Original Article

Kiwifruit Extract Attenuates Tadalafil Induced Retinal Injury in Adult Male Albino Rats: A Histological and Immunohistochemical Study

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ABSTRACT

Background: Tadalafil is a one of the commonly prescribed drugs for treatment of erectile dysfunction. Several ocular side effects have been reported with tadalafil therapy. Kiwi fruit is a popular fruit that has a wide range of pharmacological activities and a proposed role in combating serious ocular complications.

Aim: To evaluate the potential protective role of kiwi fruit extract (KFE) against tadalafil induced retinal injury in adult male albino rats.

Material and Methods: Thirty adult male albino rats were divided into four groups; control group, KFE-treated group (500 mg/kg), Tadalafil-treated group (1.8 mg/kg), and both Tadalafil & KFE-treated group. Animals were orally administered once daily for six weeks. Specimens from the retina were processed for light and electron microscopy. Immunohistochemical study was performed using antibodies against B-cell lymphoma 2 (Bcl-2).

Results: Specimens from tadalafil-treated animals showed a statistically significant decrease in the total retinal thickness and the ganglion cell number. The retinal pigmented epithelium was detached. The inner segment of the photoreceptor was irregularly oriented with interrupted outer limiting membrane. Dark pyknotic nuclei were observed in the outer and inner nuclear layers and the ganglion cell layer. Ultrastructurally, disorganized membranous discs with increased inter-disc spaces of the outer segment, cytoplasmic vacuoles and swollen mitochondria of the inner segment of the photoreceptor layer and shrunken irregular cell bodies and nuclei of the outer and inner nuclear and ganglion cell layers were observed. The immunohistochemical study showed a significant decrease in Bcl-2 immunoreaction. In contrast, minimal changes were observed in rats treated concomitantly with both tadalafil and kiwi fruit extract, with a non-significant decrease in the immunoreaction.

Conclusion: Tadalafil induced structural changes in the retina of adult albino rat that could be ameliorated by concomitant treatment with kiwi fruit extract.

INTRODUCTION

Tadalafil (Cialis) is a one of the phosphodiesterase type 5 (PDE5) inhibitors that is approved by Food and Drug Administration (FDA) to be the first line therapy in the treatment of male sexual disorders especially erectile dysfunction[1,2]. It has been also used in treatment of other vascular disorders such as pulmonary hypertension and cardiomyopathy[3,4,5,6].

Tadalafil is a popular drug that is commonly used even without prescription. However, the uncontrolled use of this drug increases the chance of development of many side effects such as headache, dyspepsia and back pain[7], in addition to many ocular side effects such as conjunctival hyperemia, subconjunctival hemorrhage, ocular pain, mydriasis and photophobia in addition to retinal vascular effects and ischemic neuropathy of optic nerve[8]. Moreover, the chronic administration of this drug alters the color vision and light perception[9,10,11].

Recently, dietary supplementation of fruits becomes beneficial in health promotion as well as prevention and management of various diseases. Kiwi fruit (Actinidia deliciosa) is one of the most common fruits that is considered to be the king of fruits because of its evident abundance of vitamin C and other strong antioxidants such as flavonoids, carotenoids, phenolics, lutein and chlorophyll[12,13]. Moreover, it is an excellent source of vitamins A and E, fructose, galactose, some minerals as potassium, magnesium, calcium and iron[14]. Kiwi fruit is also reported to be useful in the treatment of acute burn wounds[15] and many cancers[16]. Recent in-vivo and in-vitro studies have reported the antioxidant activity of kiwi fruit[17,18] in addition to its immune stimulatory activity[19].

Based on the previous data, this study was performed to evaluate the possible protective role of kiwi fruit extract against tadalafil induced retinal injury in adult male albino rat employing different histological and immunohistochemical methods.

MATERIALS AND METHODS

Chemicals

1. **Tadalafil (Cialis):** It was purchased in the form of film-coated tablets containing 20 mg each (No. 4464, Lilly icos Co.)

2. **Kiwifruit extract (KFE):** The fresh kiwifruit was obtained from a local fruit market at Tanta City, Egypt. The preparation of the used extract was done at Department of Pharmacognosy, Tanta Faculty of Pharmacy. After peeling the kiwifruit, the pulp was used for extract preparation. Small pieces of the pulp were obtained and dry-blended. At the room temperature, the blended pulp was extracted using one liter methanol/water [50/50; v/v] for ten hours. The extract was filtered and centrifuged. Under reduced pressure, the supernatant was concentrated at 40 ºC by the rotatory evaporator. Finally, the methanol crude extract of the kiwifruit was available and stored at 18 ºC[20].

Study Design

The protocol of this experimental research was approved by the Research Ethics Committee (at Tanta Faculty of Medicine) and was performed in agreement with guidelines for ethical conduct in the use and care of experimental animals (Approval code: 32682/11/18).

Thirty adult male albino rats (200- 220 grams) were used. For acclimatization, the rats were housed in well ventilated cages for one week prior to the experiment under the same environmental conditions. The rats were divided into four groups:

Group I (control group): Fifteen rats were subdivided into three equal subgroups (5 rats each); subgroup Ia received no treatment; subgroup Ib received 1 ml of distilled water (the vehicle for tadalafil and KFE) orally once daily for six weeks; and subgroup Ic received 2 ml of distilled water (the vehicle for tadalafil and KFE) orally once daily for six weeks.

Group II (KFE-treated group): Five rats were administered KFE (500 mg/kg) dissolved in 1 ml distilled water once daily orally by a gastric tube for six weeks[21,22].

Group III (Tadalafil-treated group): Five rats were administered 1.8 mg/kg of tadalafil suspended in 1 ml distilled water once daily orally by a gastric tube for six weeks. The chosen dose of tadalafil was adjusted to be similar to the recommended human oral dose 20 mg/day for treating erectile dysfunction in human[23].

Group IV (Tadalafil and KFE-treated group): Five rats were concomitantly administered both tadalafil and KFE (at the same doses and duration as in groups II and III, respectively).

At the end of the experiment, intraperitoneal pentobarbital (50 mg/ kg body weight) was used to anesthetize rats[24]. Both eyes from each rat were enucleated.

The globes were placed in the fixative and were processed for light and electron microscopic study.

For light microscopy, the whole right eye ball from each rat was immersed in 10% neutral-buffered formalin for 24 hours, washed, dehydrated, cleared and then embedded in paraffin. Sections of the paraffin blocks (5μm thickness) were stained with haematoxylin and eosin (H&E)[25].

