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Original Article	**Age-Associated Changes in the Cornea, Lens and Retina of the Albino Rat Eye: A Histological and Immuno-Histochemical Study**

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ABSTRACT

Background: Age-related changes occur throughout the body including vision. Aging has various effects on the visual system affecting both external and internal portions of the eye. Age-related visual deterioration may be due to both neural and optical factors.

Aim of the Work: To study the normal histological changes of aging on the cornea, lens and retina of albino rat eye.

Materials and Methods: In this study twenty-four male albino rats were used. They were divided into Two groups: Group (1) young-aged group (3-6 months) and Group (2) old-aged Group (22-26 months). All animals were sacrificed and their eye balls were extracted and prepared for light microscopic examination. Immuno-histochemical stain was also added to detect apoptosis.

Results: It was revealed that aging induced various histological changes in the eye of albino rat. The cornea showed irregular surface with atrophy of the epithelial cells and wide separation of corneal lamellae with loss of keratocytes. The lens subcapsular epithelial cells appeared few and disarranged. The cortical lens fibers lost their nuclei. The retina showed diminished retinal thickness, damage of some ganglion cells and retinal pigmented epithelial cells. The outer nuclear layer revealed marked diminution of its thickness with small dark nuclei. Rods and cones showed decreased density. The Bruch's membrane appeared ill-defined and irregular with some adherent deposits and blood leakage in the choriocapillaries. The immuno-histochemical stain revealed appearance of some apoptotic cells in the cornea, lens subcapsular epithelial cells and ganglion cell layer as well as pigment epithelial cells of the retina in old-aged rats.

Conclusion: Normal aging is associated with some histological and immuno-histochemical changes that could explain the visual impairment observed in elderly.

Key Words: Aging, eye histology, cornea, eye lens, retina, apoptosis, caspase-3.

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INTRODUCTION

Age-related changes occur throughout the body including vision. More than 50% of all individuals with severe vision impairment are over the age of 65. Increased rate of ocular aberrations with age, may be one of the reasons for the lower visual quality in older adults *(Ferrer-Blasco et al., 2008)*. The conjunctiva becomes more susceptible to chronic inflammations with age, the lens becomes opacified and the vitreous may contain opacities that become visible to the individual as floaters, or spots in the field of vision. The arteries and veins of the retina become narrower with reduction of the retinal blood flow *(Ehrlich et al., 2008)*.

The conjunctiva becomes more susceptible to chronic inflammations with age, the lens becomes opacified and the vitreous may contain opacities that become visible to the individual as floaters, or spots in the field of vision. The arteries and veins of the retina become narrower with reduction of the retinal blood flow *(Ehrlich et al., 2008)*. The corneal nerve terminal density was found to be significantly decreased resulting in diminished corneal sensitivity and increased incidence of dry eye in old age *(Dvorscak & Marfurt, 2008)*.

The primary function of the eye lens is to focus light on the retina. It grows by a process of epithelial cell division at its equator and the formation of generations of differentiated fiber cells. The lens can retain a high level of light transmission throughout the lifetime of

the individual, with the ability to form sharp images on the retina. Continuous growth of the lens solves the problem imposed by terminal differentiation within a closed avascular system from which cells cannot be shed *(Cvekl & Tamm, 2004; Kuszak et al., 2004)*.

The major proteins in the lens (alpha, beta, and gamma-crystallins) are constantly subjected to age-related changes which are cumulative and affect crystallin structure and function. With time, the modified crystallins aggregate, causing the lens to increasingly scatter light on the retina instead of focusing light on it and causing the lens to lose its transparency gradually and become opaque. Age-related lens opacity, or cataract, is the major cause of blindness worldwide *(Sharma & Santhoshkumar, 2009)*.

Age-related presbyopia occurs in the first five decades of life due to progressive insolubilization of the crystallins of the lens nucleus, progressive increase in lens mass, changes in the point of insertion of lens zonules and shortening of the anterior surface curvature *(Schachar, 2008)*.

One of the most common causes of irreversible vision loss in elderly is Age-related Macular Degeneration (AMD), which is a progressive degeneration of the retinal macula leading to severe decrease in fine vision and central scotoma *(Klein et al., 2004)*. Changes in the macula occur all-over time and occur more often in the elderly. The prevalence of AMD among 40 to 49 years olds is 2.1% which increases dramatically to 35% among those over 80 years of age**.** The risk factors for AMD include ethnicity, gender, hypertension, genetics, diet and sunlight exposure *(Topouzis et al., 2006)*.

The Retinal Pigment Epithelium (RPE) is always exposed to a highly oxidative environment. Mitochondrial decay, bio-energetic deficiencies and weakened antioxidant defenses in RPE cells occur as early as the age of 62 years. These factors may significantly reduce RPE function and contribute to age-related retinal anomalies *(He & Tombran-Tink, 2010)*.

Since aging has various effects on the visual system, which may induce severe vision impairments, the aim of the present work was to throw light on the normal histological changes occurring in some important components of the refractive system of the eye of old aged albino rat as well as studying the caspase-3 reactions in cornea, lens and retina in an attempt to verify the mechanism of age-associated visual impairment. Understanding the mechanism of age-related visual deterioration is of scientific and social interest and could be helpful in finding solutions to improve visual quality in old age.

MATERIALS AND METHODS

Twenty-four male albino rats were used in this study. The animals were caged in a well ventilated room under the same hygienic conditions. Rats that were (3-6)-month-old were considered as young rats, and (22-26)-month-old ones were considered as old rats *(Cavallotti et al., 2001)*. In all cases animals had free access to food and water and were maintained on a 12 h-light-12 h-dark cycle. They were divided into 2 groups as follows:

- **Group I (young aged):** Consisted of 12 animals of 3-6 months age.
- **Group II (old aged):** Consisted of 12 animals of 22-26 months age.

All animals were sacrificed and their eye balls were extracted and fixed in 10 % formal saline. Then, specimens were processed for paraffin sections of 4-6 µm and prepared for histological and Immuno-histochemical studies as follows:

Histological Study:

The Following Stains Were Used:

1. Hematoxylin and Eosin (H&E) stain: For demonstration of the general histological structure *(Drury & Wallington, 1980)*.
2. Periodic Acid Schiff's (PAS) stain: For demonstration of the PAS+ve materials *(Drury & Wallington, 1980)*.

