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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(3): 2584-2589. **OPEN ACCESS**

Histological and histochemical study of the retina in baloot muluki *Chondrostoma regium* (Heckel,1843)

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This study was aimed to recognize the histological structure of the retina of the baloot muluki (*Chondrostoma regium*). Haematoxylin – eosin and special stains were routinely used, and tissue sections were examined with the light microscope. The result showed that the retina is vascular, it consists of two layers: pigmental epithelium and neural layer, the later is composed of nine layers: visual cell layer, outer limiting membrane, external nuclear layer, external plexiform layer, internal nuclear layer, internal plexiform layer, ganglion cell layer, optic nerve fiber layer, and inner limiting membrane. The pigmented epithelium is consists of cuboidal epithelial cell, where their cytoplasmic elongations are extended toward visual cell. In addition, visual cell layer is composed of rod, single cone and double cone. Furthermore, the external plexiform layer is less thicker than the internal plexiform layer. Interestingly the number of rows of the external nuclear layer is less than that of the internal nuclear layer. It can be concluded that the differences in the density of visual cells are due to the visual acuity and light level sensitivity.

Keywords: retina, histology, baloot muluki fish

INTRODUCTION

The fish eyeballs are varies due to their wide spread in aqueous nature and the level of water in which they live. Deep sea fish tend to show some adaptations to get the greatest amount of light, such as the size of eye, lens and pupil (Gali and Dauod, 2014). The eyeball is composed of three layers: tunica fibrosa, which consist of cornea and sclera, tunica vasculosa, consists of choroid, ciliary body and iris, and the third layer is tunica interna which is called retina (Mescher, 2013). The retina is composed of three layers: photoreceptor cells layer consisting of rods and cones that intertwine with bipolar cells and horizontal cells, amacrine cells layer and ganglion cells layer (Kardong, 2012).

Because of a wide diversity of the Iraq environment, which resulted in changes in the eye tissues, several studies have been conducted on fish eyeballs (Al-jumaily, 2008; Abed and Abd Al majeed, 2012; AL-Mteewati and AL-Khaleefa, 2012), and the current study is focused on the retinal of freshwater fish to identify the histological differences and histological biochemistry in the baloot muluki fish Chondrostoma *regium* (Hecke,1843), which belongs to Class : Osteichthyes. It is an omnivore that fed on aquatic insects, eggs of other fish, algae and diatoms (Coad, 2010).

MATERIALS AND METHODS

The collection of adult fish was selected to be life or modern fishing and was obtained from the local markets of different areas in the city of Baghdad. The fish was classified by natural history research center and museum / University of Baghdad. The eye was removed from orbit and the associated muscles were removed.

Sample was fixed in Bouin's fluid solution for 4 hours, and was cut in cross-sectional acute scalpel to 1/3 and 2/3 parts, then it was left in Bouin's solution for 10 hours at room temperature, and the preparation of the tissue section was then completed. The histological slide was stained with harris's haematoxylin, and alcoholic eosin and with special stain with periodic acid-schiff solution and massons trichome technique (Suvarna et al., 2019), and finally the slides were checked and photographed by using a compound light microscope (Meiji) supplied with a camera.

RESULTS AND DISCUSSION

The results showed that the retinal vascular is consisting of two layers: pigmented epithelium and neural layer that is divided into nine layers: visual cell layer composed of rods and cones, outer limiting membrane, external nuclear layer, external plexiform layer, internal nuclear layer, internal plexiform layer, ganglion cell layer, optic nerve fiber layer and inner limiting membrane (figure–1). These results are consistent with (Al-jumaily et al., 2012). In addition, the histological study also showed their pigmented epithelium is consists of a single row of cuboidal epithelial cells with an oval nucleus shape based on the basal membrane, and several cytoplasmic elongations are extended from its surface toward the visual cells (figure–2). The epithelial cells maintains the visual cells from bright light because of their pigmented particles that absorbs the scattered and diffused light through visual cells (Moyer, 1969). The epithelial cells have many advantages such as, vitamin A storage, which contributes to the synthesis of visual pigments (Moyer, 1969), and they behave as phagocytes of infected cells to omit the external segment of visual cells, and works to support of visual cells (Donatti and Fanta, 1999).

