Effect of Aging on Cornea of Male Albino Rat and the Possible Protective Role of Vitamin C

Original Article

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ABSTRACT

Background and Objectives: Cornea is a tissue in human eye that improves quality of image formed in retina. It is located in the anterior portion of eyeball. Aging is a biological phenomenon that involves increase of oxidative stress associated with gradual degradation of structure and function of cornea. Ascorbic acid (vitamin C) has been shown to have protective effects in repair of many corneal diseases. The aim of this study was to evaluate the histological and ultra-structural changes induced by aging on cornea of albino rat and the possible protective role of vitamin C.

Materials and Methods: Fifty male albino rats were divided into 3 groups. Group I included 10 rats of 6 months old who received no medications. Group II included 20 rats of 18 months old and divided into two subgroups; IIA: rats received no medications and IIB rats received vitamin C. It was administered by gastric tube once daily (200 mg/kg body weight) for 36 days. Group III included 20 rats above 24 months old and divided into two subgroups; IIIA: rats received no medications, and IIIB rats received vitamin C of the same dose ,duration and route of administration as subgroup IIB. Histological, immune-histochemical, transmission electron microscopic and morphometric studies were performed.

Results: Aging was associated with significant separation of collagen bundles, disorganized corneal stroma and cytoplasmic vacuolation with small dark nuclei of corneal epithelial cells. Vitamin C showed significant protection of these signs of aging.

Conclusion: Aging affects the cornea of albino rats, and vitamin C has a protective effect on aging of cornea.

Received: 14 June 2020, **Accepted:** 28 September 2020

Key Words: Aging, cornea, vitamin C.

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ISSN: 1110-0559, Vol. 45, No.2

INTRODUCTION

The human eye is one of the more complex parts of the body[1]. In the human eye, the cornea is a tissue that aims to improve the quality of the image formed in the retina. It is a curved, transparent, intensely innervated and delicate film found within the front parcel of the eyeball[2,3].

The cornea is avascular and shows five layers: epithelium, Bowman's layer, substantia propria (stroma), Descemet's layer and endothelium[4].

The cornea functions as clear and transparent tissue in the human body. Its thickness is nearly 0.52 mm in the center and 0.65 mm in the periphery and it is about 12 mm in horizontal diameter. It lacks blood vessels to nourish it, but its nourishments are carried out from the tear and aqueous humor[5].

Dry eye syndrome is a disorder of cornea, the ocular surface and tears film that is more common in older persons. In dry eye patients, minimal degree of visual acuity loss can occur leading to considerable reduction in visual function and quality of life[6].

Aging is a universal phenomenon characterized by the accumulation of biological changes, which contribute to the functional degradation of the organism over time. Aging is a physiological and multifactorial process characterized by the progressive loss of anatomical and functional integrity, which leads to an increased risk of different pathologies. Age-related disability and morbidity adversely affect the quality of life; ultimately, they are associated with an increased risk of death and have dire consequences for individuals, families and society[7].

Although research on the biology of aging has attracted considerable attention recently, understanding of its underlying mechanisms is still poor. It was hypothesized that failure of mechanisms of repair leads to accumulation of cellular and molecular damage which drives aging. This was thought to happen randomly, which explains the large diversity of aging phenotypes[8].

The interplay among genetic background, environmental factors and the accumulation of irreparable damage to DNA may determine the likelihood of developing specific age-related diseases. Genomic instability, telomere attrition, proteases loss, deregulated nutrient sensing, mitochondrial

 DOI: 10.21608/ejh.2020.32288.1311

dysfunction, exhaustion of stem cells and altered cell communication have all been recognized as characteristic of aging[9].

Aging involves an increase in oxidative stress associated with a gradual deterioration of the corneal structure and function[10]. Alterations in cornea related to age and ocular surface tissues have a major effect on vision. More severe age-related illness can cause loss of transparency and then blindness[11].

All plants and most animals can synthesize vitamin C (Ascorbic acid, AA). But it is not synthesized by human. Its deficiency results in the fatal scurvy disease. It occurs in citrus fruits, green and red peppers, strawberries, tomatoes, broccoli and other leafy vegetables. Animal sources are poor in AA[12].

Vitamin Chas been reported to have curative effects in the recovery of many corneal diseases in animals and humans, such as in UV irradiation, chemical corneal burns, corneal neovascularization and inflammation[13]. However, few reviewed study literatures documented its protective role on age related corneal affections. The present study aimed at evaluating the age related histological changes on the cornea of male albino rats and the possible protective role of vitamin C.

MATERIALS AND METHODS

The present study was carried on fifty male albino rats. Their weights ranged from 100 to 250 gm and their ages were 6, 18 and above 24 months old. They were obtained from the animal house, Faculty of science, Beni-Suef University.

Study groups

The studied animals were divided into three groups as follows:

Group I (control group) (n= 10 rats, 6 months old); rats received no medications.

Group II (n = 20 rats, 18 months old); rats divided into two subgroups:(10 rats each).

- Subgroup II A: rats received no medications.
- Subgroup II B: rats received vitamin C. It was administered orally by gastric tube once daily (200 mg/kg body weight)[14] for 36 days[15]. Vitamin C was supplied in the form of Vitacid C effervescent (product of chemical industries development-Giza, Egypt).

Group III (n = 20 rats, above 24 months old); rats divided into two subgroups: (10 rats each).

- Subgroup III A: rats received no medications.
- Subgroup III B: rats received vitamin C of the same dose, duration and route of administration as those of subgroup IIB.

Housing conditions

Animals were inbred in the experimental animal unit, Faculty of science, Beni-Suef University and maintained according to the standard guidelines of Institutional Animal Care and Use Committee, Beni-Suef University and after Institutional Review Board approval.

During accommodation and growth, animals were maintained in an air-conditioned animal house with specific pathogen free conditions, at constant 12-hours of light and dark cycle. They were fed a semi-purified diet that contained: (200gm casein, 500gm sucrose, 50gm cellulose, 50gm fat blends, 10gm vitamin mix, and 35gm mineral mix) per kg[16]. A free access to water ad libitum.

Methods

All rats were anesthetized by light chloroform anesthesia, given by inhalation[17]. Then, rats were sacrificed by decapitation. The two eyes were enucleated. The corneas were extracted by circumferential excision 2mm posterior to the corneoscleral junction; the right cornea was used for light microscopic examination and the left cornea was used for electron microscopic examination.