Immuno-Histochemical Study:

Caspase-3 immuno-histochemical stain was used to demonstrate apoptosis in the corneal, subcapsular epithelial and retinal cells as well as the terminally differentiating cortical lens fiber cells as follows: Paraffin sections of 4-6

µm thickness were deparaffinized in xylene, hydrated in graded alcohol and pretreated for antigen retrieval in 10 mmol/L citrate buffer, pH 6.0 for 10 minutes. Staining was performed using polyclonal rabbit anti-human CPP32 (1:200 titer) and a Vectastatin ABC peroxidase, rabbit IgG detection Kit with 3-amino 9-ethyl carbazole as the chromogen. The slides were mounted with permount *(Fisher scientific)* and were photographed with light microscope. The apoptotic nuclei and bodies with active caspase-3 reaction were stained dark brown. To check the specificity of the immunostaining, the negative control was performed using normal rabbit serum instead of anti-CPP32 antibody *(Viktor & Eric, 2006)*.

RESULTS

Histological Results:

Group I (young-aged group):

Light microscopic examination of H&E and PAS stained eye sections revealed that the cornea was formed of five layers which were: corneal epithelium, Bowman's membrane, stroma, Descemet's membrane and corneal endothelium. The corneal epithelium was stratified squamous non-keratinizing epithelium and consisted of 5-6 layers of cells resting on a thin basement membrane. Bowman's membrane appeared as acellular layer, which was composed of fine collagen fibrils arranged in random distribution. The corneal stroma was composed of many parallel lamellae of tightly bound collagen fibers (corneal lamellae) with sparse fibrocytes (keratocytes) in-between. Descemet's membrane appeared as homogeneous, acellular membrane, which appeared magenta (PAS-positive) in the PAS stained sections. The last layer was the corneal endothelium, which was formed of simple squamous epithelium (Figs. 1, 2).

As regards the lens of young-aged rat, it was formed of an outer eosinophilic capsule, subcapsular epithelium and lens fibers. The lens capsule appeared as eosinophilic homogeneous basal lamina, which appeared PAS-positive in the PAS-stained sections. The cells of the subcapsular epithelium (or anterior lens cells) were cubical in shape with regular arrangement. They appeared reflected at the equator of the lens where they were transformed into outer nucleated cortical lens fibers. The lens fibers were formed of outer nucleated and deep non-nucleated layers. The outer nucleated cortical lens fibers were located immediately deep to the subcapsular epithelium. They were formed of elongated fibers with oval nuclei. The deep lens fibers appeared without nuclei with lateral undulation or interdigitations forming the hard lens nucleus (Figs. 3, 4, 5).

As regards the retina of young-aged rat, it was formed of ten layers which were (from inside to outside); the inner limiting membrane, the nerve fiber layer, the ganglion cell layer, the inner plexiform layer, the inner nuclear layer, the outer plexiform layer, the outer nuclear layer, the outer limiting membrane, the receptor layer of rods and cones and the pigmented epithelium. Small blood vessels were seen in the innermost part of the choroid, which was called the choriocapillary layer situated between the outer pigmented epithelium and choroid (Figs. 6, 7). The inner limiting membrane separated the retina from the vitreous body. The nerve fiber layer was formed from axons of the ganglion cells. The ganglion cell layer contained the bodies of ganglion cells. The inner plexiform layer contained synapses between the dendrites of ganglion cells and the axons of bipolar cells of the inner nuclear layer. The inner nuclear layer contained cell bodies of bipolar cells. The outer plexiform layer contained synapses between rods and cones and the bipolar cells (Fig. 8). The outer nuclear layer contained the cell bodies of rods and cones (receptor cells). The outer limiting membrane appeared as a basal lamina separating the outer nuclear layer from the rods and cones, which represented the outer segments of the receptor cells. The pigmented epithelium appeared as the outermost layer of the retina and was formed of epithelial cells resting on a well-defined PAS+ve Bruch's membrane situated between the choroid and the retina (Fig. 9).

Group II (old-aged group):

Light microscopic examination of H&E and the PAS stained eye sections of this group revealed some corneal changes. The corneal surface appeared irregular with apparent keratinization of the corneal epithelium, which exhibited shrunken deformed cells with small dark nuclei. The Bowman's membrane showed areas of detachment from the overlaying epithelium. The stroma showed wide separation of the corneal lamellae with invasion by blood vessels. The keratocytes were few, shrunken and absent in some areas (Figs. 10, 11).

As regards the lens of old-aged rat, it revealed apparent thickening of the capsule. The subcapsular epithelial cells appeared few and disarranged with ill-defined nuclei and some empty spaces or gaps. The outer cortical lens fibers appeared tightly packed together with loss of their nuclei (Figs. 12, 13).

The retina of old-aged rat appeared to be more vulnerable to aging. The Bruch's membrane appeared ill-defined, interrupted and irregular with formation of new abnormal blood vessels in the choroid and blood leakage in the choriocapillaries (Fig. 14). The ganglion cell layer revealed areas of ganglion cell loss. Some ganglion cells exhibited condensed nuclei. The outer nuclear layer revealed marked diminution of its thickness with small dark nuclei and shrunken cytoplasm as compared to that of young-aged rat. The pigmented epithelial cells showed wide areas of cell loss. Decreased density of rods and cones was observed up to their loss in many areas. Deposition of some basophilic deposits adherent to the interrupted Bruch's membrane was also noticed (Figs. 15, 16).

Immunohistochemical Results:

Group I (young-aged group):

On examination of caspase-3 stained sections of this group it was noticed that the corneal epithelial cells showed basophilic nuclei with–ve caspase-3 reaction (Fig. 17). The subcapsular epithelial cells of the lens and the outer cortical lens fibers showed also basophilic nuclei with–ve reaction (Fig. 18). Similarly, the retina revealed–ve caspase-3 reaction in the cells of all layers (Fig.19).

Group II (old-aged group):

On the other hand, examination of caspase-3 stained sections of old-aged rats showed dark brown nuclei of some corneal epithelial cells and some keratocytes with+ve caspase-3 reaction (Fig. 20). The lens also revealed some dark brown nuclei of the subcapsular epithelial cells and some apoptotic bodies with+ve reaction (Fig. 21). Moreover, retina of this group revealed some brown stained nuclei of the ganglion cells and pigment epithelial cells with+ve caspase-3 reaction (Figs. 22, 23).

