2024, 21(12 Suppl.): 4040-4049
https://doi.org/10.21123/bsj.2024.9334
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

A histological study of the black-winged kite's retina (Elanus caeruleus, Desfontaines, 1789)

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Received 09/09/2023, Revised 14/11/2023, Accepted 16/11/2023, Published Online First 20/06/2024, Published 22/12/2024

Abstract

The study showed the retina of the diurnal invasive raptor Elanus caeruleus is avascular and supplied nutrition and oxygen by the choroid and pecten oculi. The retina includes two layers: the outer layer is called the pigmented epithelium, and the inner layer is the neural layer. The neural layer consists of nine layers: visual cells layer, outer limiting membrane, external nuclear layer, external plexiform layer, internal nuclear layer, internal plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane. The visual cell layer consists of double cones, single cones and rods, and the cones are more than the rods. The external nuclear layer appears to have compact visual cells. The inner nuclear layer is characterized by its diverse and highly compact cells, which consist of bipolar cells, horizontal cells, amacrine cells, and Muller cells. The internal plexiform layer is thicker than the external plexiform layer. The retina contains the deep and shallow fovea. The deep fovea is distinguished by convex walls around the deep and wide pit. The pit of the deep fovea has only thin cones, reduced numbers of cells in the inner nuclear layer, and a lack of ganglion cells. The shallow fovea contains all the retinal layers present at the pit. However, the rows of the inner retinal layer are fewer in number than in the area adjacent to the parafovea.

Keywords: deep fovea, diurnal raptor, retina, shallow fovea, visual cells.

Introduction

Ecological diversity among diurnal raptors is highlighted regarding their adaptations for hunting in bright light with high-acuity vision[1,2]. A vertebrate eye is located in an orbit and can focus light on visual cells to form a picture of the environment. The eye has three layers: the outer tunica fibrosa, the middle tunica vasculosa, and the inner tunica interna (retina)[3,4]. The neurosensory retina contains nine layers connected to the brain via the optic nerve, and the supportive pigmented epithelium is the tenth layer[5]. In some vertebrates, the retina forms a fovea, a pit formed in the retina center that enables high-acuity vision. Composed entirely of cones, it shapes the point of sharpest focus. Although there are no rods in the fovea, they increase peripherally [3,6].

This study is aimed at revealing the tissue biochemistry and histological structure of the retina in the Black-winged Kite (*Elanus caeruleus*, Desfontaines, 1789), which is known to be an

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invasive species in Iraq (Fig. 1). The black-winged kite is one of the small diurnal invasive alien raptors[7]. This specie's population is currently expanding in numerous Iraqi locations where they have created new breeding grounds[8]. This raptor is evaluated as Least Concern[9], and feeds on small mammals, birds, insects, and reptiles[10]. The black-winged kite is characterized by gray, black, and white plumage, a large black spot on the curve of the wings, the large head is mostly white, and the iris appears deep ruby red[10,11].

Figure 1. The Black-winged Kite (*Elanus caeruleus*)

Materials and Methods

A total of 10 eyes from 5 black-winged kite (*E. caeruleus*) were collected from Al–Ghazel market for animals in Baghdad city, classified by natural history research center and Museum / University of Baghdad. Birds were anesthetic by chloroform inhalation, and the eyes were extracted from orbit. The eyes were fixed in Bouin's solution for 10 hours and then washed with 70% ethanol. The eye samples taken were routinely processed and embedded in paraffin. The eye blocks were sectioned at a thickness of 5 µm using a microtome. All sections were stained with Harris's Haematoxylin–Alcoholic Eosin (H&E), combined Alcian Blue–PAS technique (AB/PAS) and Masson's Trichrome (MT)[12]. Sections were examined and photographed by using a compound light microscope with a Canon microscope camera (EOS Kiss X7i).