For immunohistochemistry, 5μm-thick sections were dewaxed, rehydrated, and washed using phosphate buffered saline (PBS). Then, the sections were incubated overnight in a humid chamber with the diluted primary antibody in PBS at 4°C (Primary antibody: anti-Bcl-2, rabbit polyclonal antibody, Abcam, ab59348, Cambridge, USA, at a dilution of 1/100). At room temperature, it was washed in PBS buffer, and co-incubated with biotinylated secondary antibody (Dako North America, Inc., CA, USA) for one hour. Streptavidin peroxidase was added for ten minutes and was rinsed in PBS. The immunoreactivity was determined using 3, 3'diaminobenzidine (DAB)-hydrogen peroxide (a chromogen). Sections were counterstained with Mayer's haematoxylin. Preparation of negative control sections was done without adding the primary antibodies[26]. Positive control for Bcl-2 was human colon carcinoma tissue. The Bcl-2-immunostained slides were considered positive by appearance of brown cytoplasmic coloration.

For transmission electron microscopy, specimens from the retina of the left eye ball of each rat were fixed by immersion in 2.5 % phosphate buffered glutaraldehyde, processed and then embedded in epoxy resin using the routine protocol. Semithin sections (1μm thick) were examined by the light microscope. Ultrathin sections (80- 90nm) were mounted on copper grids and then stained with uranyl acetate as well as lead citrate. Examination of the obtained grids of the retinal specimens was done by using JEOL transmission electron microscope (80 KV) at the Electron Microscope Unit, Tanta Faculty of Medicine[27].

Morphometric Study

The following parameters were measured by using image analysis computer system (Leica Qwin 500 C Image analyzer computer system; Leica Imaging System LTD., Cambridge, England) at the Central Research Lab, Tanta Faculty of Medicine. Ten different non-overlapping fields at a magnification of 400 were examined in each slide for:

1. The total thickness of the rats' retina and the thickness of its outer and inner nuclear layers (in H&E stained sections).

2. The number of the ganglion cells (per 100 μm length of the ganglion cell layer) (in H&E stained slides).

3. The area percentage (area %) of Bcl-2 positive immunohistochemical reaction (in DAB stained slides).

Statistical analysis

Statistical Package for Social Sciences (SPSS) software (version 11.5; SPSS Inc., Chicago, Illinois, USA) was used to evaluate and analyze the obtained morphometric data. One-way analysis of variance (ANOVA) followed by Tukey's test was used to compare all studied groups. All obtained values were expressed as mean ± standard deviation (Mean ± SD). Differences were regarded as statistically significant if probability value ($p< 0.05$) and highly significant if ($p<0.001$)[28].

RESULTS

No animal deaths occurred in this study.

Light Microscopic Findings

Group I (control group): Rats of all control subgroups showed the same characteristic histological structure of the retina in H&E-stained sections. It was formed of ten organized layers; retinal pigment epithelium, photoreceptor layer of rods and cones, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer and the inner limiting membrane. The retinal pigment epithelium was a single layer of cuboidal cells that had pale, oval, basal nuclei. This layer was resting on Bruch's membrane which separated it from the choroid. The photoreceptor layer showed an eosinophilic parallel fibrillary striation pattern and was composed of lightly stained outer segments as well as deeply stained inner segments of the rods and cones. The outer limiting membrane was a thin membrane located at the junction between the photoreceptor layer and the outer nuclear layer. The thick outer nuclear layer was formed of several rows containing the closely packed densely stained nuclei of the cell bodies of rods and cones. The outer plexiform layer had a reticular appearance as it showed a loose network of eosinophilic fibers. The inner nuclear layer appeared much thinner than the outer nuclear layer and its cells were fewer and variable in size, shape and density. It was formed of numerous, relatively larger cells and their nuclei were paler than those of the outer nuclear one. The inner plexiform layer was much thicker than the outer plexiform layer and appeared as a loose network of eosinophilic fibers. The ganglion cell layer was composed of a single discontinuous row of the cell bodies of the ganglion cells. They were larger than most of the preceding retinal cells and had a lightly stained cytoplasm and large vesicular spherical or oval or angular nuclei. Axons of the ganglion cells extended to form the thin nerve fiber layer that was lined internally by the inner limiting membrane (Figure 1).

Group II (KFE-treated group): This group showed the same histological findings of the retinal layers of the control group.

Group III (Tadalafil-treated group): Examination of H&E-stained sections of tadalafil-treated rats showed multifocal structural changes. Focal areas displayed detachment of the retinal pigmented epithelium. The outer segment of the photoreceptors showed vacuolations. The inner segment was irregularly oriented with interrupted outer limiting membrane. Some photoreceptor nuclei appeared pyknotic. Moreover, diminished cell population of the outer and inner nuclear layers was noticed with empty spaces between their cells. Many cells in the inner nuclear layer showed dark pyknotic nuclei. Moreover, there was disorganization of the outer and inner plexiform layer with widening of the spaces between their fibers. The ganglion cell layer showed rarefaction or vacuolation and contained a few disorganized cells with pyknotic nuclei. Congested dilated blood capillaries were observed in the ganglion cell layer (Figures 2,3 and 4).

Group IV (Tadalafil and KFE-treated group): The histological findings of this group were almost similar to that of the control group. Partially preserved histological structure of the retinal layers with restoration of the cell population of the outer and inner nuclear layers as well as the ganglion cell layer was noticed. Nevertheless, focal separation of the retinal pigmented epithelium, a few vacuolations in the outer segment of the photoreceptor layer and dilated congested blood capillaries of the ganglion cell layer were observed in focal areas (Figures 5 and 6).

Immunohistochemical results

Bcl-2 immunostaining: Bcl-2-immunostained retina sections of the control group (group I) as well as the KFE-treated group II showed a moderate positive cytoplasmic immunoreaction for Bcl2 in the retinal pigmented epithelium, photoreceptors, outer and inner plexiform layers and ganglion cell layers. Tadalafil-treated group (group III) expressed a weak positive cytoplasmic immunoreaction for Bcl2 at the same previously mentioned retinal layers, while the tadalafil and KFE-treated group (group IV) showed a moderate positive cytoplasmic immunoreaction for Bcl2 at the same previously mentioned retinal layers (Figure 7).

Electron microscopic results

The Photoreceptor Layer and Outer Limiting Membrane

The photoreceptors of the control group were formed of outer and inner segments with narrow spaces in-between. The outer segments were composed of regularly arranged elongated discs having narrow spaces in-between. In tadalafil-treated group, disruption of the photoreceptor ultrastructure was observed such as wide spaces between the outer segments, interrupted membranous discs and increased interdisc spaces. As regard to tadalafil and KFE-treated group, the outer segments showed a comparatively well preserved ultrastructure. However, minimal changes were observed such as disc interruption in some of the outer segments (Figure 8).

The inner segments of the control group appeared less electron dense than the outer segments and had numerous mitochondria, rER and ribosomes. Whereas the inner segments of the tadalafil-treated group showed cytoplasmic vacuoles as well as swollen mitochondria

with disrupted cristae. In tadalafil and KFE-treated group, minimal cytoplasmic vacuoles were observed in some segments (Figure 9).