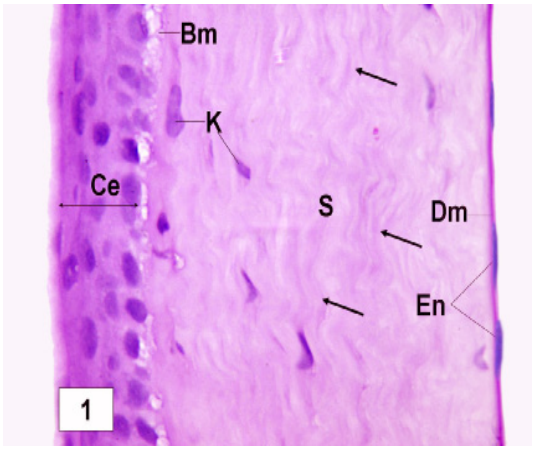

Fig. 1: A photomicrograph of a section in the cornea of young-aged rat showing the corneal epithelium (Ce), bowman's membrane (Bm), corneal stroma (S) with keratocytes (k) in-between the corneal lamellae (arrows), descemet's membrane (Dm) and the innermost endothelial cells (En). Hx.&E.; x 400

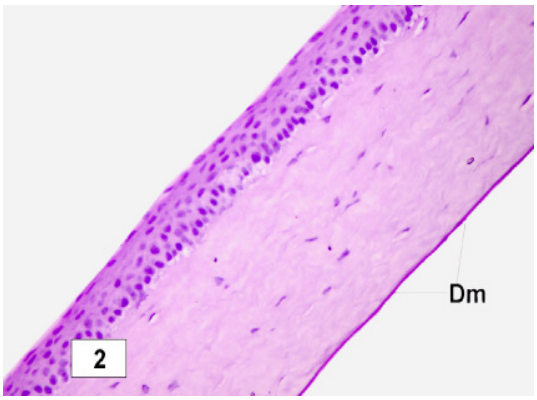

Fig. 2: A photomicrograph of a section in the cornea of young-aged rat showing PAS+ve magenta stained Descemet's membrane (Dm). PAS; x 200

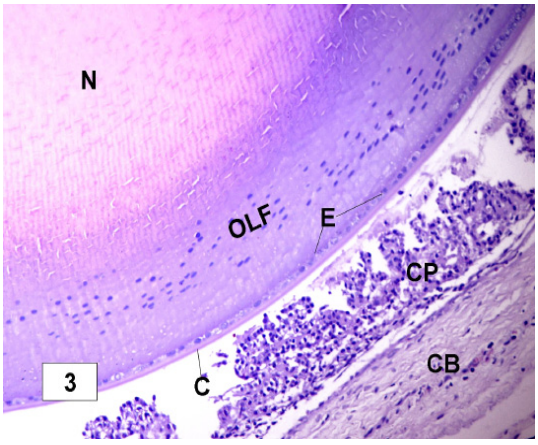

Fig. 3: A photomicrograph of a section in the lens of young-aged rat showing the capsule (C), subcapsular epithelial cells (E) and outer nucleated cortical Lens fibers (OLF) and deep lens fibers forming the Nucleus (N). notice the adjacent ciliary processes (CP) and ciliary body (CB). Hx.&E.; x 100

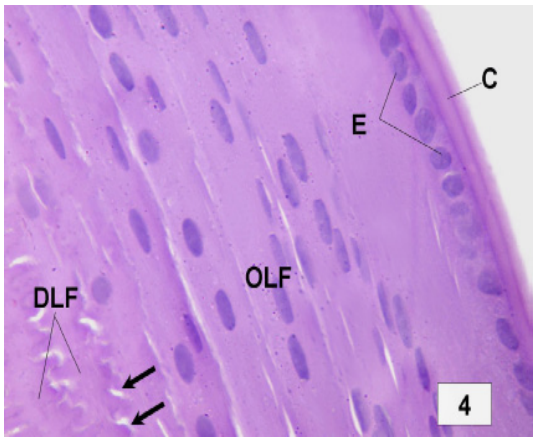

Fig. 4: A photomicrograph of a section in the lens of young-aged rat showing outer eosinophilic capsule (C), subcapsular cubical epithelial cells (E) and outer nucleated lens fibers (OLF) with oval nuclei and deep lens fibers (DLF) without nuclei. Notice the interdigitations between lens fibers (arrows). Hx.&E.; x 400

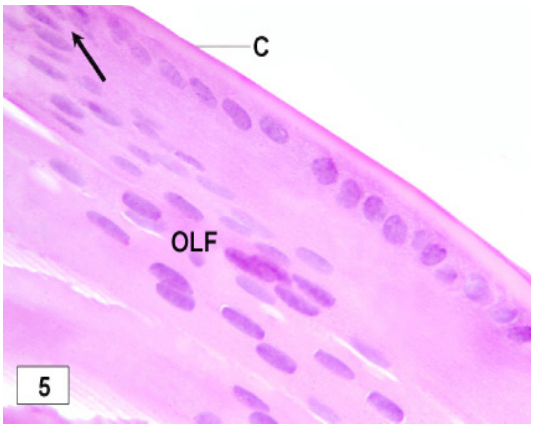

Fig. 5: A photomicrograph of a section in the lens of young-aged rat showing the PAS+ve magenta stained capsule (C). Notice the reflection of the subcapsular epithelial cells (arrow) at the equator to form the outer nucleated cortical lens fibers (OLF). PAS; x 400

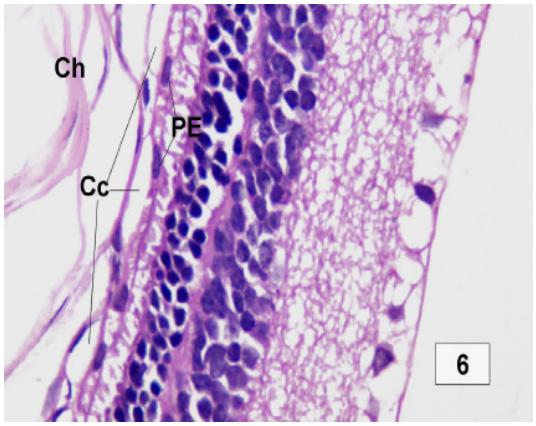

Fig. 6: A photomicrograph of a section in the retina of young-aged rat showing its different layers and the choriocapillaries layer (Cc) between the outer pigmented epithelium (PE) and the choroid (Ch). Hx.&E.; x 400