Corneal Examination

Light microscopic examination: Samples were prepared as follows: the corneas were fixed in 10% phosphate buffered formalin (pH 7.4), processed for paraffin embedding and serial 5µm thick sections were cut. The sections were stained by: Hematoxylin and Eosin stain[18]. Immuno-histochemical study using caspase 3 for detection of apoptosis[19] and then examined by light microscopy.

Immunohistochemistry for caspase-3

Sections were dewaxed, rehydrated and autoclaved at 120°C for 10 minutes in 10 Mm citrate buffer. After washing with PBS, endogenous peroxidase was blocked using 0.3% H_2O_2 in methanol for 15 min. Slides were washed in PBS again and blocking was performed by addion of blocking buffer, then incubated for 30 min. at room temperature. Caspase-3 antibody is a rabbit polyclonal antibody was used for staining (3015-100; Biovision, Milpitas, California, USA).The primary antibody first was diluted at (1:20), then applied on the sections for 60 min. Thereafter, the sections were incubated together with a secondary antibody;Biotinylated goat anti-polyvalent "Ultravision detection system" (TP-015-HD, ready to use; Thermo Scientific, USA,). The site of the reaction was seen with diaminobenzidine tetrahydrochloride. Therafter, sections were counterstained with hematoxylin. For negative control, the primary antibody was omitted. Rat brain sections were used as positive control. Sections stained by caspase3: positive (apoptotic) cells showed brown cytoplasmic deposits with some nuclear staining that was classified into; mild, moderate and strong immune reaction[19].

Electron Microscopic examination (Transmission electron microscopy)

Samples were prepared as follows: The corneal tissue was prefixed in glutaraldehyde 1% in PBS at 4C, post-fixed in buffered osmium tetroxide 2% for 2 hours and dehydrated rapidly in a graded series of ethanol. Samples were embedded in epoxy resin (Epon 812, Merck), and sectioned with a Reichert-Jung ultratome (OmU2, Reichert, Austria)[20]. Toluidine blue semi-thin sections (1 to 2 µm thick) were examined under a light microscope. This was used to select the areas for ultrathin sections. Ultrathin sections (around 100 nm thick) were cut and double-stained with uranyl acetate and lead citrate and then evaluated with an EM-10 C transmission electron microscope (according to the protocol of E.M. unit Assiut Unit) (Carl Zeiss Meditec) (Germany)[21,22].

Morphometric study

Morphometric measurements were performed at Faculty of veterinary Medicine, Beni-suef University, using Leica Qwin 500 image analyzer computer system (Leica Imaging Systems, Cambridge, England). The image analyzer consisted of a colored video camera; Panasonic wv. GP 210, colored monitor, hard disk of Leica IBM personal computer connected to a BX41 Olympus microscope (Tokyo, Japan) and controlled by LeciaQwin 500 software.

The measurements included:

1. The epithelial height was measured in H&E stained of corneal sections of control and experimental groups, on each slide, ten non-overlapping fields were assessed to measure the corneal epithelial thickness (um). Corneal thickness of epithelium was studied at magnification of X 200 .
2. The area percent (%) of caspase 3 immuno reactivity in corneal section of control and experimental groups, on each slide, tennon-overlapping fields were assessed to measure the area percent of caspase 3 immuno-reactivity. The area percent of caspase 3 immuno-reactivity was studied at magnification of X 400.

Statistical analysis

Comparison between the different groups in morphometric results was calculated by analysis of variance (ANOVA) followed by post hoc tukey test using the statistical package SPSS (Statistical Package for the Social Sciences) version 24[23,24]. The results were expressed as mean ± SD. The difference was considered statistically significant when *P value* was <0.05 and highly significant when $p<0.001$[23,24].

RESUL

No deaths were observed in all rats.

A- Histological results

Light Microscopy with hematoxylin and eosin stain

The control group (6M old) showed the normal histological architecture of cornea that denoted stratified squamous non-keratinized corneal epithelium with smooth regular upper surface formed of flattened squamous cells resting on Bowman's membrane. The stroma was formed of parallel arranged collagen bundles with numerous spindle-shaped keratocytes in between. The innermost endothelial cells were observed (Figure 1A). In old age (18, above 24 M old groups) showed irregular corneal surface, exhibiting degenerated superficial corneal epithelial cells, separation of corneal stroma from bowman's membrane and from each other with disorganized corneal stroma. Less numerous keratocytes in corneal stroma was observed. The innermost endothelial layer was observed (Figures 1 B1, 2,D). In treated group (18 M old) showed similar details as that of 6 M old (Figure C), but in treated group (above 24 M old) showed mild separation of corneal stroma from bowman's membrane and from each other with mild disorganized corneal stroma (Figure 1E).

Immunohistochemical staining with caspase 3

The control group (6M old) showed negative caspase 3 reaction of nuclear epithelial cells (Figure 2A). In old age 18 M old non treated group showed moderate positive caspase-3 reactionof nuclear epithelial cellsof basal epithelial cells manifesting apoptosis (Figure 2B) compared to 18 M old treated group that showed mild positive caspase-3 reaction ofnuclear epithelial cells (Figures 2C1, 2). In above 24 M old non treated group, it revealed strong positive caspase-3 reaction of nuclear epithelial cells (Figure 2D) when compared to above 24 M old treated group that showed moderate positive caspase-3 reaction of nuclear epithelial cells (Figure 2E).

Ultrastructural study of the cornea (TEM)

The control group (6M old) showed the normal architecture of cornea that revealed stratified squamous epithelium of 3-5 cell layers. The basal cells were cuboidal, having large euchromatic nucleus and less electron dense cytoplasm contain variable sized vacuoles. The other upper layers appeared flattened with euchromatic elongated nucleus and increased electron density of the cytoplasm. The epithelial layer is situated on a thin Bowman's membrane (Figure 3A).In 18 and above 24 M old non-treated groups, they showed the presence of numerous variable sized vacuoles in the intercellular space and numerous fine granular globules in the subepithelial area, it may be apoptotic bodies (Figures 3 B1,2,D).While in 18 and above 24 M old treated groups, they showed gradual improvement in the histological changes observed in the non-treated groups (Figure 3 C,E).

In 18 and above 24 M old treated and non-treated groups showed the presence of interconnecting desmosomes and numerous vacuoles in between the cells (Figures 3 B1,B2,C,D&E).

The control group (6M old) revealed collagenous bundles arranged in regular parallel lamellae and long slender fibroblasts present in corneal stroma among the collagen bundles (Figure 4A). In 18 and above 24 M old non-treated groups, the stroma appeared disorganized. The corneal stroma showed variable amount of less electron dense homogenous edematous material in between the collagen bundles with small electron dense fragments in between (Figure 4 B,D). In 18 and above 24 M old treated groups the histological changes were less than that occurred in non-treated rats (Figures B1,2,D).