The outer limiting membrane of control rats was an electron dense continuous membrane. It was formed by the junction between inner segments and processes of Müller cells. Tadalafil-treated group showed an irregular or undulant thin discontinuous outer limiting membrane. On the other hand, tadalafil and KFE-treated group revealed renewal of the outer limiting membrane (Figure 9).

The Outer Nuclear Layer

In control group, the outer nuclear layer was composed of the cell bodies of rods and cones. Rod nucleus was more electron dense than cone nucleus and had a large central mass of heterochromatin. In tadalafil-treated group, the cell bodies of rods and cones were irregular and shrunken having wide spaces in-between. Rods and cones nuclei were irregular and shrunken. On the other hand, tadalafil and KFE-treated group revealed intact rods and cones nuclei with narrowing of the spaces between their cell bodies (Figure 10).

The Outer Plexiform Layer

The outer plexiform layer of the control rats was formed by synapses between the synaptic processes of the photoreceptors (rods and cones) and the dendrites of bipolar cells of the inner nuclear layer. The synaptic terminals are closely adherent to each other and contain mitochondria and neurofibrils with normal density. In tadalafil-treated group, reduced density of the synaptic terminals of the photoreceptors with wide spacing of the swollen synaptic terminals, rarefaction of their cytoplasm and cristolysis of their mitochondria in addition to loosening of the synaptic junction was observed in this layer. Tadalafil and KFE-treated group showed nearly normal density of most photoreceptors synaptic terminals however a few synaptic terminals showed reduced density (Figure 11).

The Inner Nuclear Layer

In control group, this layer was formed by cell bodies of the retinal neurons which contained large vesicular rounded or oval nuclei, mitochondria, rER and ribosomes. The angulated Müller cells were found between the retinal neurons and had large elongated angular dark nuclei.

Regarding tadalafil-treated group, some neurons of this layer were shrunken with shrunken dense

nuclei, cytoplasmic vacuoles, dilated rER and swollen mitochondria with destroyed cristae. In tadalafil and KFE-treated group, the cell bodies appeared to some extent intact. However, minimal changes were still present such as cytoplasmic vacuoles, damaged mitochondria, dilated perinuclear space and rER in a few cells (Figure 12).

The Inner Plexiform Layer

In control rats, this layer was formed by synapses between axons of the neurons of the inner nuclear layer and dendrites of the ganglion cells. The neurons synaptic terminals showed normal density of the neurofibrils. Tadalafil-treated group showed many swollen synaptic terminals having widely spaced neurofibrils. In tadalafil and KFE-treated group, a few synaptic terminals were swollen with some disrupted neurofibrils (Figure 13).

The Ganglion Cell Layer

The ganglion cells of the control group contained large euchromatic nuclei, rER, mitochondria and lysosomes. On the other hand, some ganglion cells of tadalafil-treated group showed cytoplasmic vacuoles, many swollen mitochondria with disrupted cristae and dilated rER. Other ganglion cells were shrunken having dark shrunken irregular nuclei, wide perinuclear spaces and many lysosomes in their cytoplasm. Moreover, congested blood vessels were noticed in the ganglion cell layer. However, the ganglion cells of tadalafil and KFE-treated group appeared more or less intact. They contained less electron dense nuclei, a few swollen mitochondria with disrupted cristae and lysosomes (Figure 14).

Morphometric and Statistical Results

The mean total retinal thickness, the mean thickness of the outer and inner nuclear layers and the mean number of the ganglion cells showed a statistically significant decrease in the tadalafil-treated group (Group III) compared to the control group. Moreover, tadalafil and KFE-treated group (group IV) showed a non-significant decrease of the same previously mentioned parameters compared to the control (Table 1, Histogram 1 and 2).

The mean area percentage of Bcl2-positive immunoreaction in the tadalafil-treated group (Group III) showed a statistically significant decrease compared with the control group, whereas the tadalafil and KFE-treated group (group IV) showed a non-significant decrease compared with the control (Table 1 and Histogram 3).
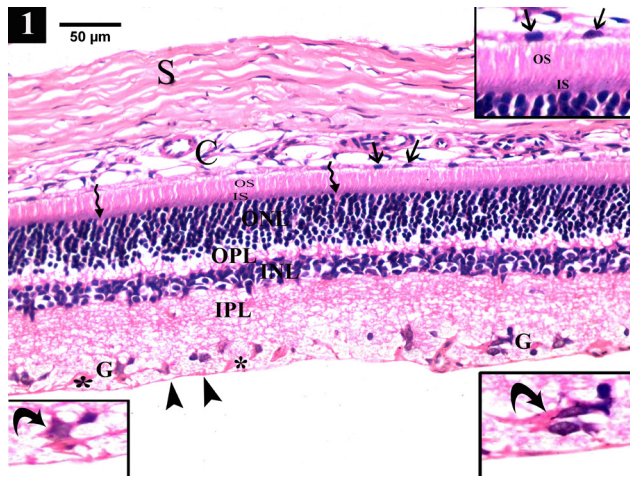
Fig. 1: A photomicrograph of a section in the retina of an adult male albino rat from the control group showing the retinal layers from outside inwards; the retinal pigmented epithelium (arrow), outer (OS) and inner (IS) segments of photoreceptors, outer limiting membrane (wavy arrow), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (G), nerve fiber layer (asterisks) and inner limiting membrane (arrow head). The underlying choroid (C) and sclera (S) can be also seen. The upper inset shows a higher magnification of the retinal pigmented epithelium with its pale oval basal nuclei (arrow) and the eosinophilic parallel fibrillary striation pattern of the photoreceptors which is composed of lightly stained outer segments (OS) and deeply stained inner segments (IS) of the rods and cones. The lower insets show the cell bodies of the ganglion cells (curved arrow) with a lightly stained cytoplasm and large vesicular spherical or oval or angular nuclei. (H&E X 400, Insets X1000).
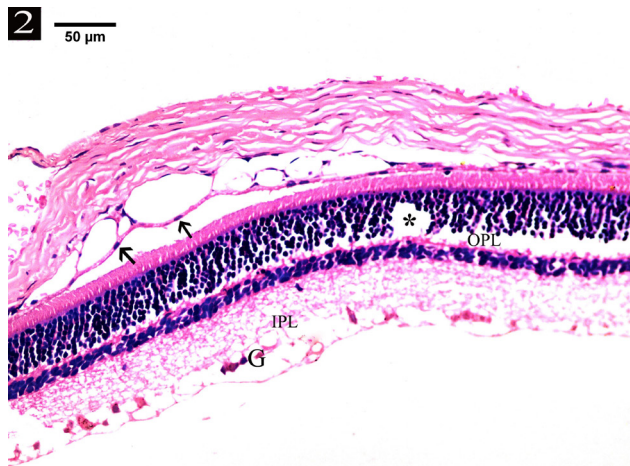