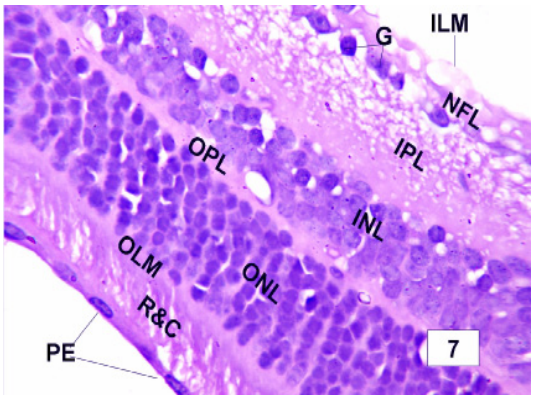

Fig. 7: A photomicrograph of a section in the retina of young-aged rat showing its ten layers; the inner limiting membrane (ILM), the nerve fiber layer (NFL), ganglion cell layer (G), inner plexiform Layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), outer limiting membrane (OLM), receptor layer of rods and cones (R&C) and pigmented epithelium (PE). Hx.&E.; x 400

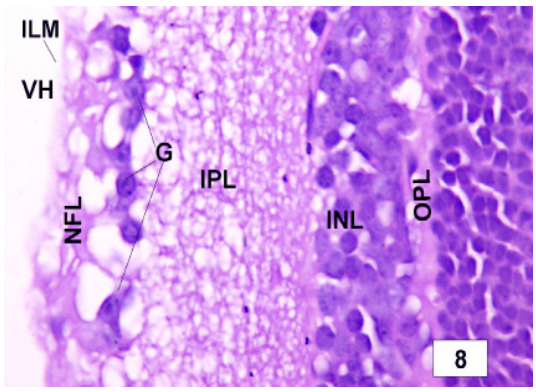

Fig. 8: A photomicrograph of a section in the retina of young-aged rat showing the inner limiting membrane (ILM) separating the retina from the vitreous humer (VH), the nerve fiber layer (NFL) formed from axons of ganglion cells (G). The inner plexiforml ayer (IPL) contains synapses of ganglion cells dendrites with bipolar cells of the inner nuclear layer (INL). The outer plexiform layer (OPL) contains synapses of rods and cones with bipolar cells. Hx.&E.; x 400

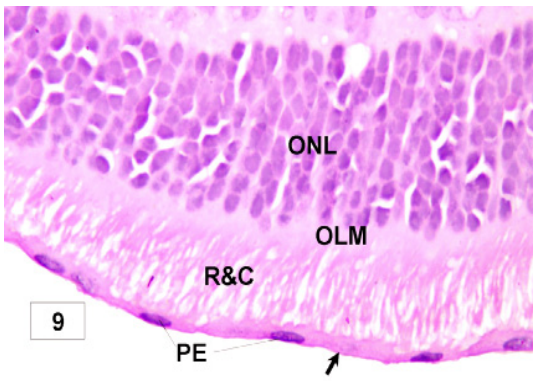

Fig. 9: A photomicrograph of a section in the retina of young-aged rat showing the outer nuclear layer (ONL) contains the cell bodies of receptor cells, the Outer limiting membrane (OLM),rods and cones (R&C) and the Pigmented Epithelial cells (PE) resting on a well defined magenta Bruch's membrane (arrow). PAS; x 400

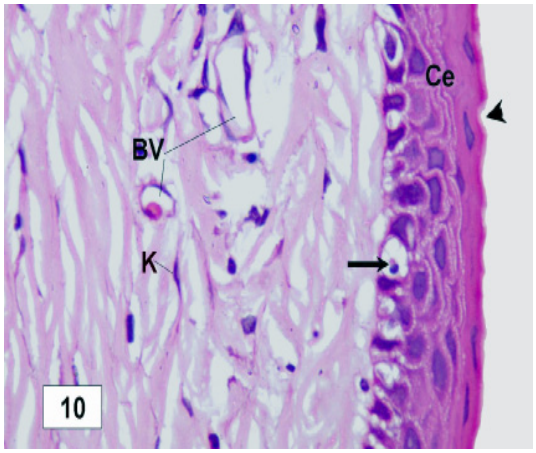

Fig. 10: A photomicrograph of a section in the cornea of old-aged rat showing irregular corneal surface (arrow head) with apparent keratinization of the corneal epithelium (Ce) exhibiting shrunken deformed corneal cells with small dark nuclei (arrow). Notice shrunken keratocytes (K) and invasion of the corneal stroma with blood vessels (BV). Hx.&E.; x 400

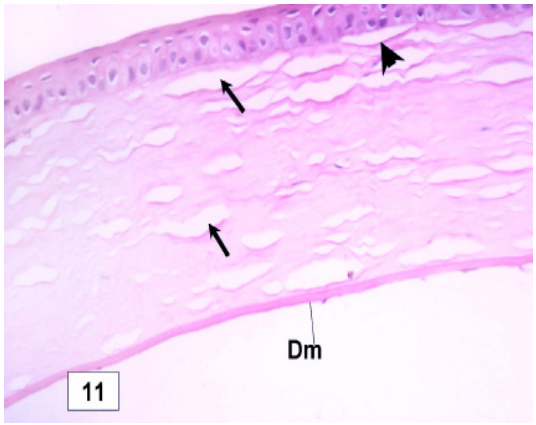

Fig. 11: A photomicrograph of a section in the cornea of old-aged rat showing magenta stained descemet's membrane (Dm), area of detached bowman's membrane (arrow head) and wide separation (arrows) of the corneal lamellae with absence of keratocytes. PAS; x 200