The neural retina consists of nine layers: the visual cell layer consisting of long rods, single cone and double cone, each of these cells consists of an outer segment that interacts with the pigmented epithelium and inner segment. The distribution of cones and rods is varies, from the outer part of the cones is more conical and wider than the rods where the later are cylindrical and thin, moreover, cones are more than rods (figure–3). The retina in the baloot muluki fish represents a type of retina that contains of two types of receiving light cells, but there is a clear difference in the form and the structure of these cells, as in most bony fish, particularly shallow waters and surface water fish (AL-Mteewati and AL-Khaleefa, 2012). Furthermore, the layer of visual cells in the *Sardinella aurita* retina consists of long single cones, short single cones and double cones (Salem, 2016), whereas, the visual cell layer in the *Silurus triostegus* retina is composed of long rods and single cones, while in the retina of fish *Liza abu* contains rods and long single cones, short single cones and dwarf single cones as well as fourth types of twin cones (Al-jumaily et al., 2012). The difference in the shape and the structure of the visual cells may be due to the fact that the fish is live at different levels of freshwater depth and to the variation in the proportion of light in the aquatic environment.

In addition, the outer limiting membrane is a light color and clear, and is separate the visual cell layer from the external nuclear layer (Figure–3). This membrane is a general characteristic of the retina in the vertebrate (Garica and De-Juan, 1999). The external nuclear layer consists of the visual cell bodies and has a cell number of 4 – 5 (Figure–4). This result is not consistent with the study of (Garcia et al., 2017). This difference in the number of external nuclear layer rows may be due to the differences in the visual cell density. The external plexiform layer is narrow and intertwined with the axons of the visual cells with the dendrites of both bipolar and horizontal cells.

Furthermore, the internal nuclear layer consists of bipolar cells, horizontal cells, and long-stranded amacrine cells with a range of 5–8 (Figure–4), and this is not consistent with the study of (Garcia et al., 2017), this may be due to differences in size, shape, location and cells function. The function of the large bipolar cell is to binds several rods with a single ganglion cell and the small bipolar cell connects one cone to one ganglion cell (Collin, 1988).

In Figure–4, the internal plexiform layer is thicker than that of the external plexiform layer. It consists of the intertwining of the axes of both bipolar and amacrine cells with ganglion cells forming one row of the ganglion cell layer. The ganglion cells are characterized by large size and clear nucleus (Figure–4). There is a difference in the thickness of the external plexiform layer between *Silurus triostegus* and *Liza abu*, which due to the density of visual cells (Al-jumaily, 2008).The axons of the ganglion cells are clustered to form the optic nerve fiber layer, which grows thicker as it moves toward the back to the optic nerve. The inner limiting membrane separates the retina from the vitreous humor. It is a base plate of muller cells that extend between the outer and inner limiting membrane and its nucleus lies within the internal nuclear layer (figure–5), this result is consistent with (Al-jumaily et al., 2012; Garcia et al., 2017).