Results and discussion

The histological sections showed that the black-winged kite tunica interna (retina) is avascular and lines the choroid from the inside. The choroid is a highly vascularized pigmented layer that receives blood vessels from arterial branches after penetrating the sclera (Fig. 2A, B). Pecten oculi are located above the optic nerve and have folds rich in blood vessels (Fig. 3A, B). The black-winged kite is a diurnal invasive bird of prey that needs a vascular choroid and large pecten oculi to provide nutrition and oxygen to the retina. Perhaps this bird has a binocular vision that allows it to focus its eyes on prey and capture it from long distances. The variety of blood vessel density in the choroid and pecten oculi between birds, like the northern bald ibis (*Geronticus eremita*)[13]. The density of blood vessels in the choroid may be related to the eyeball's need for blood supply, the type of bird, and the density of the light environment. The pecten oculi of various bird species suggest that they contribute to keen vision during migration and hunting in addition to supplying nutrients to the retina[14].

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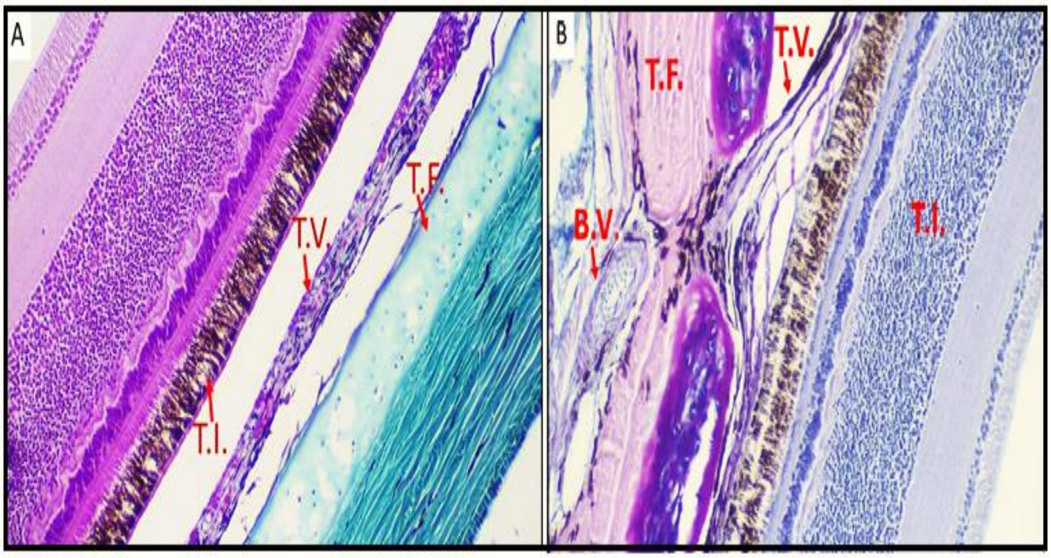

Figure2. Cross section of eye layers in a black-winged kite (A:200X (MT stain) &B:200X (AB/PAS stain)). The eye layers involved Tunica Interna (T.I.), Tunica Vasculosa (T.V.), Tunica Fibrosa (T.F.), Blood Vessel (B.V.)