The control group (6M old) showeda thick moderately electron dense non cellular Descemet's membrane of about 3.75 µ with cross electron-dense striations, interposed between the collagenous connective tissue, the endothelial cells contained many vacuoles and small electron dense lysosome (Figure 5A). In old age, increasing thickness of Descemet's membrane was observed. It was about 5 µ thick in 18 M old non-treated group (Figure 5B). In 18 M old treated group was about 4.2 µ thick (Figure 5C). It was about 4.5 µ thick in above 24 M old non-treated group (Figure 5D)compared to above 24 M old treated group that was about 3.45 µ thick (Figure 5E).In 18 M old and above 24 M old treated groups (Figures 5 C,E)showed the posterior endothelial cell of simple squamous type having elongated nucleus and the Descemet's membrane appeared as thick moderately electron dense membrane.

Morphometric results

Highly significantly decrease in mean value of epithelial height was recorded in group IIIA compared to control group (I), Non-significant decrease in mean value of epithelial height was recorded in group IIA compared to control group (I) and marked improvement of epithelial height in treated groups (IIB,IIIB) compared to non- treated groups (IIA,IIIA) (Figure 6, Table 1).

Highly significantly increase in mean value of the area percent of caspase3 immuno-reactivity was recorded in group IIA, IIIA compared to control group (I) and decrease in the area percent of caspase3 immuno-reactivity in treated groups (IIB, IIIB) compared to non-treated groups (IIA, IIIA) (Figure7, Table 2).

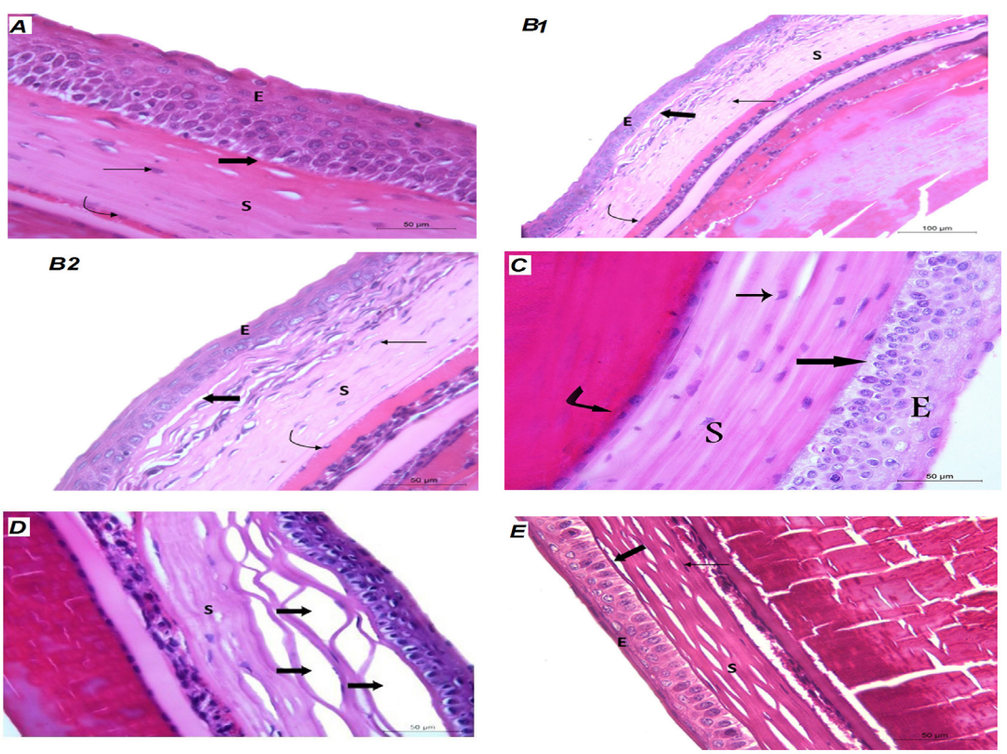

Fig. 1: Photomicrographs of histological sections show: 6 month old ,control group (A) with the stratified squamous non-keratinized corneal Epithelium with smooth regular upper surface formed of flattened squamous cells (E) resting on Bowman's membrane (thick arrow). The Stroma (S) formed of parallel arranged collagen bundles with numerous spindle-shaped keratocytes in between (thin arrow). The innermost endothelial cells (curved arrow) were also observed. (H& Ex 400) 18 M old non treated groups (B1, B2) show irregular surface of corneal epithelium (E), separation of corneal stroma from bowman's membrane (thick arrow) , and less numerous keratocytes (thin arrow) in corneal stroma (S) are also observed and the innermost endothelial cells (curved arrow) are seen . (B1: H& E x 100, B2: H& Ex 400) 18 M old treated group(C) showing similar details as that of 6 M old rats. (H& E x 400)
Above 24 M old non treated(D) group showing separation of collagen bundles (thick arrows) and disorganized corneal stroma (S). (H& E x 400) Above 24 M old treated group(E) showing mild separation of corneal stroma from bowman's membrane and from each other with mild disorganized corneal stroma (H& E x 400).