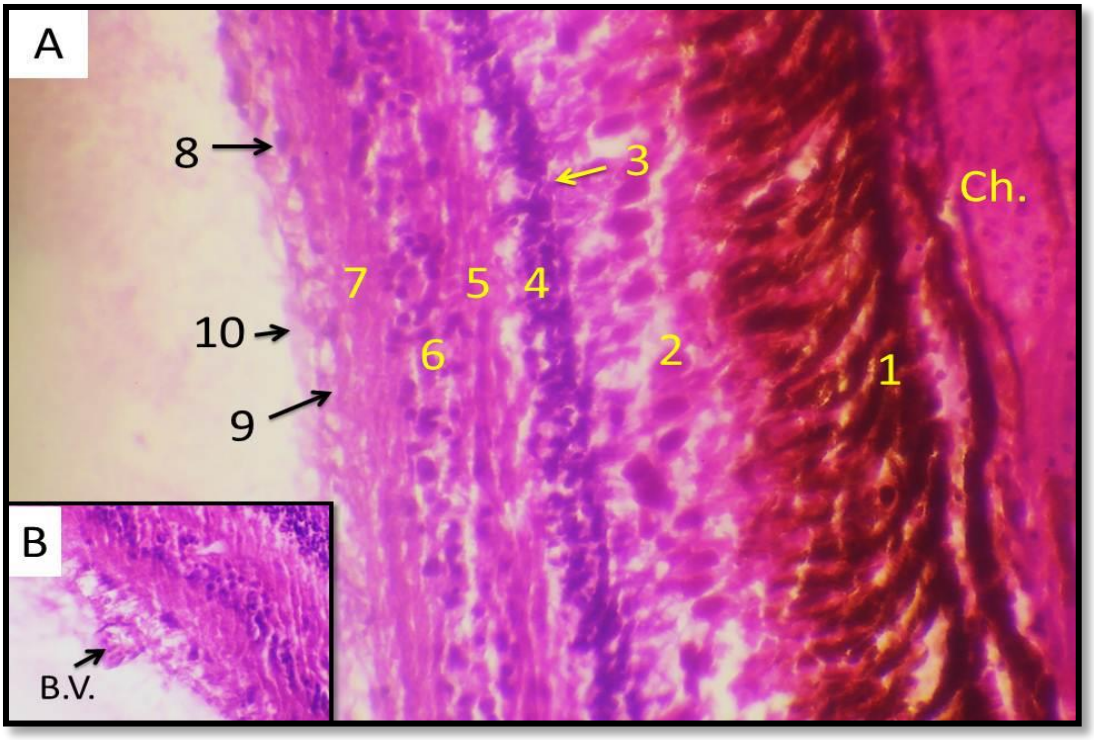

Figure 1: longitudinal section showing retinal layers in the baloot muluki fish eye A&B: 400x (H&E Stain). (1) Pigmented epithelium, (2) Visual cells layer, (3) Outer Limiting membrane, (4) External nuclear layer, (5) External plexiform layer, (6) Inner nuclear layer, (7) Inner plexiform layer, (8) Ganglion cells layer, (9) Optic nerve fiber layer, (10) Inner limiting membrane, (B.V.) Blood Vessel, (Ch.) Choroid

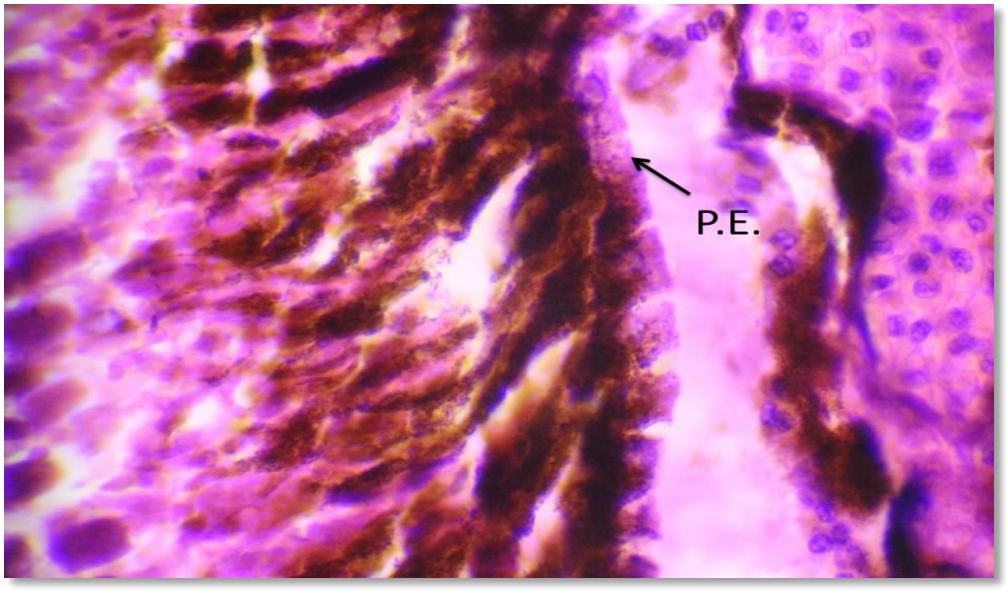

Figure 2: longitudinal section shows the Pigmented Epithelium layer in the retina (H&E Stain) 1000x. (P.E.) Pigmented Epithelium