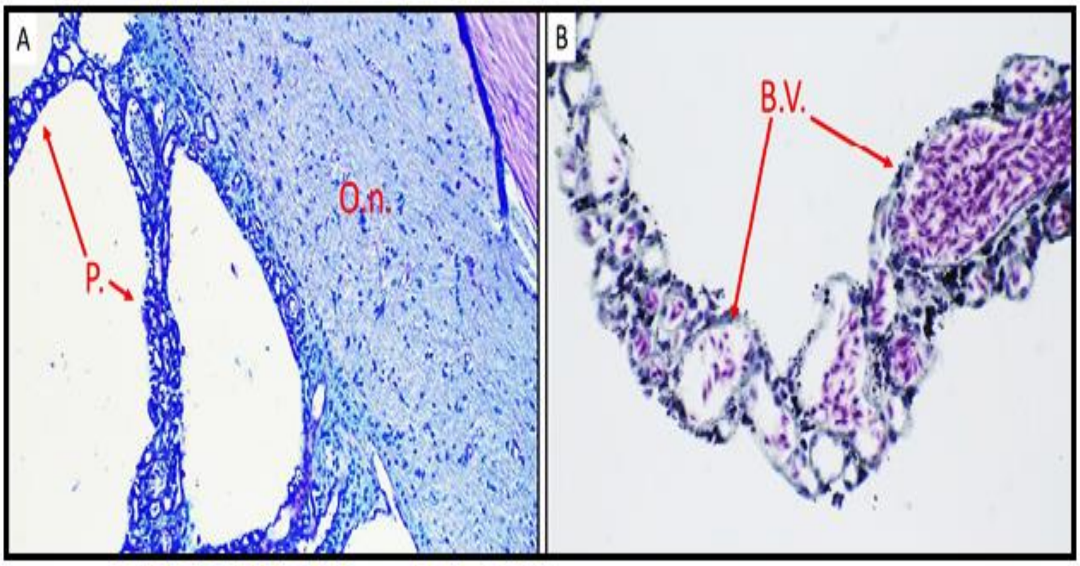

Figure3. Cross section of eye in a black-winged kite showing the structure of pecten oculi and its connection to the optic nerve (A: 200X (AB/PAS stain) & B: 400X (MT stain)). Optic nerve (O.n.), Pleat (P.), Blood Vessel (B.V.)

The retina in the black-winged kite is composed of two layers: the pigmented epithelial layer and the neural layer, which consists of nine layers: photoreceptor layer, outer limiting membrane, external nuclear layer, external plexiform layer, internal nuclear layer, internal plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane (Fig. 4). This similar to most vertebrate retina[5,15,16]. The retina's function is to accurately transport information from outer environmental light stimuli to the brain, where nerve impulses are translated into vision[5].

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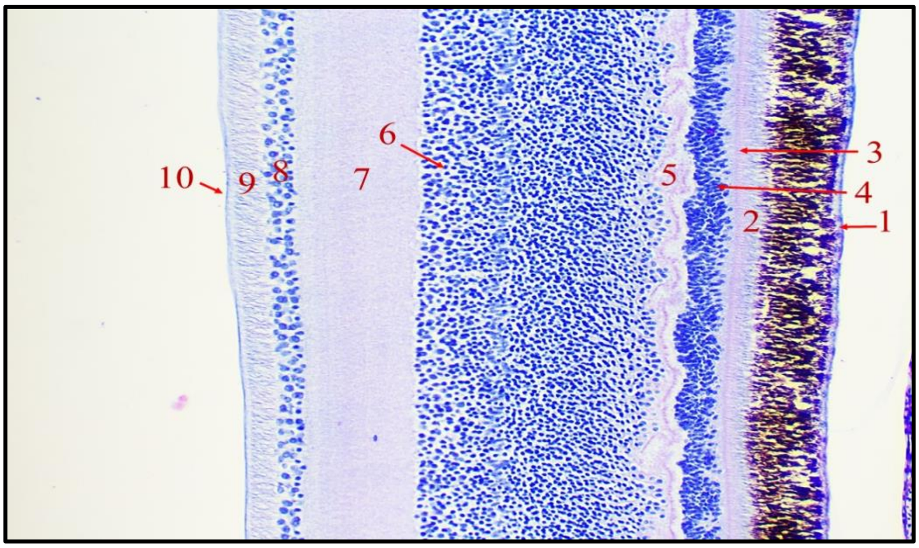

Figure 4. Cross section of eye in a black-winged kite showing the retinal layers (AB/PAS stain (200X))(1) pigmented epithelium, (2) visual cells layer, (3) outer limiting membrane, (4) external nuclear layer, (5) external plexiform layer, (6) internal nuclear layer, (7) internal plexiform layer, (8) ganglion cell layer, (9) nerve fiber layer, (10) inner limiting membrane

The pigmented epithelium of the black-winged kite is composed of a single layer of cuboidal epithelial cells with oval nuclei. This layer is more adherent to the choroid and depends on the basement membrane. Each cell sends numerous cytoplasmic elongations inward to surround the photoreceptor cells and is characterized by containing pigmented granules (Fig. 5). Perhaps the black-winged kite's pigmented epithelium has a high density of pigmented granules, particularly at the apical projections, which might protect the external segment of photoreceptors from the impacts of intense light. According to Sultan *et al*., the pigmented epithelium of the laughing dove (*S. senegalensis*) has numerous highly functional factories for lipid biosynthesis, which are necessary for esterification and storage of vitamin A substances for acute vision[15]. The pigmented epithelium maintains photoreceptors and visual functions, including phagocytosis and removal of shed photoreceptor outer segment debris[17].