Fig. 2: A photomicrograph of a section in the retina of an adult male albino rat from the tadalafil-treated group (Group III) showing focal separation of the retinal pigmented epithelium (arrow), diminished cell population of the outer nuclear layer with empty spaces between their cells (asterisk) and disorganization of the outer (OPL) and inner (IPL) plexiform layers with widening of the spaces between their fibers. The ganglion cell layer shows rarefaction or vacuolation with a few disorganized cells (G) (H&E X 400).
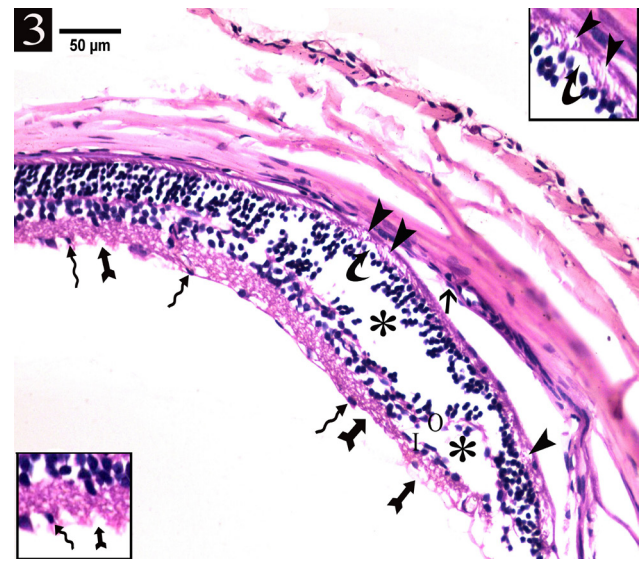

Fig. 3: A photomicrograph of a section in the retina of an adult male albino rat from the tadalafil-treated group (Group III) showing focal separation of the retinal pigmented epithelium (arrow), vacuolations of the outer segment and irregularly oriented inner segment (arrow head) of the photoreceptors with interrupted outer limiting membrane (curved arrow). Diminished cell population of the outer and inner nuclear layers with empty spaces between their cells and many pyknotic nuclei (asterisk) are observed. Notice disorganized outer (O) and inner (I) plexiform layers and pyknotic nuclei of many ganglion cells (wavy arrow) with interrupted inner limiting membrane (notched arrow). (H&E X 400, Insets X1000).
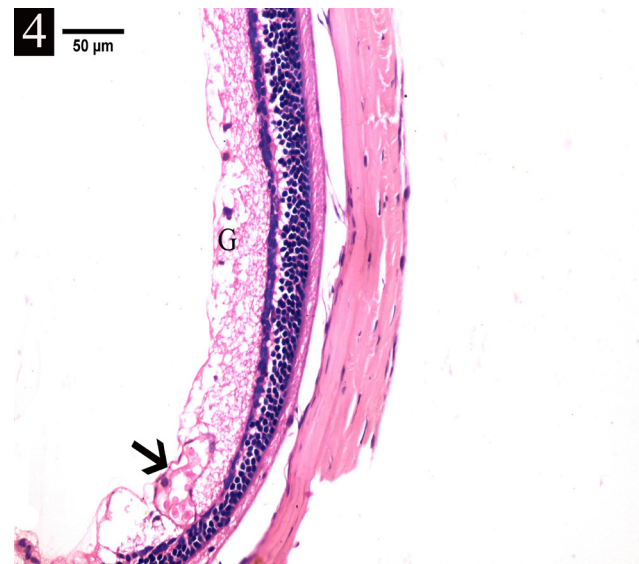

Fig. 4: A photomicrograph of a section in the retina of an adult male albino rat from the tadalafil-treated group (Group III) showing diminished cell population of the ganglion cell layer (G) with dilated congested blood capillary (arrow) (H&E X 400).
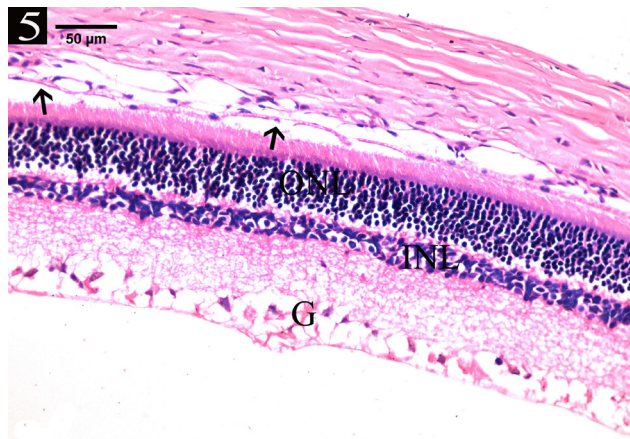
Fig. 5: A photomicrograph of a section in the retina of an adult male albino rat from the tadalafil & KFE-treated group (group IV) showing partially preserved histological structure of the retinal layers with restoration of the cell population of the outer (ONL) and inner (INL) nuclear layers as well as the ganglion cell layer (G). Focal separation of the retinal pigmented epithelium can be seen (arrow). (H&E X 400).
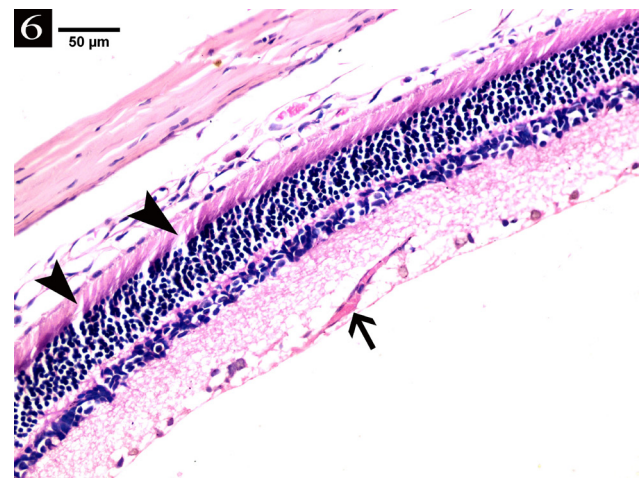