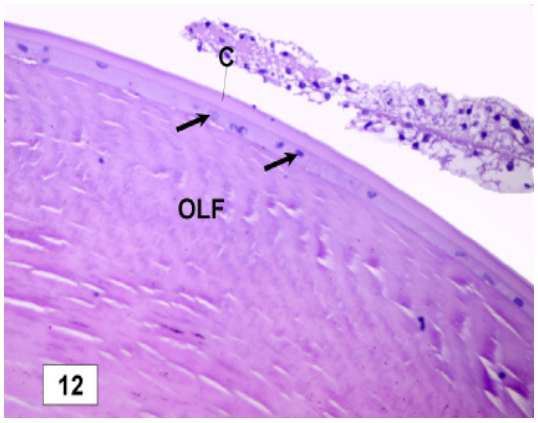

Fig. 12: A photomicrograph of a section in the lens of old-aged rat showing outer eosinophilic capsule (C) and few scattered subcapsular epithelial cells (arrows) and the outer cortical lens fibers (OLF) with loss of their nuclei. Hx.&E.; x 200

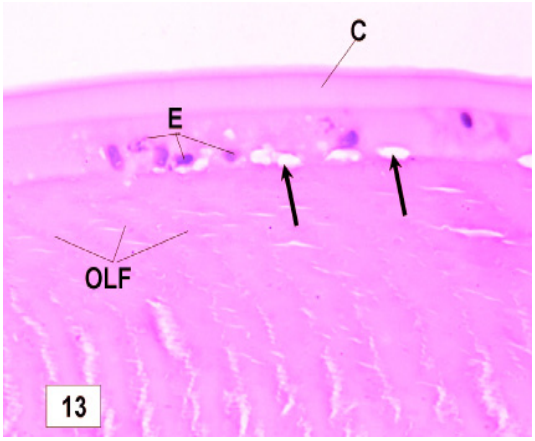

Fig. 13: A photomicrograph of a section in the lens of old-aged rat showing magenta stained apparently thickened capsule (C) with few and disarranged subcapsular epithelial cells (E) exhibiting ill-defined nuclei and some empty spaces (arrows). notice tightly packed outer cortical Lens fibers (OLF) with loss of their nuclei. PAS; x 400

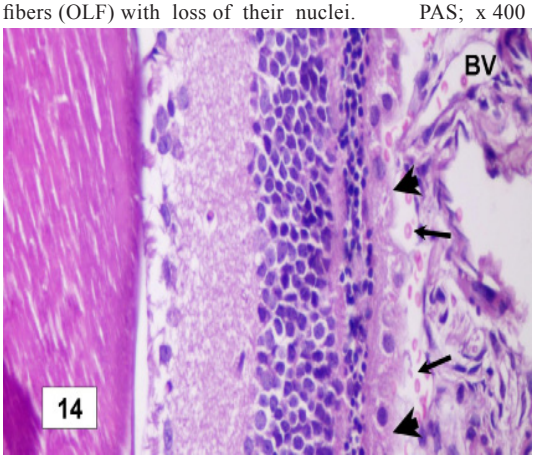

Fig. 14: A photomicrograph of a section in the retina of old-aged rat showing new abnormal blood vessels (BV) with blood leakage (arrows) in the choriocapillaries. Notice ill-defined irregular Bruch's membrane (arrowheads). Hx.&E.; x 200

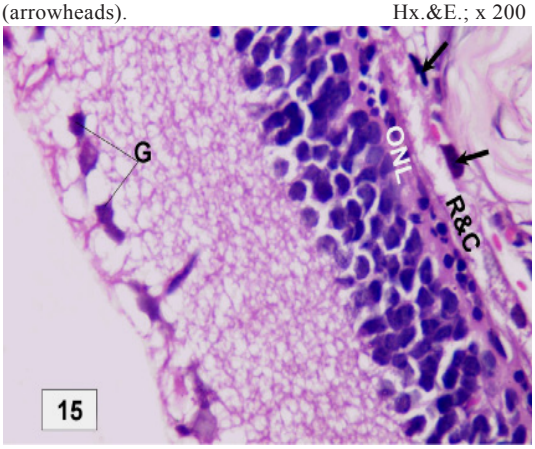

Fig. 15: A photomicrograph of a section in the retina of old-aged rat showing some ganglion cells (G) with condensed nuclei, diminished thickness of the outer nuclear layer (ONL) with small dark nuclei and diminished density of rods and cones (R&C). Notice the appearance of some basophilic deposits (arrows) adherent to Bruch's membrane. Hx.&E.; x 400

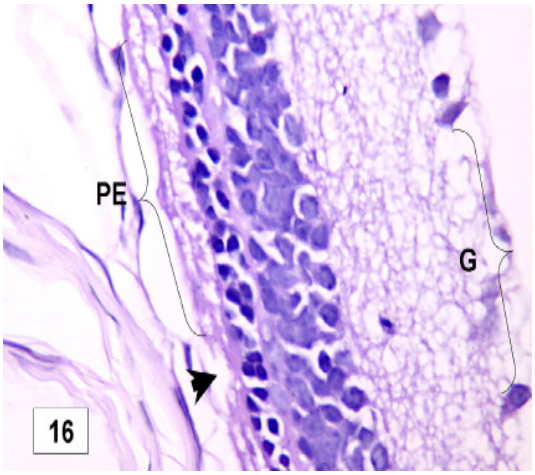

Fig. 16: A photomicrograph of a section in the retina of old-aged rat showing interrupted Bruch's membrane (arrowhead). notice areas of loss of ganglion cells (G) and pigmented epithelial cells (PE). PAS; x 400

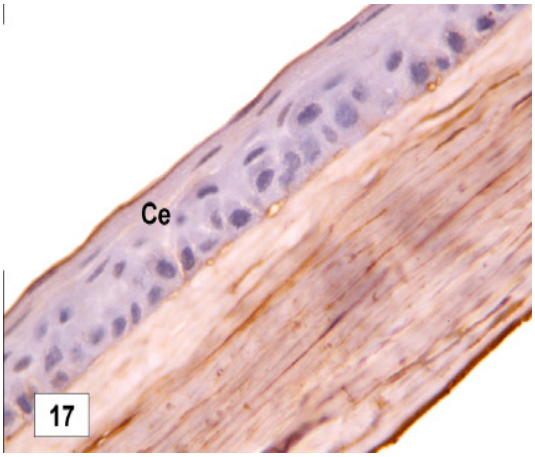

Fig. 17: A photomicrograph of a section in the cornea of young-aged rat showing basophilic nuclei of the corneal epithelial cells (Ce) with–ve caspase-3 reaction. Caspase-3; x 400