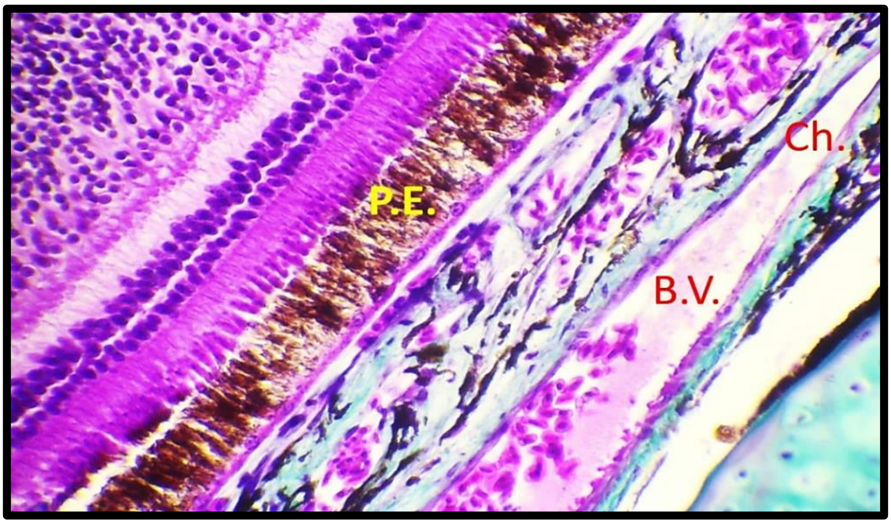

Figure 5. Cross section of eye in a black-winged kite showing Pigmented Epithelium (MT stain (400X)). Pigmented Epithelium (P.E.), Choroid (Ch.), Blood Vessel (B.V.)

The neural retina of *E. caeruleus* includes a layer of visual cells consisting of elongated rods, double cones, and single cones. Each of these photoreceptor cells has an outer segment that overlaps with the extensions of the pigmented epithelial cells and an inner segment. The outer segment of the cones is conical in shape and broad, while the rod's outer segment is long and cylindrical. In this layer, the cones and rods are close together, and more cones than rods (Fig. 6A, B). The visual cells of *E. caeruleus* adapt to light. This diurnal raptor may hunt prey through the high-resolution and color eyesight of the cones. Bird vision depends on cone photoreceptors for color vision and rods for vision in low light[18]. The central regions of the yellow-legged gull's retina have a higher density of cones, which enables the bird to see the prey without moving the eyes or the head[19].

The outer limiting membrane of *E. caeruleus* is a light color that separates the visual cell layer from the external nuclear layer and is indistinct in some areas (Fig. 6A). This is consistent with the magpie (*Pica pica*)[20]. The outer limiting membrane of *E. caeruleus* may be due to the numerous Muller cell extensions that space between the photoreceptor cells. The external nuclear layer of *E. caeruleus* consists of the visual cell bodies and their nuclei, which appear more compact and have rows 2–5(Fig. 6A). The presence of numerous visual cell's bodies in the external nuclear layer is perhaps because the black-winged kite is a diurnal bird of prey that depends on acute vision to see prey. The density of the visual cells in *E. caeruleus* may result in a difference in the number of these cells in the external nuclear layer rows.

The external plexiform layer of *E. caeruleus* is tight and consists of the terminals of visual cell axons that synapse with the dendrites of bipolar and horizontal cells (Fig. 6A). The internal nuclear layer of the black-winged kite is composed of diverse and compact cells and contains the cell bodies and nuclei of bipolar cells, horizontal cells, amacrine cells, and muller cells. The number of nuclei rows in this layer ranges between 4 – 30 (Fig.7). The retina of the diurnal raptor (*Elanus caeruleus*) is distinguished by a thick inner nuclear layer, which may be due to the connectivity patterns of neural cells. The inner nuclear layer of *E. caeruleus* has a number of nuclei rows that is contrary to other avian, such as *Pica pica*[20]. According to Marc, the difference in the number of nuclei rows of these cells in the internal nuclear layer is due to the differences in size, shape, location, and function of the cells between the species[21].