Fig. 6: A photomicrograph of a section in the retina of an adult male albino rat from the tadalafil & KFE-treated group (group IV) showing a few vacuolations of the outer segment of the photoreceptor layer (arrow head) and a dilated congested blood capillary in the ganglion cell layer (arrow). (H&E X 400).
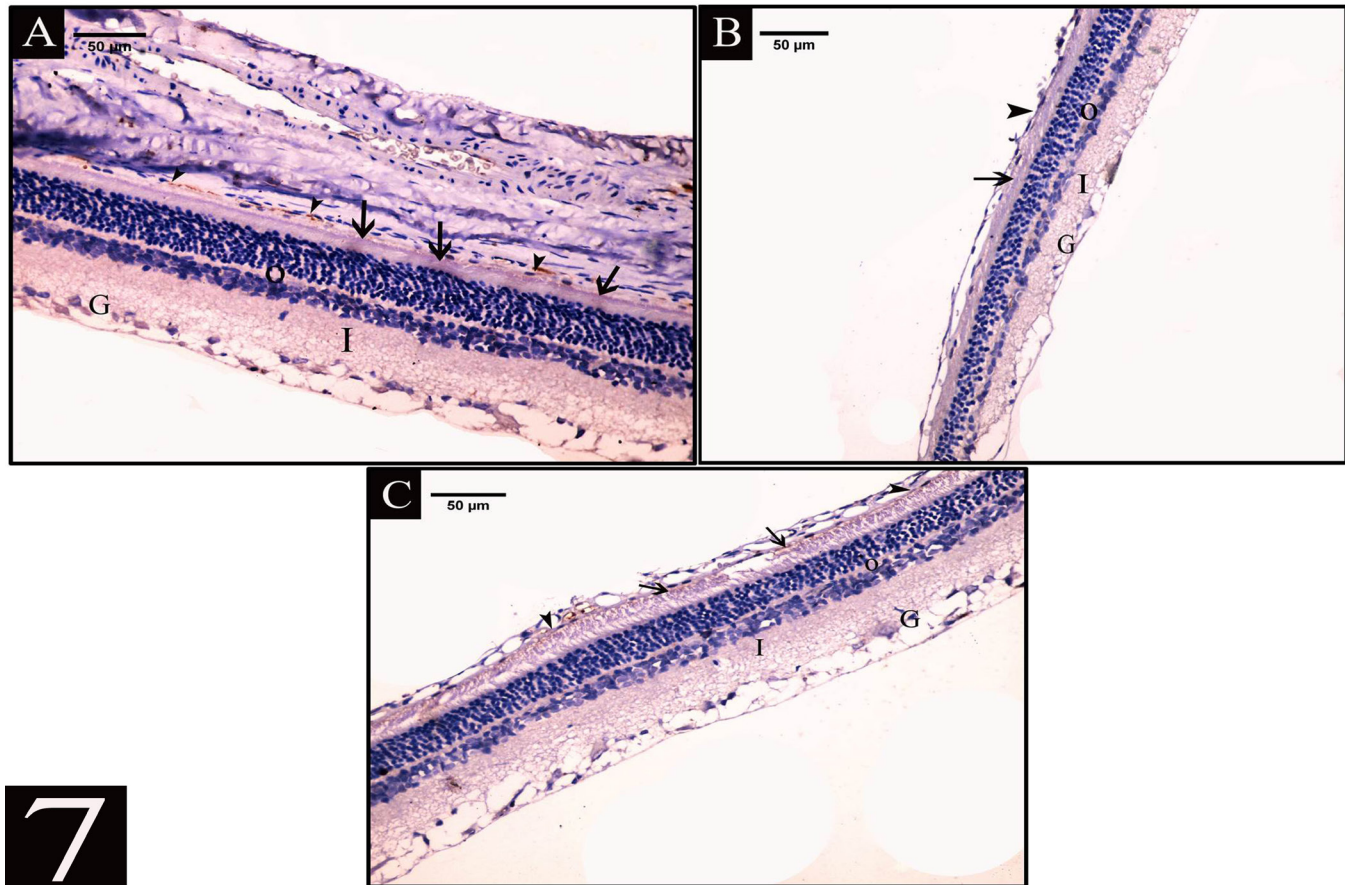