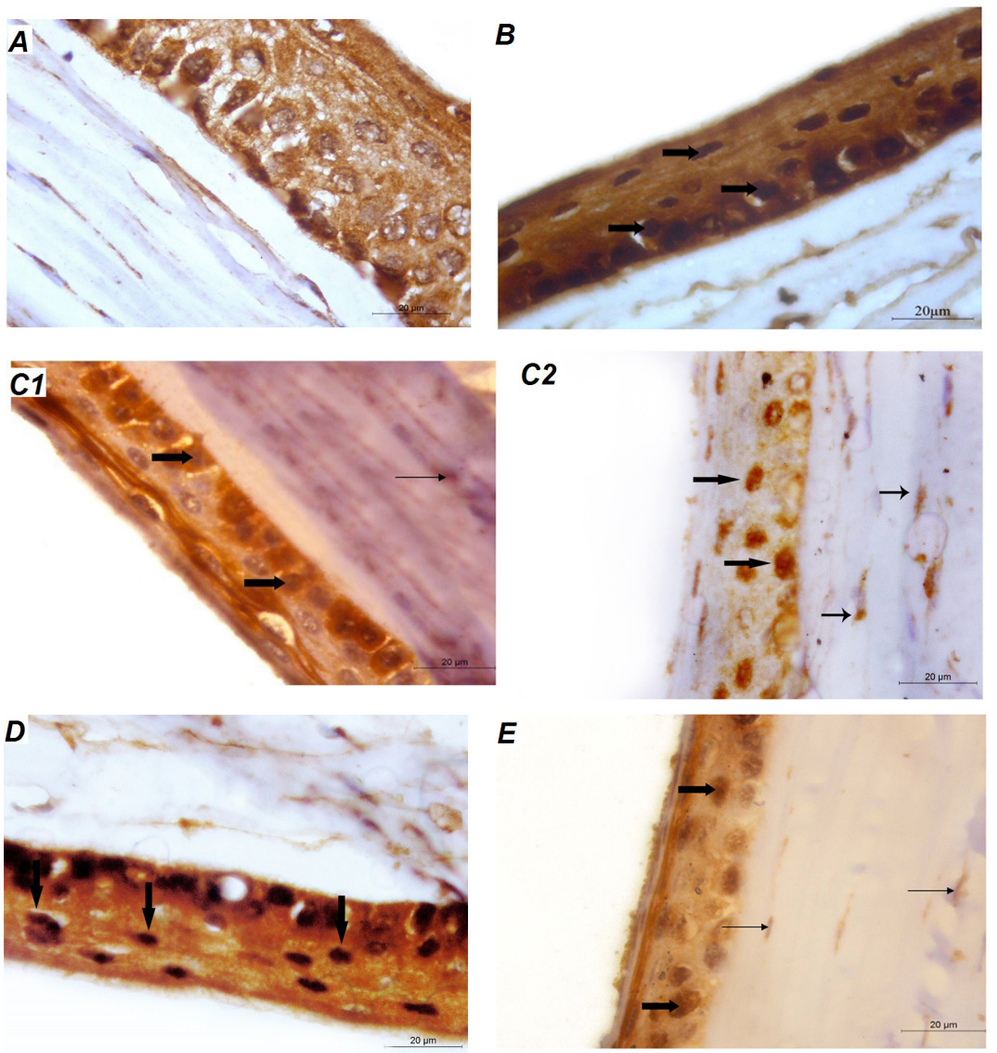

Fig. 2: Photomicrographs of histological sections show: 6 month old ,control group (A) with negative caspase-3 reaction of nuclear epithelial cells. In 18 M old non treated rats(B) show moderatly positive caspase-3 reaction of nuclear epithelial cells (arrow) . In 18 M old treated rats(C1-C2) show mild positive caspase-3 reaction of nuclear epithelial cells(thick arrow)and keratocytes in stroma (thin arrow) . In above 24 M old non treated rats (D)show strong positive caspase-3 reaction of nuclear epithelial cells (thick arrow). In above 24 M old treated rats(E) show moderatly positive caspase-3 reaction of nuclear epithelial cells (thick arrow) and keratocytes in stroma (thin arrow).(Caspase-3 immunostaining; x 1000).