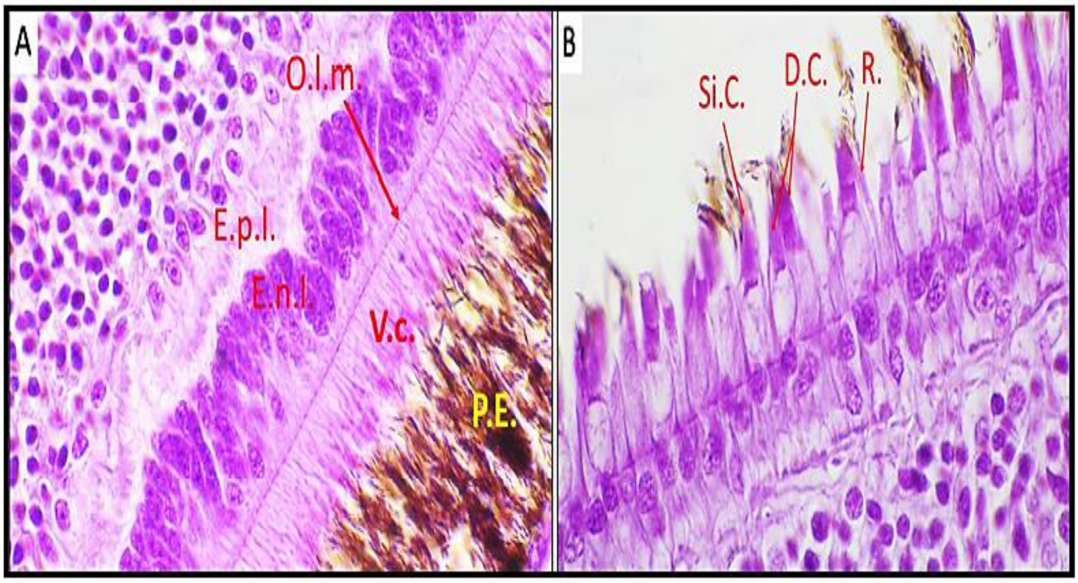

Figure 6. Cross section of eye in a black-winged kite showing the shapes of visual cells in the external nuclear layer (E.n.l.), the outer limiting membrane (O.l.m.), and the external plexiform layer (E.p.l.) of the retina as well as Pigmented Epithelium(P.E.), Visual cell (V.c.), Double Cone (D.C.), Single Cone (Si.C.), and Rod (R) were observed (A&B:1000X (MT stain).

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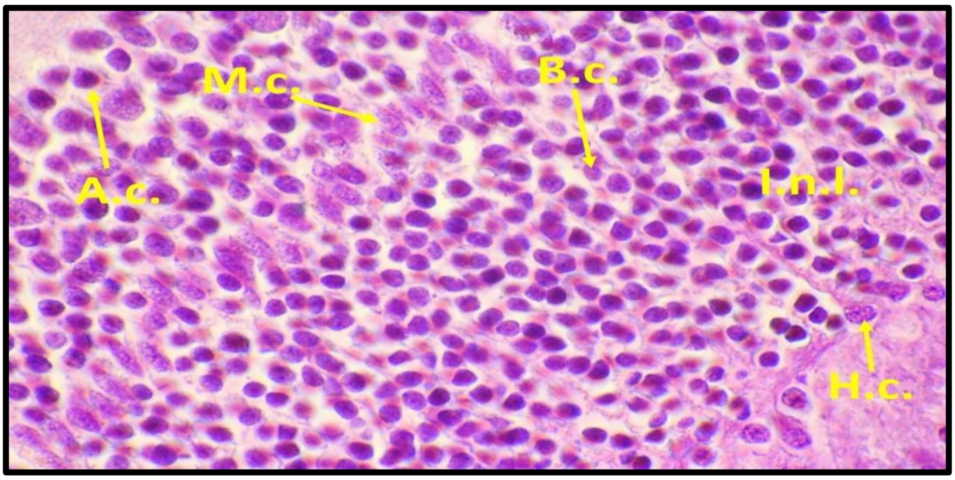