Fig.7: Photomicrographs of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).
- A: showing a moderate positive cytoplasmic immunoreaction for Bcl2 in the retinal pigmented epithelium (arrow head), photoreceptors (arrow), outer (O) and inner (I) plexiform layers and ganglion cell layers (G).
- B: showing a weak positive cytoplasmic immunoreaction for Bcl2 in the retinal pigmented epithelium (arrow head), photoreceptors (arrow), outer (O) and inner (I) plexiform layers and ganglion cell layers (G).
- C: showing a moderate positive cytoplasmic immunoreaction for Bcl2 in the retinal pigmented epithelium (arrow head), photoreceptors (arrow), outer (O) and inner (I) plexiform layers and ganglion cell layers (G).
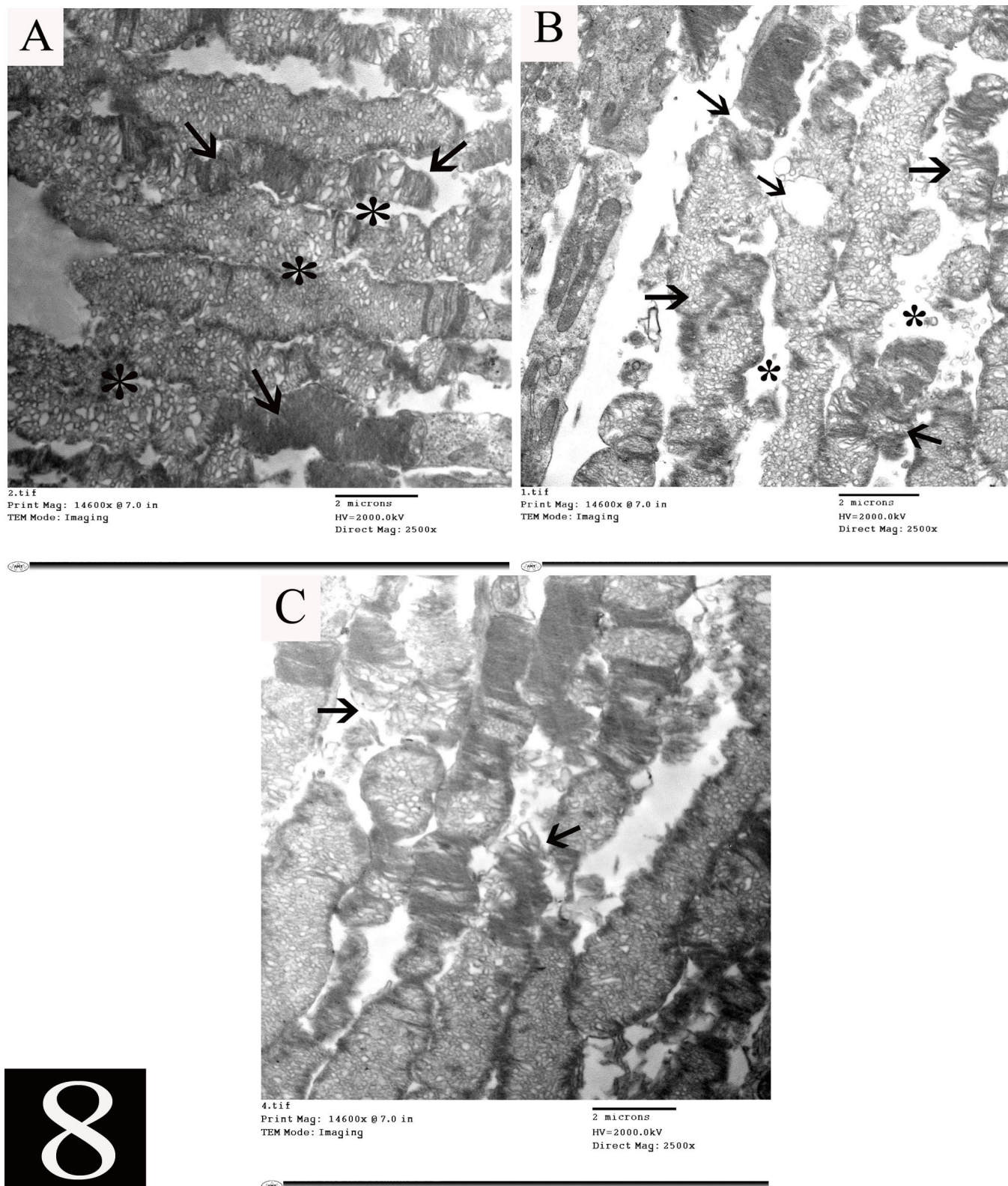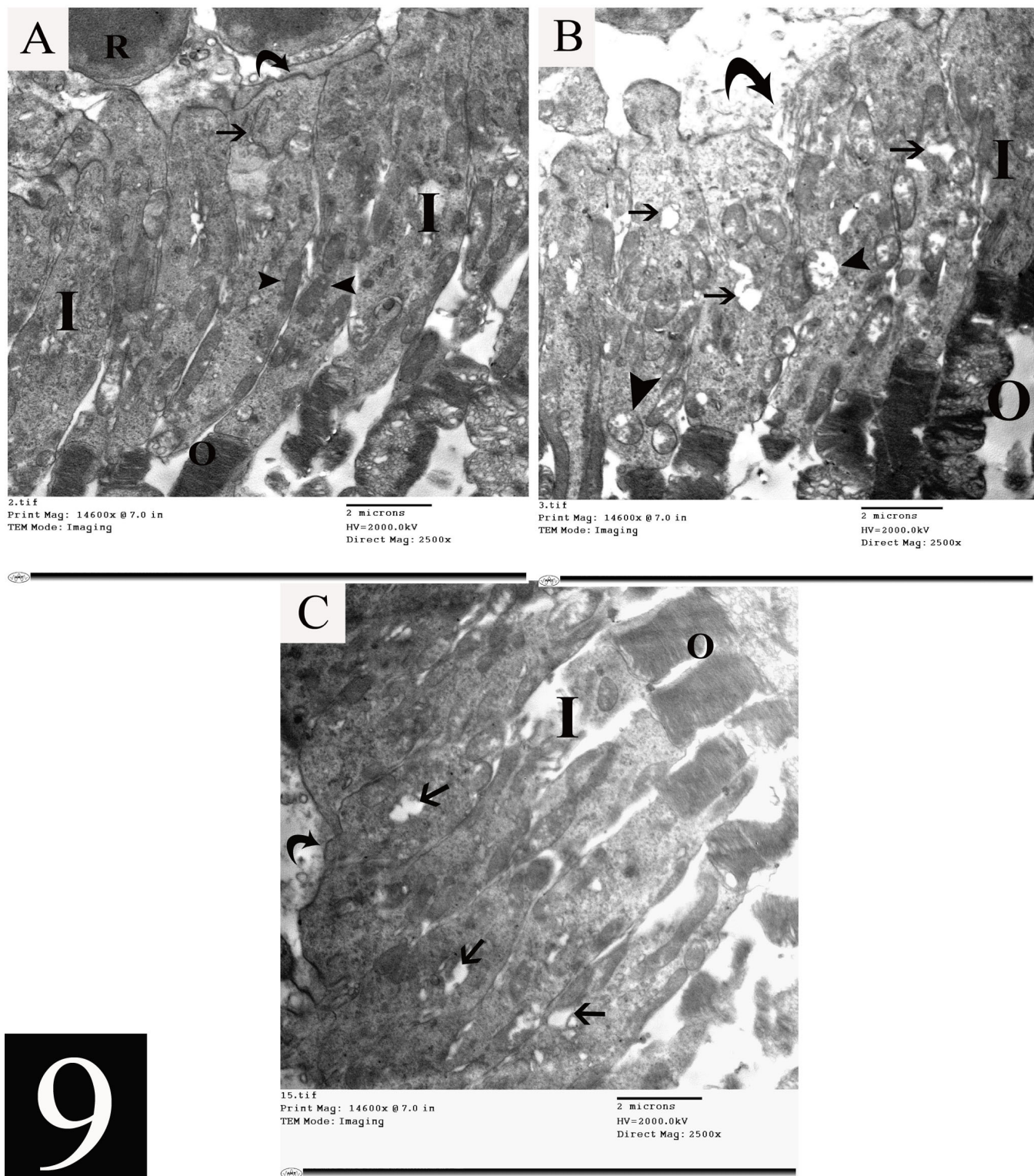
(Bcl2 immunostaining X 400).

Fig. 8: Electron micrographs of the outer segment of photoreceptors of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).

- A: showing narrow intersegment spaces (asterisk) and regularly arranged elongated discs having narrow interdisc spaces (arrow).
- B: showing wide spaces between the outer segments (asterisk), interrupted membranous discs and increased interdisc spaces (arrow).
- C: showing a comparatively well preserved ultrastructure. Notice minimal disc interruption (arrow).

(TEM X 14600).

Fig. 9: Electron micrographs of the inner segment (I) of photoreceptors of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).