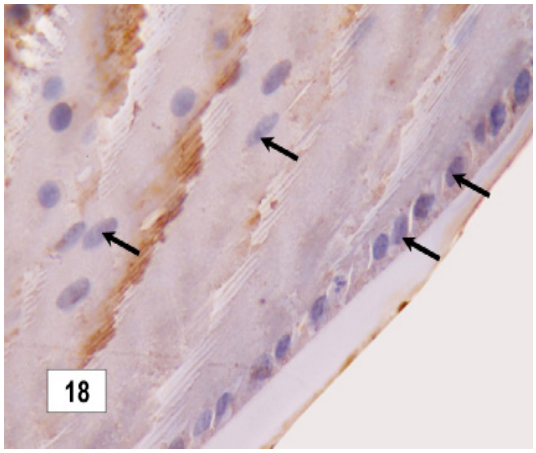

Fig. 18: A photomicrograph of a section in the lens of young-aged rat showing basophilic nuclei (arrows) of the subcapsular epithelial cells and outer cortical lens fibers with–ve caspase-3 reaction. Caspase-3; x 400

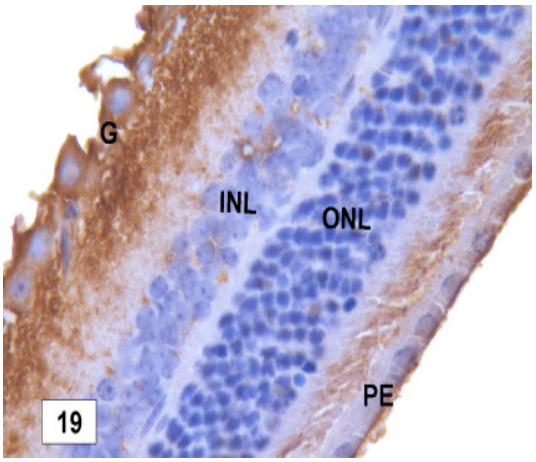

Fig. 19: A photomicrograph of a section in the retina of young-aged rat showing normal basophilic nuclei of the ganglion cells (G), inner nuclear (INL), outer nuclear (ONL) and pigmented epithelial cell (PE) layers with–ve caspase-3 reaction. Caspase-3; x 400

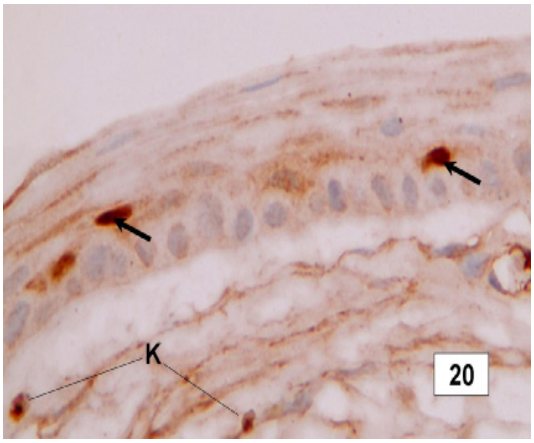

Fig. 20: A photomicrograph of a section in the cornea of old-aged rat showing dark brown nuclei (arrows) of some corneal epithelial cells and some keratocytes (k) with+ve caspase-3 reaction. Caspase-3; x 400

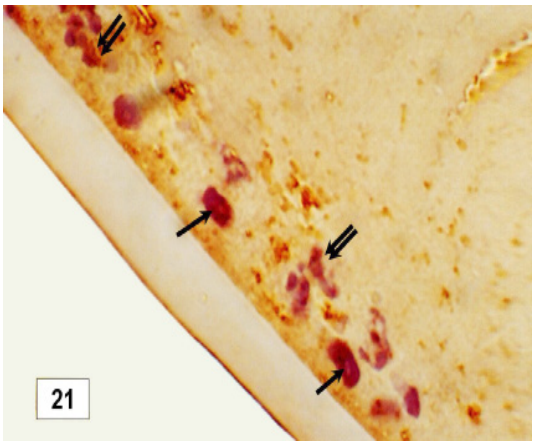

Fig. 21: A photomicrograph of a section in the lens of old-aged rat showing dark brown nuclei (arrows) of the subcapsular epithelial cells and some apoptotic bodies (double arrows) with+ve caspase-3 reaction. Caspase-3; x 400

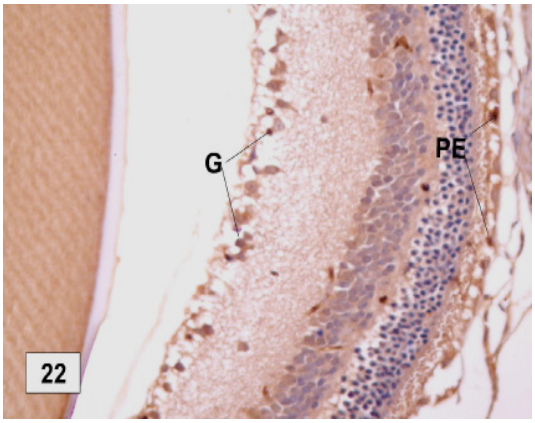

Fig. 22: A photomicrograph of a section in the retina of old-aged rat showing some brown nuclei of ganglion cells (G) and retinal pigmented epithelial cells (PE) with+ve caspase-3 reaction. Caspase-3; x 100

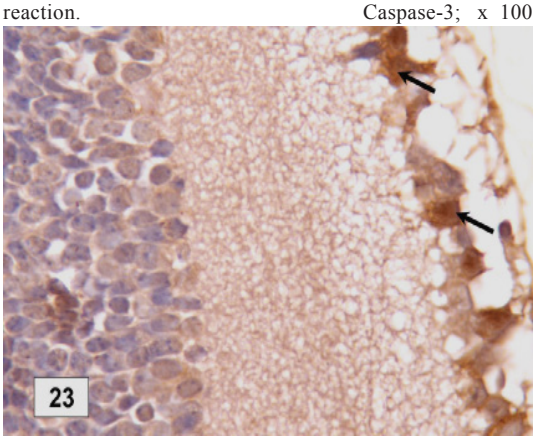

Fig. 23: A photomicrograph of a section in the retina of old-aged rat showing brown nuclei (arrows) of some ganglion cells with+ve caspase-3 reaction. Caspase-3; x 400

DISCUSSION

Aging has various effects on the visual system. In this study it was found that the cornea of old-aged rats showed atrophy and keratinization of the corneal epithelium. These findings are in agreement with *Konomi and Joyce (2007)* who found a significant decrease in the cell proliferative capacity and intensity of the corneal epithelium with aging. Moreover, *Kamiya et al. (2009)* demonstrated that all biomechanical parameters of the cornea were significantly decreased by aging.