Figure 7. Cross section of eye in a black-winged kite showing cell shapes in the inner nuclear layer (I.n.l.) of the retina as well as Horizontal cell (H.c.), Bipolar cell (B.c.), Amacrine cell (A.c.), Muller cell (M.c.) (H&E stain (1000X))

The internal plexiform layer of *E. caeruleus* consists of the synapses of the axons of the bipolar cells and amacrine cells, with the dendrites of the ganglion cells forming the ganglion cell layer (Fig. 8). This layer is thicker than the external plexiform layer in the retina of the black-winged kite, which may be due to the high density of the neural cells in the internal nuclear layer and their synapses with ganglion cells. The ganglion cell layer of *E. caeruleus* contains ganglion cells of large size with clear nuclei, and the number of nuclei rows ranges between 1 – 6 (Fig. 8). The difference in the number of ganglion cell rows in a specific region of the black-winged kite retina may be due to the axons of ganglion cells forming rows in the nerve fiber layer surrounded by muller cell processes. The number of nuclei rows in the ganglion cell layer is different from those in the yellow-legged gull (*Larus michahellis*) and the magpie (*Pica pica*)[19,20].

In the black-winged kite retina, the ganglion cell axons are collected to form a layer of nerve fiber. The nerve fiber layer increases in thickness towards the center of the eye to form the optic nerve. (Fig. 9). The inner limiting membrane is a basement membrane formed by the terminations of muller cells that separates the retina from the vitreous humor (Fig. 8,9). In the black-winged kite retina, the nerve fiber layer's well-organized and thick axon of ganglion cells suggests sharp visual acuity. These results were consistent with[15]. The muller cells maintain homeostasis and cell survival in the entire retina[22].

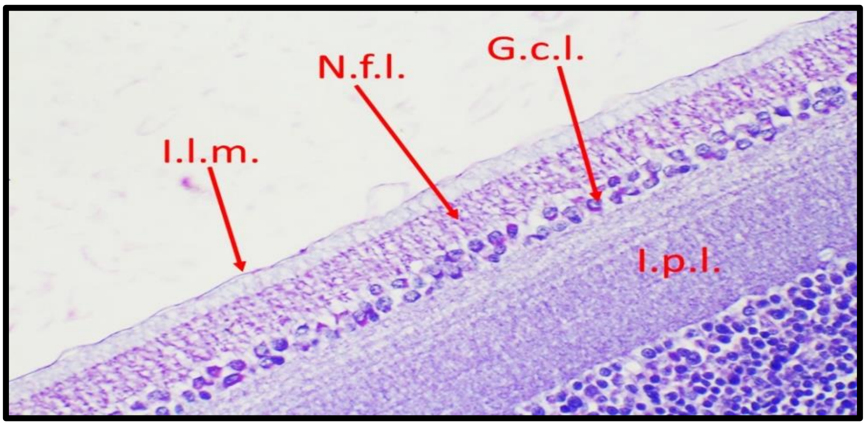

Figure 8. Cross section of eye in a black-winged kite showing internal plexiform layer (I.p.l.), ganglion cell layer (G.c.l.), nerve fiber layer (N.f.l.), and inner limiting membrane (I.l.m.) of the retina (AB/PAS stain (400X))

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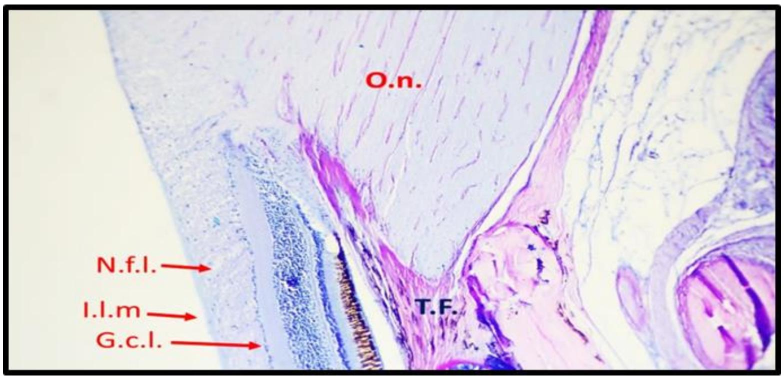