- A: showing numerous mitochondria (arrow head), rER and ribosomes (arrow). Notice the regularly arranged elongated discs of the outer segment (O), a continuous outer limiting membrane (curved arrow) between the inner segment (I) and the outer nuclear layer (R).
- B: showing cytoplasmic vacuoles (arrow) and swollen mitochondria with disrupted cristae (arrow head). Notice the disrupted membranous discs of the outer segment (O) and an irregular or undulant thin discontinuous outer limiting membrane (curved arrow).
- C: showing minimal cytoplasmic vacuoles (arrow) in some segments (I). Notice the regularly arranged elongated discs of the outer segment (O) and a continuous outer limiting membrane (curved arrow).
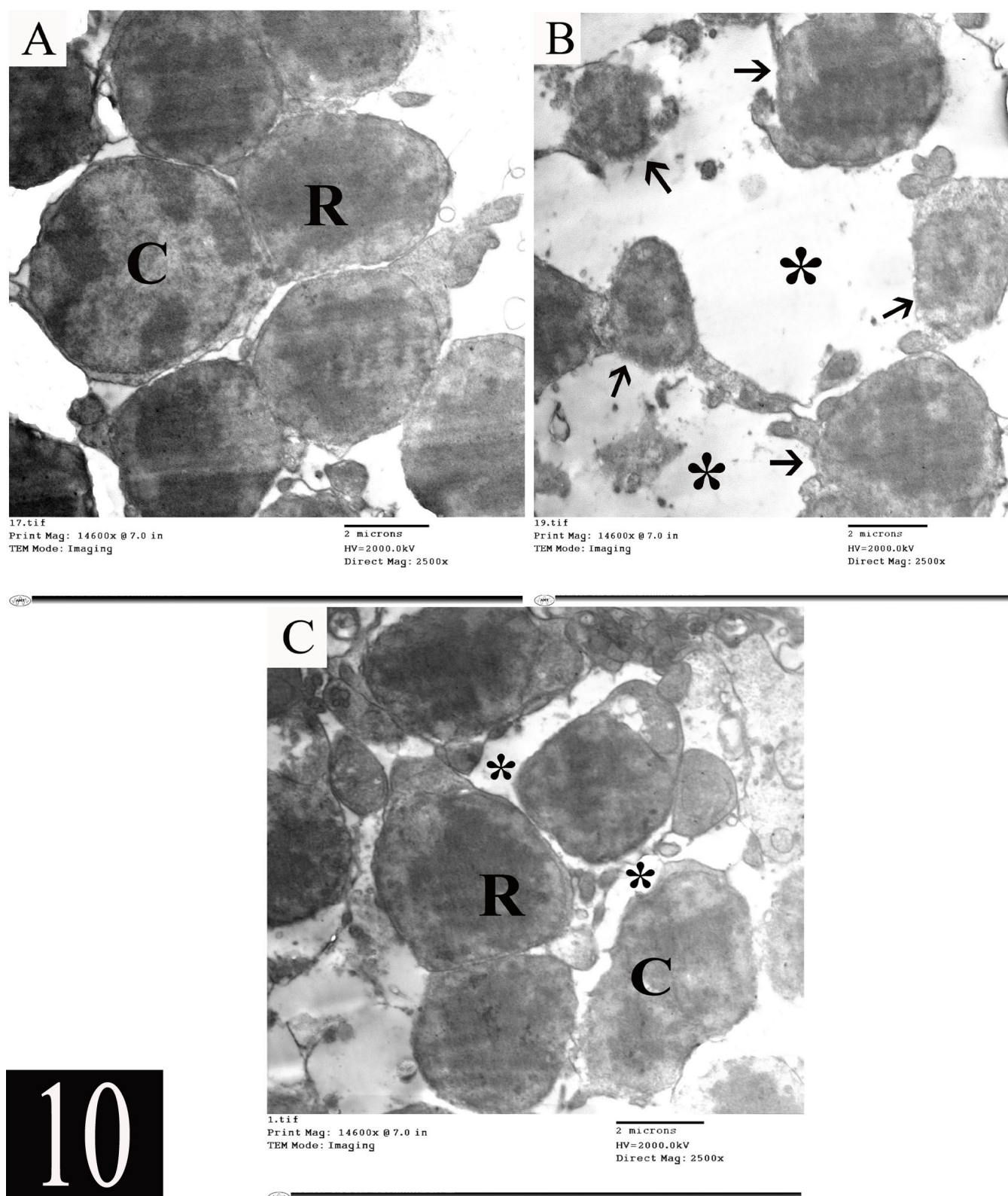
(TEM X 14600).

Fig. 10: Electron micrographs of the outer nuclear layer of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).
- A: showing the cell bodies of rods (R) and cones (C). Rod nucleus was more electron dense than cone nucleus and had a large central mass of heterochromatin.
- B: showing shrunken and irregular cell bodies and nuclei of rods and cones (arrow) and widening of the spaces (asterisk) between them.
- C: showing intact rods (R) and cones (C) nuclei with narrowing of the spaces (asterisk) between their cell bodies.
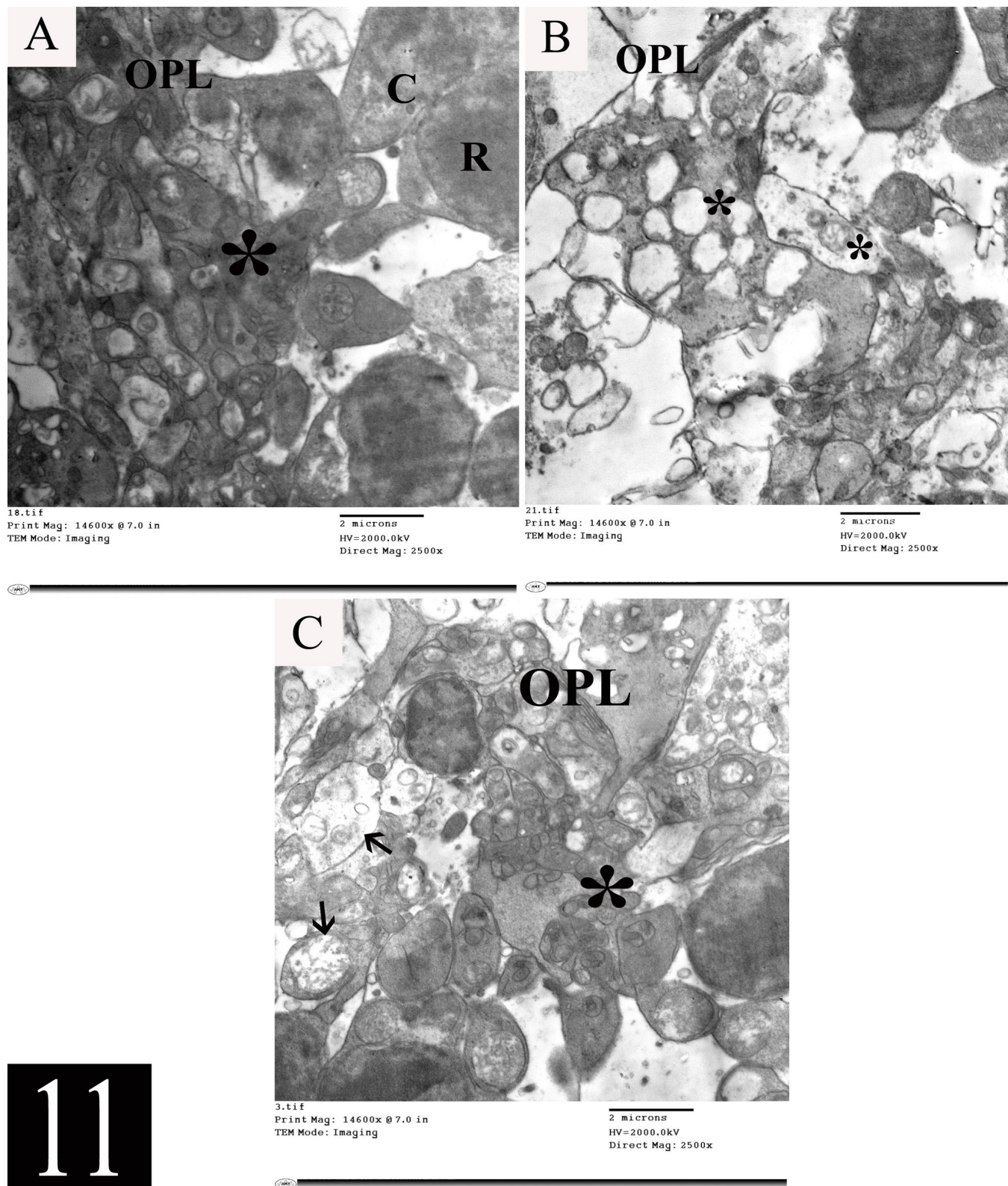
(TEM X 14600).