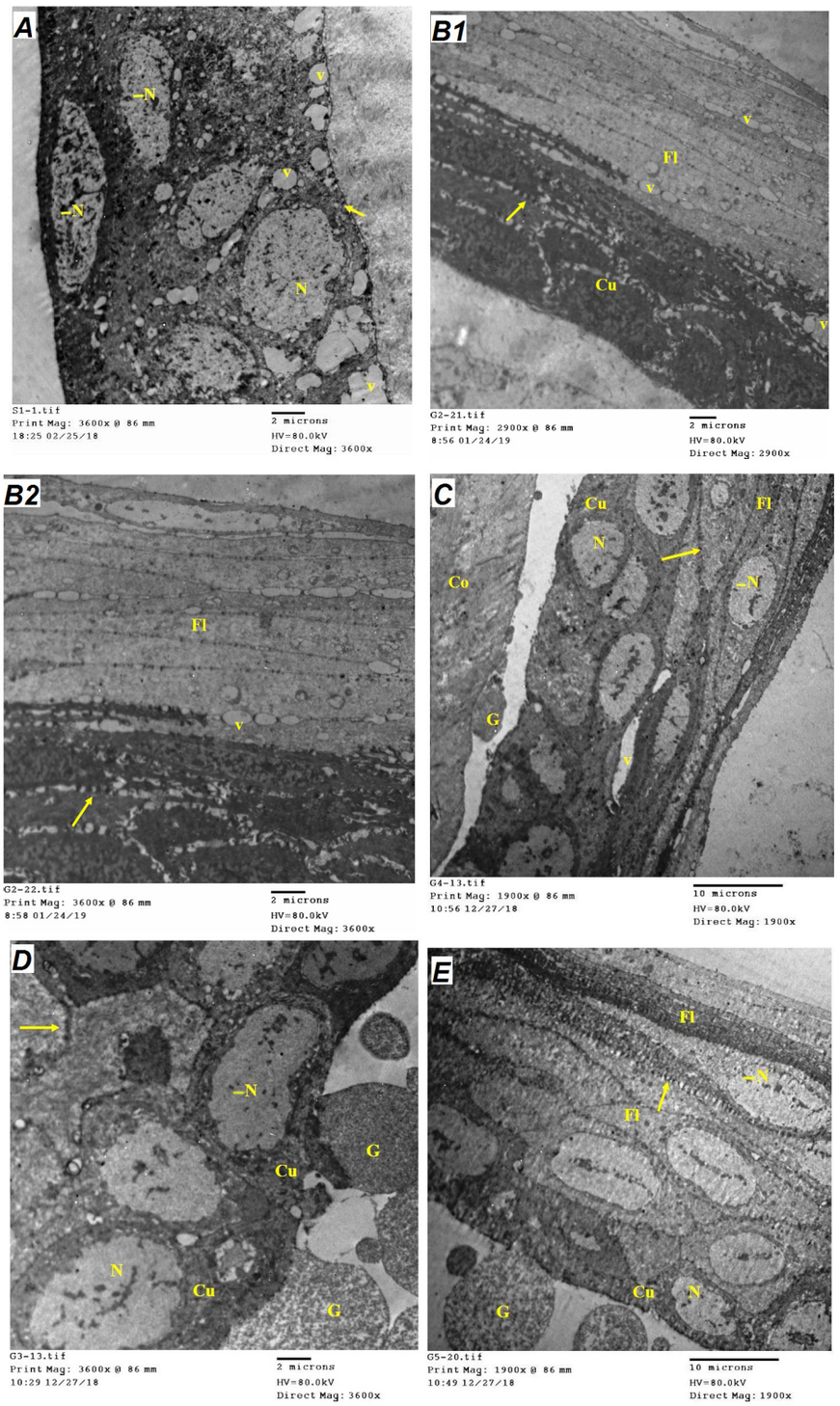

Fig. 3: TEM of epithelium of the cornea of 6 M old rats (A x 3600) show ŝtratified squamous epithelium of 3-5 cell layers. The basal cuboidal cell, has large euchromatic nucleus (N) and electron lucent cytoplasm contain variable sized vacules (v). The other upper layers appeared flattened with euchromatic elongated nucleus (-N) with increased electron density of the cytoplasm. The epithelial layers are situated on a thin Bowman's membrane (arrow). In 18 (B1x2900),(B2x3600) and above 24 (Dx3600) old non-treated rats show the presence of numerous variable sized vacuoles (v) in the intercellular space and numerous fine granular globules in the subepithelial area it may be apoptotic bodies (G), the superficial layers gradually flattening (Fl). In 18(Cx1900) and above 24 M old treated(Ex1900) old treated rats show the basal layer of cuboidal type (Cu), the second polyhedral and the other layers become gradually flattened (Fl). Fine granular globules (G) are observed in the separated area of the epithelial layers. Notice corneal collagenous ŝtroma (Co). Notice that B1, B2, C, D, &E sections show the interconnecting desmosomes (arrow) and numerous vacuoles (v) are present in between the cells.

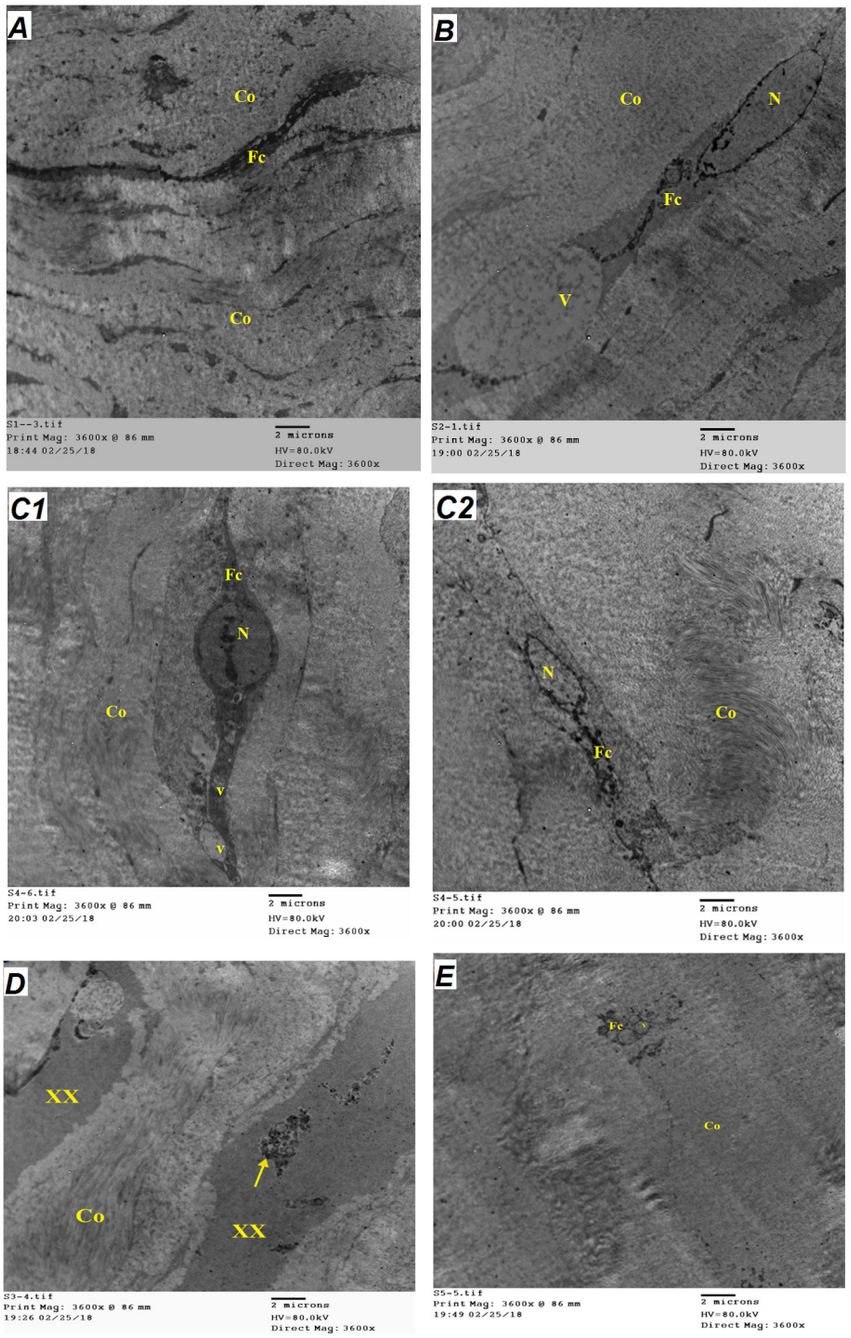

Fig. 4: TEM of stroma of cornea of 6 month old rats (group A) show collagenous bundles arranged in regular parallel lamellae (Co) and long slender keratocytes (Fc) present among the collagen bundles. In 18(B) and above 24 (D)M old non-treated rats, the stroma appear disorganized. The corneal stroma shows variable amount of less electron dense homogenous edematous material (XX) in between the collagen bundles (Co) with small electron dense fragments (arrow) in between. In 18(C1,C2) showing fibroblast cell (Fc) in between the bundles having euchromatic nucleus (N) and numerous vacuoles (v) in its cytoplasm. In 18(C1,C2) and above 24(E) M old treated rats the histological changes were less than that occurred in non-treated rats, collagenous bundles arranged in regular parallel lamellae (Co) and long slender fibroblasts (Fc) present among the collagen bundles. (X 3600).