Figure 9. Cross section of eye in a black-winged kite showing the nerve fiber layer (N.f.l.) forming the optic nerve (O.n.), Ganglion cell layer (G.c.l.), Inner limiting membrane (I.l.m.), Tunica Fibrosa (T.F.) (AB/PAS stain (100X))

The retina of the black-winged kite contains two foveae. First, the deep central fovea has convex walls around the deep and wide pit. The fovea pit consists of only cones that are thinner and longer, and it contains a few Muller cells, bipolar cells, and amacrine cells in the inner nuclear layers, but ganglion cells are absent. In contrast, the ganglion cells in the parafovea were arranged in up to six rows (Fig. 10A, B, C). Second, the shallow temporal fovea consists of all layers of the retina in the pit. However, the rows of the inner retinal layer are fewer in number than in the area adjacent to the parafovea (Fig. 11).

The presence of deep and shallow fovea in the retina of *E. caeruleus* may be due to its ability to greater image magnification, high visual acuity for detecting movements within the visual field without the need to move the eye. Perhaps this diurnal bird of prey is adapted to high-acuity vision in bright light by higher densities of cone and ganglion cell densities in the fovea.

Birds of prey are adapted to predation because they have a central fovea used to detect prey, a temporary fovea to fixate on the prey at the moment of capture, and an enlarged binocular field to position during prey capture[1]. The researcher[23] suggested that Muller's cell contributes to the foveal center image becoming magnified and light focusing into a point within and ring around the foveal center. The deep fovea in *Larus michahellis*, which offers the greatest resolution and color distinction, can be used to see distant objects[24]. According to Potier *et al.*, raptors have the best eyesight, including different arrangements of the fovea, whose depth and width correlate greatly with eye size[25].

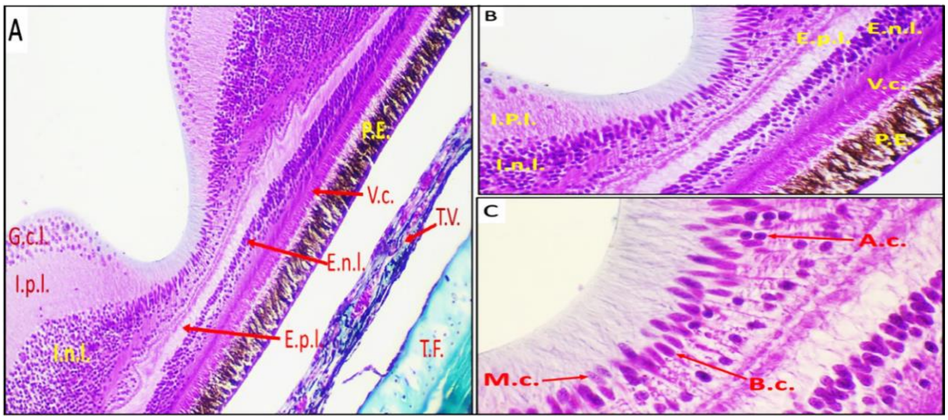

Figure 10. Cross section of eye in a black-winged kite showing deep fovea of the retina. Tunica Fibrosa (T.F.), Tunica Vasculosa (T.V.), Pigmented Epithelium (P.E.), Visual cell (V.c.), external nuclear layer (E.n.l.), external plexiform layer (E.p.l.), Internal nuclear layer (I.n.l.), Internal plexiform layer (I.p.l.), Ganglion cell layer (G.c.l.), Bipolar cell (B.c.), Amacrine cell (A.c.), Muller cell (M.c.) (A:200X, B: 400X & C: 1000X (MT stain))

