright light; (16–18) showing BAX immunostaining. Strong reaction appeared in different retinal layers of BL and DL retina more than in normal retina. (NL) Normal light, (BL) bright light, (DL) dim light.

immunohistochemistry, leading to an increase in the rate of apoptosis compared to a weak expression of immune reaction in the NL exposed retina. However, DL exposure following BL decreased the immune reaction and confirmed a partial recovery.

Also, glial fibrillary acidic protein (GFAP) is a protein situated in glial cells of the central nervous system that may be secreted as an inhibitory factor as a result of oxidative stress, abnormal metabolism, blood brain barrier damage, injury and inflammatory reaction (Sofroniew 2009). GFAP has a protective

effect on the neuronal injury (Lefrançois et al. 1997). It inhibits the oxidative stress (Cheon et al. 2016) and reduces the inflammatory response to cell injury (de Pablo et al. 2013). It is also the marker predicting astrocyte damage associated oxidative stress (Pekny and Pekna 2004, Zhang et al. 2017, Trautz et al. 2019). The observed finding revealed increased immunohistochemistry of the GFAP after exposure to BL and DL compared the ones that were not exposed. Under DL exposure, the immunohistochemical staining reaction of the GFAP decreased significantly,

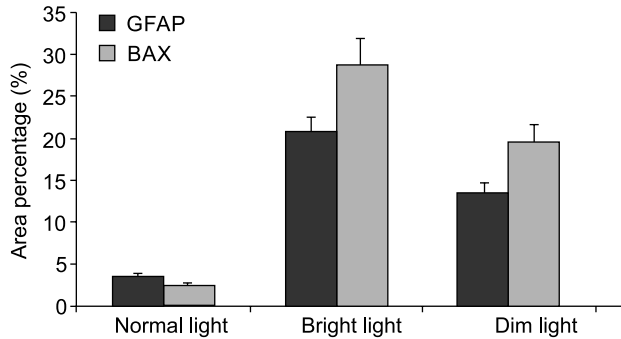


Figure 19. Means of area percentage of GFAP and BAX. Each result represents the mean \pm SD (n = 7). All estimated values of GFAP and BAX are significant at $p < 0.05$ among the different groups.

suggesting a reduced oxidative stress. Similar results were detected by Bian et al. (2016) who found a prominent reaction of the GFAP in the retina of mice exposed to BL.

Also, BL and DL exposure increased the oxidative stress assessed by an increase of MDA content compared to the NL exposed group. This finding is consistent with the work of Tsikas (2017) and Jovanović et al. (2010). Vakifahmetoglu-Norberg et al. (2017) reported that, if the amount of ROS exceeds the capability of antioxidant protection system, ROS induces reactions by oxidizing macromolecules in the cells as proteins and lipids, which increase cell damage and disrupt cellular activities, resulting in apoptosis.

Also, the sharp rise of LDH activity in the retina during BL exposure may have disrupted the integrity of the cell membrane, increasing lipid peroxidation (Jovanović et al. 2010). This was confirmed by a depletion of the antioxidant enzymes such as SOD (Fang et al. 2016).

In addition, exposure to BL decreased the retinal activity of the G6PDH more than exposure to DL. G6PDH is the main enzyme in the oxidative pentose pathway, and plays an important role in the production of nicotinamide adenine dinucleotide phosphate (NADPH) (Hsu et al. 2014, Wang et al. 2014), which keeps glutathione (GSH) in its reduced form. Reduced glutathione facilitates scavenging the reactive oxygen species (Margis et al. 2008, Pereira et al. 2016) and also apoptosis associated with perturbed NADPH (Kim et al. 2007). The observed biochemical analysis and isoenzyme electrophoresis in the eye exposed to DL revealed a significant decrease in the level of LDH and MDA and a significant increase in the level of G6PDH and SOD compared to the group exposed to BL, which confirms a significant improvement and reduction of oxidative stress. Hitchcock et al. (2004) reported that the retina of teleost fish grows continuously by neurogenesis throughout the fish's life. Otteson et al. (2001) and Stenkamp et al. (2007) explained that the recovery of the photoreceptors is due to the formation of rods and cones at the germinal zone of the retinal boundary.

The present study revealed that, exposing adult specimens of *C. gariepinus* to BL induced dramatic changes in the cornea

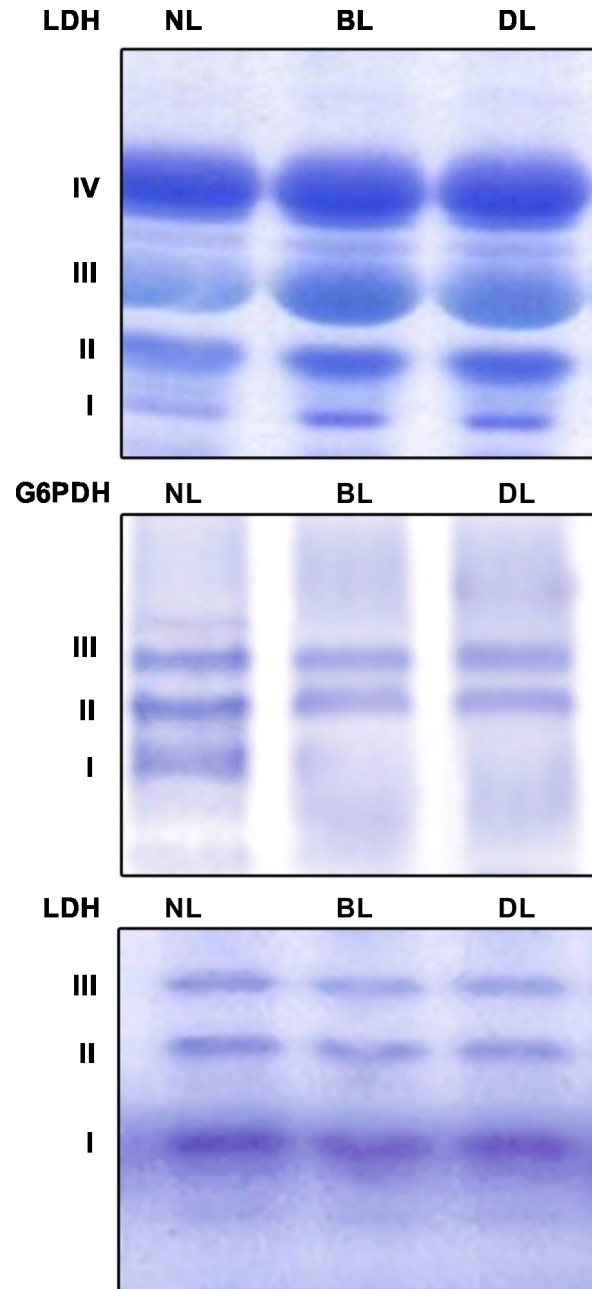


Figure 20. Isoenzyme electrophoresis of *Clarias gariepinus* retina of lactic dehydrogenase (LDH), glucose- 6-phosphate-dehydrogenase (G6PDH), Super oxide dismutase (SOD). (NL) Normal light, (BL) bright light, (DL) dim light.

and retina associated with impairing the antioxidant enzymes and increased oxidative stress and apoptotic markers. However, exposure to dim light following the bright light restored the damaged organelles to some extent through an increase in the antioxidant enzymes and decrease of oxidative stress. This shows

the influence of light conditions on the ordinary structure and function of the cornea and retina and illustrates the plasticity that can enable *C. gariepinus* to survive in different aquatic light conditions of various environments.

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