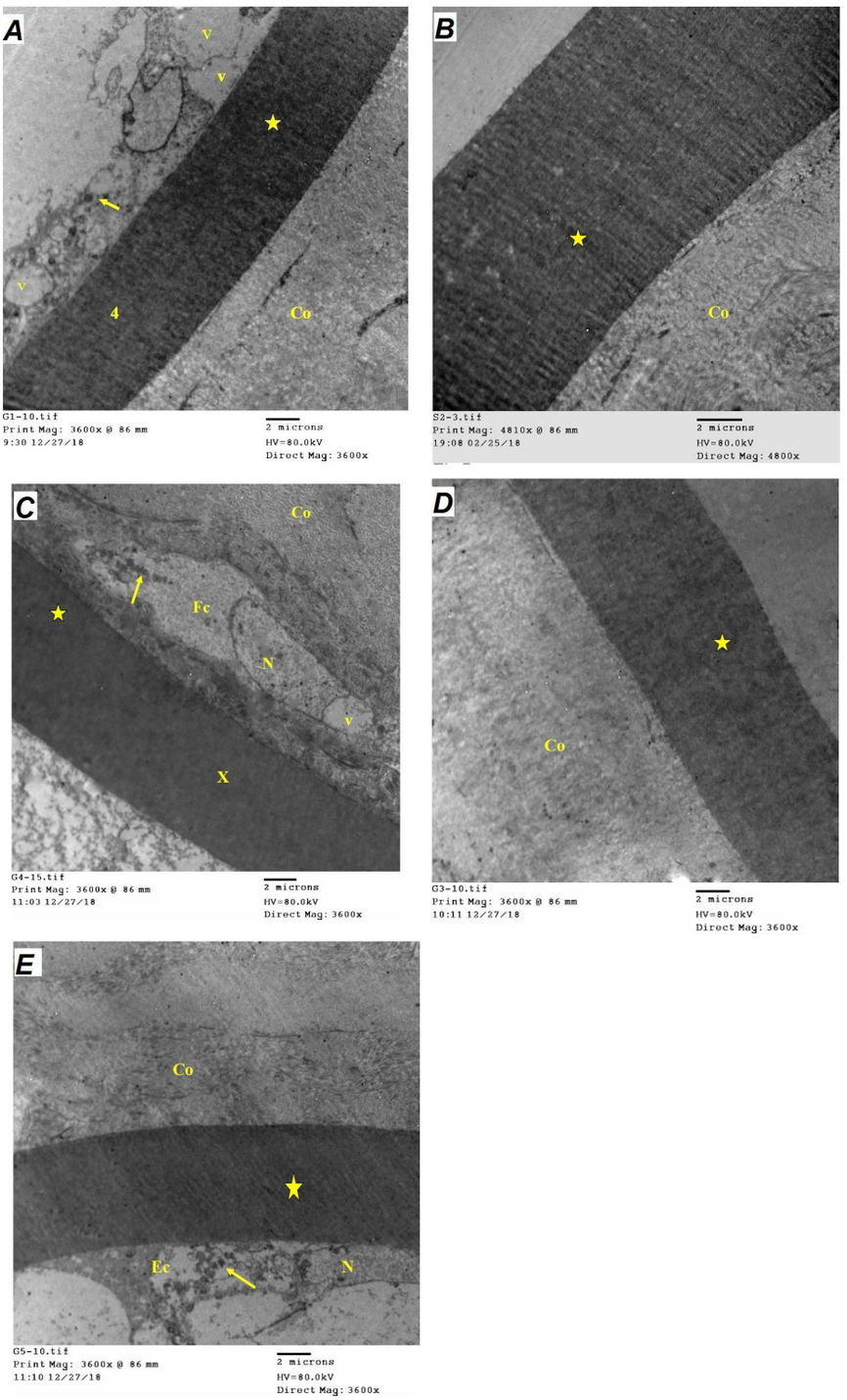

Fig. 5: TEM of Descemet's membrane of the cornea of 6 M old rats (A x 3600)showing a thick non cellular moderately electron dense membrane of about 3.75 µ with cross electron-dense striations (star), interposed between the collagenous connective tissue (Co), the endothelial cells contain many vacuoles (v) and small electron dense lysosome (arrow). 18 M (B x 4800) old non-treated rats, showing the thickness of Descemet's membrane that is about 5 µ thick. 18 M (C x 3600) old treated rats is about 4.2 µ thick, fibroblast cell (Fc) in the stroma is seen. It is about 4.5 µ thick in above 24 M (D x 3600) old non-treated rats compare to above 24 M (Ex 3600) old treated rats that is about 3.45 µ thick. 18 M (C x 3600) and above 24 M (E x 3600) old treated rats showing the posterior endothelial cell (EC) of simple squamous type having elongated nucleus (N) and the Descemet's membrane appear as thick moderately electron dense (X) membrane.

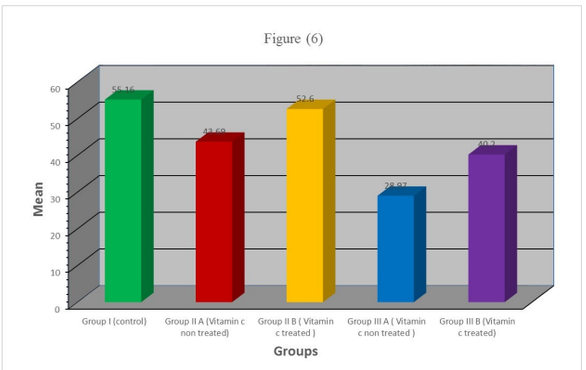

Fig. 6: Histogram showing comparison between all groups according to the mean value of epithelial height in corneal sections.

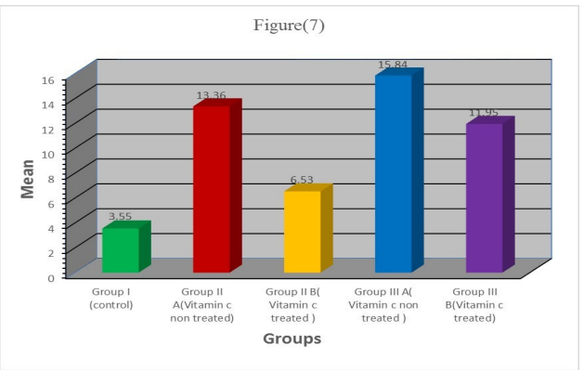

Fig. 7: Histogram showing comparison between all groups according to the mean value of area percent of caspase3 immuno-reactivity in corneal sections.

Table 1: Mean value of epithelial height by um in corneal sections ± standard deviation in all groups

Group	Control group (I)	Experimental non treated (IIA)	Experimental treated (IIB)	Experimental non-treated (IIIA)	Experimental treated(IIIB)
Mean height %±SD	55.16±15.4	43.69±17.2	52.62±8.64	28.97±4.12	40.20±2.51
P-value		0.106 (NS)	0.488 (NS)	0.001 (HS)	0.016 (S)

SD: Standard deviation
NS = Non-significant (*P-value* > 0.05) S = Significant (*P-value* < 0.05)
HS = Highly significant (*P-value* < 0.01) *P-value* = Probability of chance

Table 2: Mean value of area percent of caspase3 immuno-reactivity in corneal sections ± standard deviation in all groups

Group	Control group (I)	Experimental non treated (IIA)	Experimental treated (IIB)	Experimental non-treated (IIIA)	Experimental treated(IIIB)
Mean height %±SD	3.55±0.757	13.36±1.14	6.53±0.744	15.84±2.28	11.95±0.875
P-value		0.001 (HS)	0.104 (NS)	0.001 (HS)	0.001 (HS)

SD: Standard deviation
NS = Non-significant (*P-value* > 0.05) S = Significant (*P-value* < 0.05)
HS = Highly significant (*P-value* < 0.01) *P-value* = Probability of chance

DISCUSSION

The cornea forms the anterior surface of the eye in an area largely corresponding to the pigmented iris, which is visible behind the cornea[1].