The present work revealed wide separation of the corneal lamellae of the stroma in eye sections of old rats suggesting impairment of the corneal transparency. These results are confirmed by *Nishida (2008)* who reported that good vision requires maintenance of the transparency and proper refractive shape of the cornea. He explained that the corneal fibrils must be separated from each other by less than ½ of a wavelength of light to remain transparent. Hence wide separation of the corneal fibrils results in corneal edema, which renders the cornea cloudy.

Keratocytes are known to be developmentally derived from neural crest cells and migrate to settle in the mesenchyme. Once settled in the stroma, keratocytes start synthesizing collagen molecules of different types and keratan sulfate. By the moment of eye opening after birth the proliferation of keratocytes come to an end and most of them are in the quiescent state *(West-Mays & Dwivedi, 2006)*. Quiescent keratocytes synthesize the so-called crystallins. Corneal crystallins, like those of the lens, are thought to help maintenance of corneal transparency and optimal refraction *(Jester, 2008)*. They are also part of the corneal antioxidant defense mechanism *(Lassen et al., 2008)*. On the other hand, Keratan sulfate produced by keratocytes is thought to help maintenance of optimal corneal hydration *(Funderburgh, 2000)*.

The current study showed reduction of corneal keratocytes in the stroma of old-aged rats. Similar finding was previously reported by *Niederer et al. (2007)* using laser scanning in vivo confocal microscopy. They revealed that increasing age was associated with a significant and relatively linear reduction in keratocyte number, endothelial cell density and corneal sub-basal nerve fibers density. Similarly, *Patel et al. (2001)* found that the number of keratocytes declines with age, at a rate approximately of 0.45% per year.

In this study, it was observed that the lens of old-aged rats showed apparent thickening of its capsule, few disarranged subcapsular epithelial cells with ill-defined nuclei and some empty spaces. These findings are supported by *Tkachov et al. (2006)* who reported a significant decrease of the mean cell density in both central and germinative zones of the lens epithelium with aging. These morphological changes were observed before cataract formation and increased with aging. Moreover, *Starodubtseva (2009)* clarified that with ageing, the cells lose their ability for rapid functional re-arrangement of the cellular skeleton.

Truscott (2005) stated that eye lenses from young rats or mice can synthesize reduced Glutathione (GSH) from methionine or N-acetylcysteine while lenses from old animals can not synthesize it. Proper GSH content protects the lens against oxidative stress and has important role in the prevention of cataractogenesis. On the other hand, *Santhoshkumar et al. (2008)* attributed age-related cataractogenesis to protein aggregation in the lens.

The present work showed that cortical lens fibers of old-aged rats appeared tightly packed with loss of their nuclei. This finding is consistent with *Al-Khudari et al. (2007)* who found that cortical fibers undergo a greater degree of age-related compaction than nuclear fibers suggesting that lens fibers compaction is simply one of the factors that contribute to the overall decreased transparency in aged rabbit lenses.

The retina of old-aged rats in this study showed various changes. Areas of loss of ganglion cells were observed. The outer nuclear layer revealed marked diminution of its thickness with small dark nuclei as compared to the retina of young-aged rats. These results are in agreement with *Cavallotti et al. (2001)* who hypothesized that rat retina is particularly sensitive to developmental changes and to senile decay. They added that the retinal thickness was significantly decreased with age and ganglion cells seemed to be more vulnerable to age-related loss than other retinal cells.

Cavallotti et al. (2004) stated that human retina also undergoes specific changes with aging. They observed a significant decrease in all retinal parameters between the young and old subjects including the mean retinal thickness, mean number of ganglion cells and intercellular connections area. Similarly, *Sung et al. (2009)* proved that all macular thickness parameters were significantly decreased with increasing age, except for the central fovea sector.

The present study revealed areas of pigmented epithelial cell loss in old-aged rats. This result is in agreement with *Szaflik et al. (2009)* who attributed vision loss in individuals over the age of 55 in the Western world to the damage of Retinal Pigment Epithelial (RPE) cells mainly due to oxidative stress, which also affects their DNA and promotes genome instability in these cells. They suggested that these effects may coincide with the decrease in the efficacy of DNA repair with age.

On examination of retinal photoreceptors of old-aged rats in the present work, it was observed that rods and cones exhibited decreased density with areas of loss. This result is supported by *Panda-Jonas et al. (1995)* who reported that the annual cell loss of photoreceptors was approximately 0.2% to 0.4% accompanied by loss of retinal ganglion cells and pigment epithelium cells. They also added that the decline in photoreceptor count affected more rods than cones.

In the current study, the retina Bruch's membrane appeared ill-defined and irregular with adherent deposits. These observed data can be supported by *Spraul et al. (1999)* who found early calcification and fragmentation of Bruch's membrane with soft, diffuse and large extracellular as well as basal laminar deposits**.** Moreover, *Crabb et al. (2002)* described these extracellular deposits (drusen), as a mixture of blood proteins, extracellular proteins and intracellular proteins.

However**,** *Janik-Papis et al. (2009)* and *García-Castiñeiras. (2010)* attributed these deposits to oxidative stress. They suggested that retinal pigment epithelial cells can be exposed to Reactive Oxygen Species (ROS) induced by accumulation of iron ions in these cells, sunlight exposure and tobacco smoke. Moreover, oxidized polyunsaturated fatty acids are not correctly cleaved in the lysosomes of RPE and are accumulated in the form of lipofuscin, which is deposited in Bruch's membrane in the form of drusen.