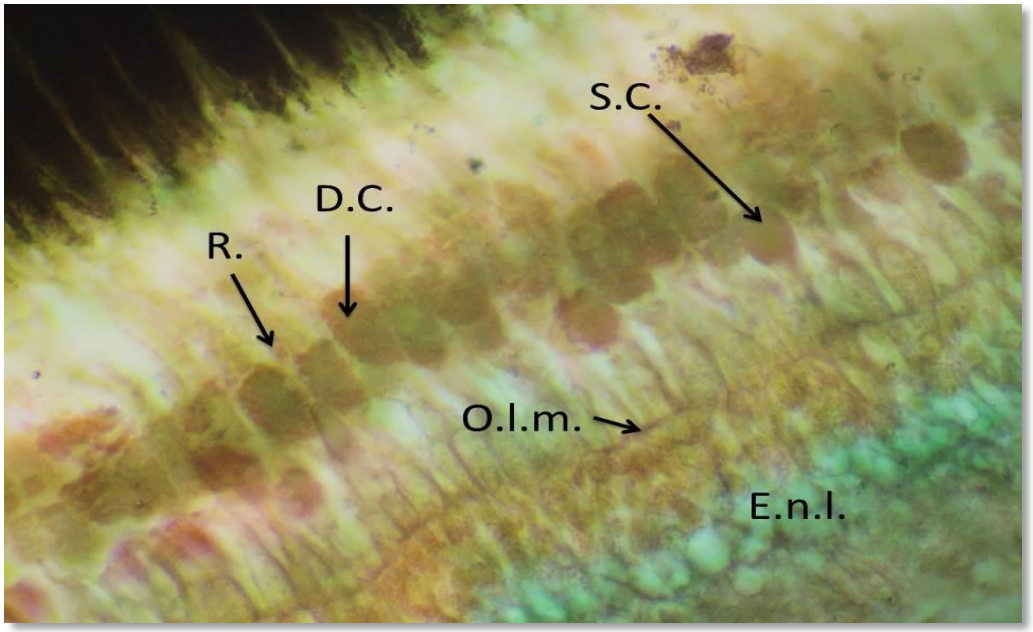

Figure 3: longitudinal section shows the forms of visual cells, the outer limiting membrane and the external nuclear layer in the retina (M.T. Stain) 1000x. (R.) Rod, (S.C.) Single Cone, (D.C.) Double Cone, (O.l.m.) Outer limiting membrane, (E.n.l.) External nuclear layer