Aging is abiological phenomenon that involves internal physiological deterioration of the multicellular organisms[10]. In humans, aging is often evaluated in relation to time, which makes it difficult to differentiate between time-dependent biological changes and environmental damage[3]. Changes in aging occur from the eyelids to the macula causing different effects, the incidence of age-related maculopathy, vitreous liquefaction and cataract increases with age[10].

In the current study, aging of the cornea was studied histologically by examining corneas of albino rats of different age groups: 6 months, 18 months and above 24 months. Moreover, the possible protective effect of vitamin c on aging of cornea was examined in two different ages 18 months and above 24 months.

The results of the current study showed that on light microscopic examination of corneas of 6 months of aged rats (control group), the corneal cell layers were normally oriented with characteristic non-keratinized stratified epithelium and their basal layer appeared columnar. The corneal epithelium appeared resting on a uniform basement membrane that was the Bowman's layer. The corneal stroma consisted of regularly oriented collagen fibrils infiltrated by flattened keratocytes. The regularity of the collagen fibers account for the transparency of the cornea. Descemet's membrane with underlying endothelial layer are located beneath the stroma. The results of current study were in concurrence with El-Sayyad *et al*., (2015)[10] who observed that the normal pattern of corneal epithelium is appeared in the form of stratified squamous non keratinized layer having several cell layers thick, the stroma formed of regularly organized collagen fibrils infiltrated by keratocytes[10].

The current work found that aging 18 months aged rats, were associated with irregular corneal surface with degenerated corneal epithelial cells. Moreover, separation of corneal stroma from Bowman's membrane and less numerous keratocytes in the corneal stroma were observed. The above 24 months aged rats, were associated with

significant separation of collagen bundles, disorganized corneal stroma and cytoplasmic vacuolation with small dark nuclei of corneal epithelial cells. These findings were not observed in 6 months aged rats where the cornea showed normal histological architecture with no signs of atrophy. These results were in accordance with those found by Halawa, (2011) who also observed decrease in the cell proliferative capacity of the corneal epithelium with aging and impairment of the corneal transparency[25].

The results of present study demonstrated that the corneal epithelium of old-aged rats was desquamated at 18 months and appeared flattened in 24 months-old. Moreover, 24 months-old group demonstrated considerable reduction of the corneal epithelium. Many of the epithelial cells appeared vacuolated, the corneal stroma displaying the collagen bundles arranged in lamellae. Variable amount of less electron dense edematous material is present in between the bundles. These results are confirmed by other researchers who also observed in some areas the keratocytes were reduced and absent[10,25].

The findings of the current study showed the highest epithelial height of corneal rats aged 6 months. The epithelial height of treated and untreated 18-month-old corneal rats were statistically insignificant compared with the 6-month-old corneal rats. On the other hand, the above 24-month-old cornea of untreated rats was highly significant compared with the 6-month-old corneal rats and the above 24-month-old cornea of treated rats were significant compared with the 6-month-old corneal rats regarding their corneal epithelial height. These results were consistent with Yang *et al*., (2014) who showed a significant reduction in the corneal epithelial density associated with aging[26].

In the present study, examination of caspase-3 stained sections of cornea of above 24 months aged corneal rats showed strong positive caspase-3 reaction, whereas 18 months aged corneal rats showed moderate positive caspase-3 reaction, but on the other hand examined caspase-3 stained sections of 6 months aged corneal rats showed negative caspase-3 reaction.

The results of current study showed that the 6 months aged corneal rats had the lowest area percent of caspase3 immuno-reactivity. The 18 months aged cornea of non-treated rats were statistically high significant when compared to the 6 months aged corneal rats as regards area percent of caspase 3 immunoreactivity, while 18 months aged cornea of treated rats which were non- significant when compared to the 6 months aged corneal rats .On the other hand, the area percent of caspase3 immuno-reactivity of above 24 months aged corneal rats treated and non-treated were statistically high significant when compared to the 6 months aged corneal rats. These findings were in apart in accordance with El-Sayyad *et al*., (2015) study who revealed an intense expression of caspase 3 was detected within the basal epithelium reflecting higher apoptotic rates in above 24 months old rats[10]. Our findings were supported by the work of Joyce *et al*. (2011) who mentioned that age-related increase in oxidative nuclear DNA damage by forming DNA damage repair foci with DNA damage-signaling genes. which explained the positive nuclear reaction of caspase3 immuno-reactivity[27].

On ultra-structural examination, the results of current study showed that the cornea of 6 months aged rats were formed of stratified epithelial layer and the basal cells of cuboidal type having large euchromatic nucleus. The upper layers appeared flattened and have large electron lucent euchromatic nuclei and their cytoplasm were variable in electron density but mostly electron lucent. The remaining epithelial layer appeared flattened with flatten nucleus of the same electron density .The epithelial cells were interconnected with desmosomes, on the other hand 18 months aged cornea was characterized by presence of numerous variable size vacuoles and fine granular globules in the sub epithelial area, it may be apoptotic bodies .Such results agree in part with the results of Abdel Salam (2007), who found that the aged cornea showed weakness in the adhesion of surface epithelium of cornea to the underlying stroma, thickened and distorted basement membrane[28].

In the current study corneal stroma of above 24 months aged rats was formed of collagen bundles arranged in lamellae with long slender fibroblasts having oval euchromatic nuclei as well as variable sized vacuoles and fine granules seen in their cytoplasm. A variable amount of homogenous edematous material and small fragments are present in between the bundles and the corneal Descemet's membrane appeared thick. Similar findings were detected by other studies who showed that aged cornea revealed apparent degenerative changes in the endothelial cells, which appeared distorted, shrunken with increased in thickness of the Descemet's membrane[10,28].