Coinciding with the previous results *Wang et al. (2009)* detected formation of basal laminar deposits on Bruch's membrane early in the course of Age-related Macular Degeneration (AMD), which is a major cause of loss of the central vision in elderly. They added that the aged RPE may contribute to the formation of basal laminar deposits via release of intracellular proteins. Molecular and cellular changes in the old RPE may also underlie susceptibility to genetic mutations, which may be associated with the pathogenesis of AMD in the elderly. On the other hand,

Jarrett et al. (2008) found an increasing evidence to support an association between mitochondrial dysfunction and a number of retinal pathologies including age-Related Macular Degeneration (AMD), diabetic retinopathy and glaucoma. They added that the susceptibility of mitochondrial DNA to oxidative damage together with deficits in mitochondrial DNA repair pathways are important contributors in the pathogenesis of retinal degeneration.

The present work revealed formation of new abnormal blood vessels with blood leakage in the retinal choriocapillaries of old rats. This finding was previously observed by *Salminen et al. (2010)* who explained that exudative age-related macular degeneration involves choroidal neovascularization in the form of new abnormal blood vessels in the choriocapillaries through Bruch's membrane. These vessels have a greater tendency of leakage and bleeding into the macula, ultimately leading to irreversible damage to the photoreceptors if left untreated.

Concerning the adult ocular lens, a unique type of cell death plays a role in maintaining its supramolecular order and metabolism, which is called terminal differentiation. Terminal differentiation is considered a specialized type of apoptosis. Terminally differentiating adult lens fibers undergo denucleation but remain viable as anucleated fiber cells. Terminal differentiation of lens fibers resembles the apoptotic process in that organelles are lost, DNA is fragmented and changes in membrane morphology occur. However, unlike classically apoptotic cells, aging lens fibers are compressed into the center of the lens, where they undergo cell-cell fusion with formation of specialized membrane interdigitations *(Lee et al. 2001; West-Mays et al., 2010)*.

The current work revealed appearance of some apoptotic cells with+ve caspase-3 reaction in the corneal epithelium, ganglion cell layer and pigmented epithelial cells of the retina. These findings coincide with *Dunaief et al. (2002)* who proved that cell loss in AMD could occur by apoptosis of the retinal pigment epithelial cells followed by apoptosis of photoreceptors. In a previous study three tissues were examined for cell death, involving young (Five months) and old (Two years) guinea pigs. It was found that some of the factors contributing to aging process might be responsible for the enhanced amount of damaged DNA in older tissues undergoing cell death *(Gagna et al.. 2001)*.

Furthermore, *Kim et al. (2010)* reported increased caspase-3 activity in human RPE cells exposed to oxidative stress. They added that increased caspase-3 activity could be significantly inhibited by clusterin as determined by absence of apoptotic bodies.

On the other hand, the caspase-3 stained eye sections of old-aged rats in this work revealed+ve reaction in the lens subcapsular cells and some cortical fibers cells with appearance of some apoptotic bodies. These findings are consistent with *Gagna et al. (2001)* who demonstrated that the amount of terminal differentiation in the lens and apoptosis in the cornea were increased in older tissues as compared to younger ones.

In conclusion, this study revealed that normal aging is associated with histological and immuno-histochemical changes in the rat eye involving the cornea, lens and retina, which appeared to be more vulnerable to aging. These changes may explain the mechanism of visual impairment observed in elderly. Understanding these changes may be helpful in finding solutions to improve visual quality in old age.

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التغيرات العمرية المصاحبة لتقدم السن في القرنية وعدسة العين والشبكية لعين الجرذ الأبيض: دراسة هستولوجية وهستوكيميائية مناعية

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ملخص البحث

إن التغيرات المرتبطة بتقدم السن تحدث في جميع أنحاء الجسم بما في ذلك الرؤية. فالشيخوخة لها تأثيرات مختلفة على الجهاز البصري والتي تؤثر على كل من الأجزاء الخارجية والداخلية للعين. وقد ينتج التدهور البصري المرتبط بتقدم العمر عن عوامل عصبية أو بصرية .لذلك فإن الهدف من البحث هو دراسة التغيرات الهستولوجية الطبيعية لتقدم العمر على كل من القرنية وعدسة العين والشبكية في عين الجرذ الأبيض.

وقد تم استخدام أربعة وعشرون جرذ في هذا البحث والتي تم تقسيمها إلى مجموعتين: مجموعة (١) صغيرة السن والتي تبلغ من العمر (٣-٦ أشهر). ومجموعة (٢) كبيرة السن وتبلغ من العمر (٢٢-٦٢ شهر). وقد تم التضحية بكل الجرذان واستخراج مقلة العين لكل منها وإعدادها للفحص بالميكروسكوب الضوئي. وقد تم إضافة الصبغة الهستوكيميائيه المناعيه لاكتشاف الفناء المبرمج للخلايا.

وقد أوضحت النتائج أن تقدم العمر قد يؤدى إلى تغيرات هستولوجيه متعددة في عين الجرذ الأبيض حيث أظهرت القرنية ضمورا في الخلايا الطلائية واتساع المسافات بين رقائق القرنية مع فقدان للخلايا القرنية. كما بدت الخلايا الطلائية تحت المحفظة الكبسولية لعدسة العين قليلة وغير مرتبة بينما فقدت الألياف القشرية للعدسة أنويتها. أما الشبكية فقد أظهرت نقصا في سمكها مع تلف لبعض الخلايا العقدية والخلايا الصبغية الطلائية بها. وأوضحت الطبقة النووية الخارجية نقصا بالغا في السمك مع وجود أنويه صغيرة وداكنة اللون. أما خلاياها العصوية والمخروطية فقد أظهرت نقصا في الكثافة. وقد ظهر الغشاء الفرشى (براش) غير محدد المعالم ومتعرج مع وجود بعض الترسبات الملتصقة به و تسرب دموي خارجه. وقد أوضحت الصبغة الهستوكيميائيه المناعيه ظهور بعض خلايا الفناء المبرمج في القرنية والخلايا الطلائية تحت المحفظة الكبسولية لعدسة العين وكذلك بعض الخلايا العقدية والخلايا الصبغية الطلائية للشبكية في الجرذان كبيرة السن.

ويتضح من هذه الدراسة أن تقدم العمر الطبيعي قد يسبب بعض التغيرات الهستولوجيه والهستوكيميائيه المناعيه التي قد تفسر ضعف الإبصار الملاحظ في كبار السن.