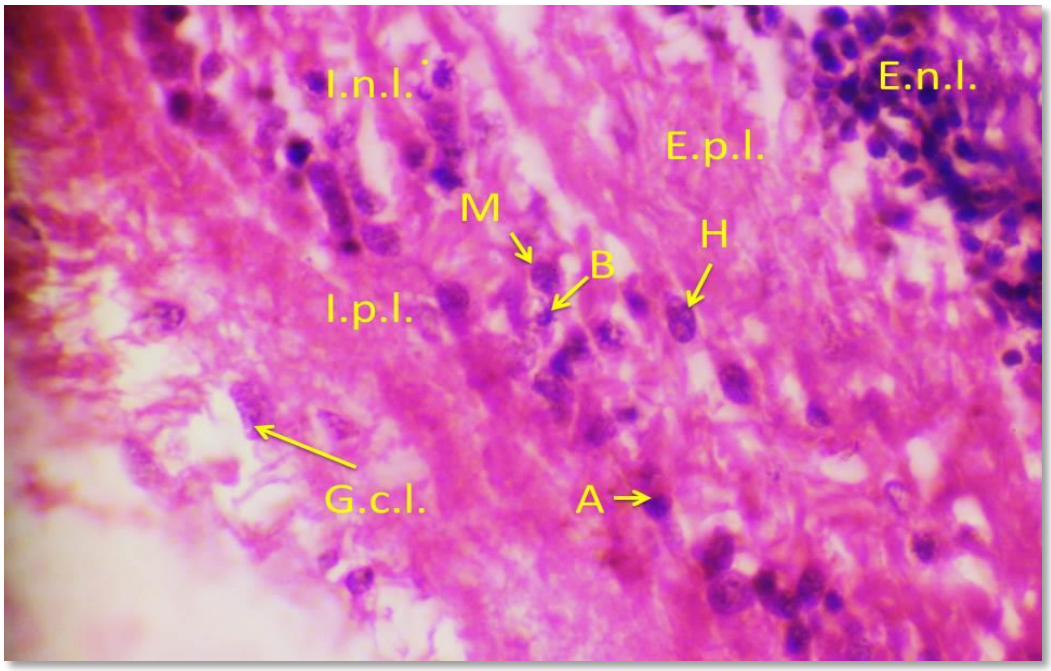

Figure 4: longitudinal section shows the external nuclear layer, the external plexiform layer, the cell forms in the internal nuclear layer, the internal plexiform layer and the ganglion cell layer (H&E Stain) 1000x. (E.n.l.) External nuclear layer, (E.p.l.) External plexiform layer, (I.n.l.) Inner nuclear layer,(H) Horizontal cell, (B) Bipolar cell, (A) Amacrine cell, (M) Muller's cell, (I.p.l.) Inner plexiform layer, (G.c.l.) Ganglion cell layer

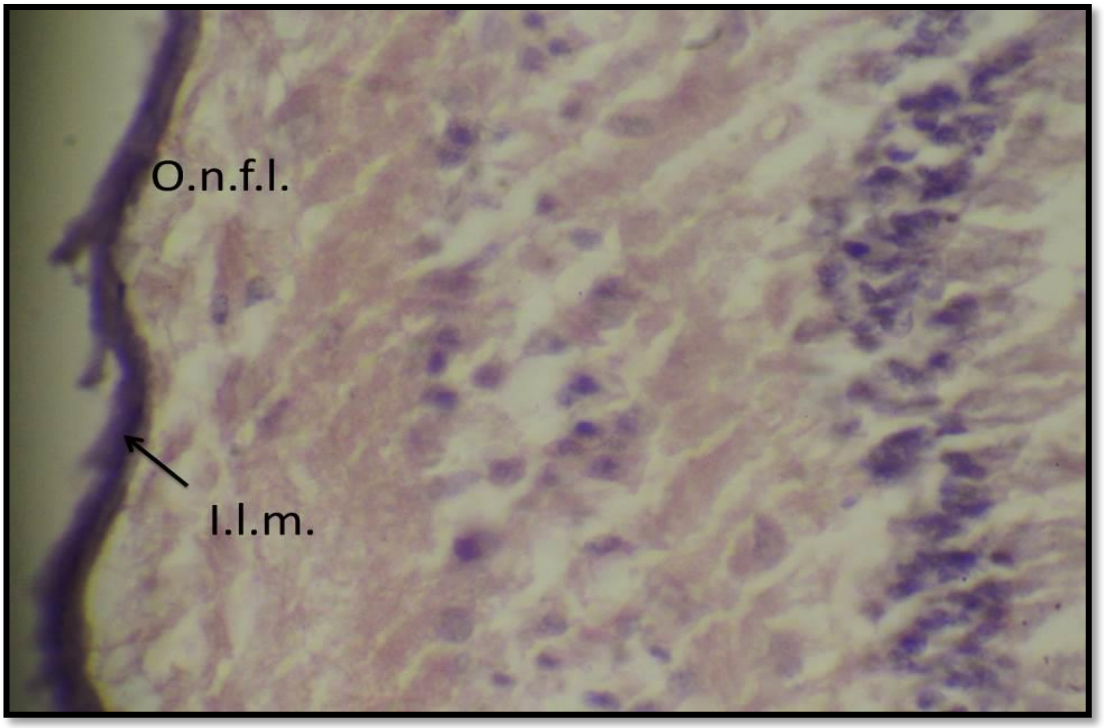

Figure 5: longitudinal section shows the Optic nerve fiber Layer and Inner limiting membrane (PAS Stain) 1000x.(O.n.f.l.) Optic nerve fiber layer, (I.l.m.) Inner limiting membrane

CONCLUSION

The presence of cones more than rods and the difference in the density of visual cells are due to visual acuity and light level sensitivity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was supported by Department of Biology, College of Science for Women.

AUTHOR CONTRIBUTIONS

There are no participants in this search. The Research was carried out at my personal expense.

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