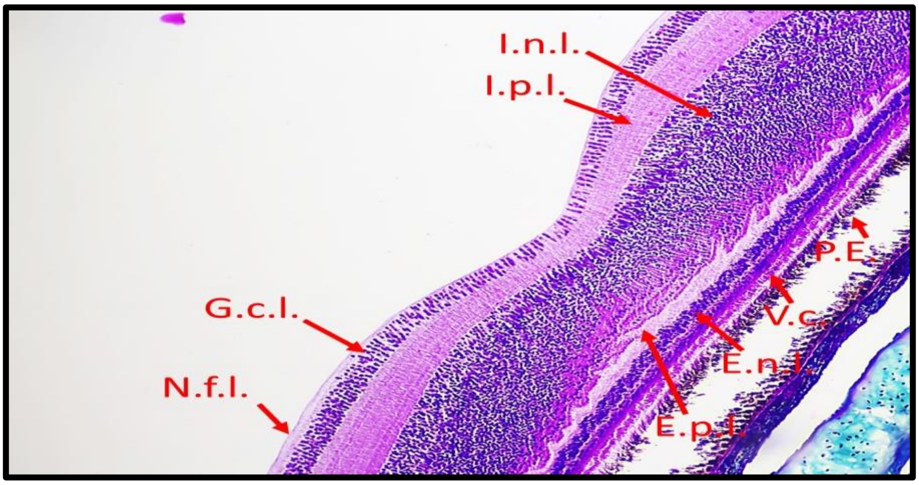

Figure 11. Cross section of eye in a black-winged kite showing shallow fovea of the retina. Pigmented Epithelium (P.E.), Visual cell (V.c.), external nuclear layer (E.n.l.), external plexiform layer (E.p.l.), Internal nuclear layer (I.n.l.), Internal plexiform layer (I.p.l.), Ganglion cell layer (G.c.l.), Nerve fiber layer (N.f.l.) (200X (MT stain)

Conclusion

The density of cones in the visual cell layer is due to the increase in high visual acuity, which enhances the black-winged kite's ability to hunt prey. The deep and shallow fovea are areas of high sharpness in *Elanus caeruleus*, so they need to absorb a large amount of light to increase visual severity and color vision to search for prey from long distances.

Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

S.A.A prepared the draft manuscript. M.K.H. supervised the study and reviewed the manuscript. All authors discussed the result and approved the final manuscript.

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دراسة نسجية لشبكية عين الحدأة سوداء الجناح (*Elanus caeruleus*, Desfontaines, 1789)

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قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

أظهرت الدراسة أن شبكية عين الطائرالجارح النهاري الغازي *Elanus caeruleus* ، هي لاوعائية وتزود بالتغذية والأكسجين عن طريق المشيمية وممشط العين. تتكون شبكية العين من طبقتين: الطبقة الخارجية تسمى الظهارية المخضبة ، والطبقة الداخلية هي الطبقة العصبية. تتكون الطبقة العصبية من تسع طبقات: طبقة الخلايا البصرية ، الغشاء المحدد الخارجي ، الطبقة النووية الخارجية ، الطبقة الضفيرية الخارجية ، الطبقة النووية الداخلية ، الطبقة الضفيرية الداخلية ، طبقة الخلايا العقدية ، طبقة الألياف العصبية ، الغشاء المحدد الداخلي. تتكون طبقة الخلية البصرية من مخاريط مزدوجة ، ومخاريط مفردة وقضبان ، وتكون المخاريط أكثر من القضبان. أن الطبقة النووية الخارجية تحتوي على خلايا بصرية متراصة. تتميز الطبقة النووية الداخلية بأن خلاياها متنوعة ومتراصة بشكل كبير وتتألف من خلايا ثنائية القطب، والخلايا الافقية، و الخلايا عديمة البروزات الطويلة، وخلايا مولر. وتكون الطبقة الضفيرية الداخلية أكثر سمكًا من الطبقة الضفيرية الخارجية. تحتوي شبكية العين على الحفيرة العميقة والحفيرة الضحلة. تتميز الحفيرة العميقة بجدران محدبة حول النقرة العميقة والواسعة. تحتوي النقرة في الحفيرة العميقة على مخاريط رفيعة فقط ، وعدد قليل من الخلايا في الطبقة النووية الداخلية ، وعدم وجود الخلايا العقدية. تحتوي الحفيرة الضحلة على جميع طبقات الشبكية الموجودة في النقرة. ولكن ان صفوف الطبقة الشبكية الداخلية اقل عدد مما كانت عليه في المنطقة القريبة من الحفيرة.

الكلمات المفتاحية: الحفيرة العميقة، الطائر الجارح النهاري، الشبكية، الحفيرة الضحلة، الخلايا البصرية.