Several reports have explained possible mechanisms that affect cornea ageing. Oxidative stress is involved in many ocular diseases and injuries. The imbalance between oxidants and antioxidants in favour of oxidants; oxidative stress may be highly involved in ocular aging processes[29]. Oxidative stress lead to cell damage, through several mechanisms; such as lipid peroxidation of membranes, oxidative changes in proteins, and oxidative damage to DNA[30]. In humans, oxidative stress is involved in many eye disorders, including cataract, uveitis, retinopathy of prematurity, age-related macular degeneration[31,32].

The results of the current study showed that treatment with vitamin c improved the thickness of cornel epithelium and decreased cell loss in corneal endothelium in all treated groups. This was further verified by the morphometric measurement of corneal epithelial height that was statistically insignificant in 18 months aged groups and significant in above 24 months aged groups. This was in accordance with Takenori, *et al* (2017)[33] who revealed that there was a decrease in corneal epithelial height with aging in mice. In addition, Padua *et al*. (2017), demonstrated that showed that ascorbic acid minimizes cellular losses in the corneal endothelium[34].

In the present study treatment of vitamin c for 36 days improved the arrangement of collagen bundles and also increased thickness of corneal stroma of rats. No comparable results in rats were found in the reviewed literatures. However, the results of current study were in accordance with those observed by Guo *et al*., (2007) in humans who illustrated that human keratocytes cultured with ascorbic acid for 5 weeks could automatically assemble organized ECM with parallel arrays of fibrils, which are morphologically similar to the corneal stroma[35].

Furthermore, the results of current study showed that significant protection of vitamin C on the signs of aging in18 months aged group. This was confirmed by the morphometric measurement of area percentage of caspase 3 immuno-reactivity that was statistically non- significant in 18 months aged groups and highly significant in above 24 months aged groups. These were in line with El-Sayyad *et al*., (2015) who showed intense caspase 3expression in the old aged rats as compared to the younger aged-rats[10].

Furthermore, Cho *et al*., 2014 showed that systemic (oral or intravenous) vitamin C supplementation had a more beneficial effect on corneas of the younger aged-patients as compared to the older aged-counterparts[13].

There is no accurate mechanism by which vitamin C affects the cornea. However, several reports have explained possible mechanisms; first, it has been shown that vitamin C accelerates the proliferation of corneal epithelial cells and cures epithelial defects[36].

Another reason is that vitamin C has impacts on the amalgamation of collagen. Vitamin C is a critical collagen generation modulator which acts as a cofactor in procollagen for the hydroxylation of proline and lysine residues. Vitamin C is also known to inhibit angiogenic factors, including vascular endothelial growth factor and

Matrix Metalloproteinases (MMPs). Then eventually, vitamin C applies antioxidant and defensive impacts on the inflammatory reaction of the eye by scavenging reactive oxygen radicals and metabolites, such as myeloperoxidase, that are discharged by penetrating inflammatory cells[13].

CONCLUSION

From the present study, it can be concluded that aging affects the cornea of albino rats, and that vitamin C has a potential protective effect on corneal aging.

RECOMMENDATION

Based on our findings in this study and in conjunction with that from previous studies, we suggested carrying out the following: additional studies on large number of cases in association with assessment of other parameters to evaluate the protective role of vitamin C on corneal aging. Further studies about cellular pathways that becomes activated or inhibited in the corneal cells of aged rats due to vitamin C administration and performing clinical trials to evaluate the effect of the protective role of vitamin C on corneal aging.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الشيخوخة على قرنية ذكر الجرذ الابيض والدور الوقائي المحتمل لفيتامين ج

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الخلفيه والاهداف : القرنية هي نسيج في العين البشرية تعمل على تحسين جودة الصورة التي تتكون في شبكية العين. وهي تقع في الجزء الأمامي من مقلة العين. الشيخوخة هي ظاهرة بيولوجية تنطوي على زيادة الإجهاد التأكسدي المرتبط بالتدهور التدريجي لهيكل القرنية ووظيفتها.تبين أن حمض الأسكوربيك (فيتامين ج) له خواص وقائية في إصلاح العديد من أمراض القرنية في الحيوانات والبشر.

كان الهدف من هذه الدراسة هو تقييم التغيرات النسيجية والتركيبية الفائقة الناجمة عن الشيخوخة على قرنية الجرذان البيضاء والدور الوقائي المحتمل لفيتامين ج.

الادوات والطرق: وقد شملت هذه الدراسه إستخدام خمسين من ذكورالجرذان البيضاء وتم تقسيمهم الى ٣ مجموعات على النحو التالي:

المجموعة الاولي (المجموعة الضابطة): ١٠ جرذان وعمرهم ٦ أشهر ولا يتلقوا أي أدوية.

المجموعة الثانية (مجموعة تجريبية) : ٢٠ جزذا وعمرهم ١٨ شهر وتم تقسيمها إلى مجموعتين فرعيتين: (أ) لا تتلقى أي أدوية و(ب) تتلقي جرعات من فيتامين ج عن طريق أنبوب المعدة

مرة واحدة يوميا (٢٠٠ ملجم / كجم من وزن الجسم) لمدة ٣٦ يوما.

المجموعة الثالثة (مجموعة تجريبية):٢٠ جزذا ،عمرهم فوق ٢٤ شهرا وتم تقسيمها إلى مجموعتين فرعيتين: (أ) لا تتلقي أي أدوية و(ب) تتلقي جرعات من فيتامين ج بنفس الجرعه ولنفس المده و طريقة الاستعمال مثل المجموعه الفرعيه الثانيه ب .

وفى نهاية التجربة تم التضحيه بالجرذان ثم استئصال القرنية واستخدمت العين اليمني للدراسة النسيجية المجهرية باستخدام المجهر الضوئي (لإعداد قطاعات شمعية وتجهيزها وتم صبغها بالهيماتوكسيلين والايوسين وصبغة الهستوكيميائية المناعيه باستخدام ال Caspase٣) واستخدمت العين اليسري للدراسة المجهرية الإلكترونية باستخدام المجهر الإلكتروني الناقل ثم تم عمل دراسة كمية قياسية.

ومن نتائج هذه الدراسة تبين ما يلي: ارتبطت الشيخوخة بفصل كبيرلحزم الكولاجين،وسدى القرنية غيرالمنتظم، والإجهادالسيتوبلازمي مع نوى داكنة صغيرة للخلايا الطلائيه للقرنية وأظهرفيتامين ج حماية كبيرة من علامات الشيخوخة.

الخلاصه: ومن النتائج السابقة يمكن استنتاج أن الشيخوخة تؤثر على قرنية الجرذان البيضاء وأن فيتامين ج يلعب دورا مؤثرا في حماية القرنية ضد الشيخوخة.