Fig. 11: Electron micrographs of the outer plexiform layer (OPL) of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).

- A: showing synapses (asterisk) between the synaptic terminals of rods (R) and cones (C) and the dendrites of bipolar cells of the inner nuclear layer. The synaptic terminals are closely adherent to each other and contain mitochondria and neurofibrils with normal density.
- B: showing reduced density of rods and cones synaptic terminals with wide spacing of the swollen synaptic terminals, rarefaction of their cytoplasm and cristolysis of their mitochondria in addition to loosening of the synaptic junction (asterisk).
- C: showing nearly normal density of most rods and cones synaptic terminals (asterisk). Notice a few synaptic terminals showed reduced density (arrow).
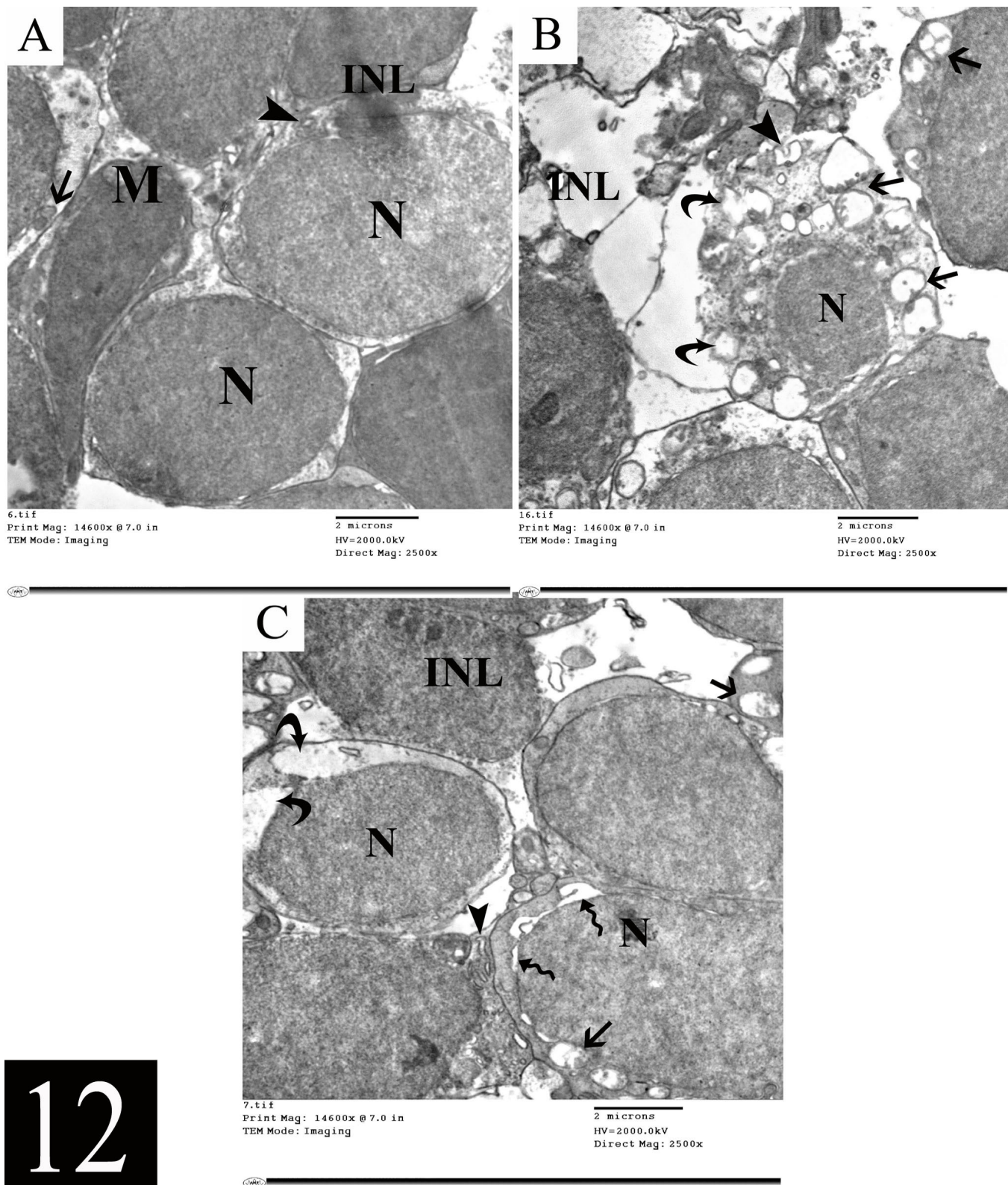
(TEM X 14600).

Fig. 12: Electron micrographs of the inner nuclear layer (INL) of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).

- A: showing the cell bodies of the retinal neurons with large vesicular rounded or oval nuclei (N), mitochondria (arrow), rER and ribosomes (arrow head). Notice the angulated Müller cell between the retinal neurons with its large dark elongated angular nuclei (M).
- B: showing a shrunken retinal neuron with a shrunken dense nucleus (N), cytoplasmic vacuoles (curved arrow), swollen mitochondria with destroyed cristae (arrow) and dilated rER (arrow head).
- C: showing nearly intact retinal neurons (N). Notice minimal changes as cytoplasmic vacuoles (curved arrow), damaged mitochondria (arrow), dilated perinuclear space (wavy arrow) and rER (arrow head) in a few cells.

(TEM X 14600).

Fig. 13: Electron micrographs of the inner plexiform layer (IPL) of rat retina from (A, control group; B, tadalafil-treated group; C, tadalafil & KFE-treated group).
- A: showing synapses between the axons of inner nuclear layer neurons and the dendrites of the ganglion cells. Notice the normal density of the neurofibrils in the synaptic terminals (asterisk).
- B: showing many swollen synaptic terminals with widely spaced neurofibrils (asterisk).
- C: showing nearly normal density of most synaptic terminals (asterisk). Notice a few swollen synaptic terminals with some disrupted neurofibrils (arrow).

(TEM X 14600).

A
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2 microns
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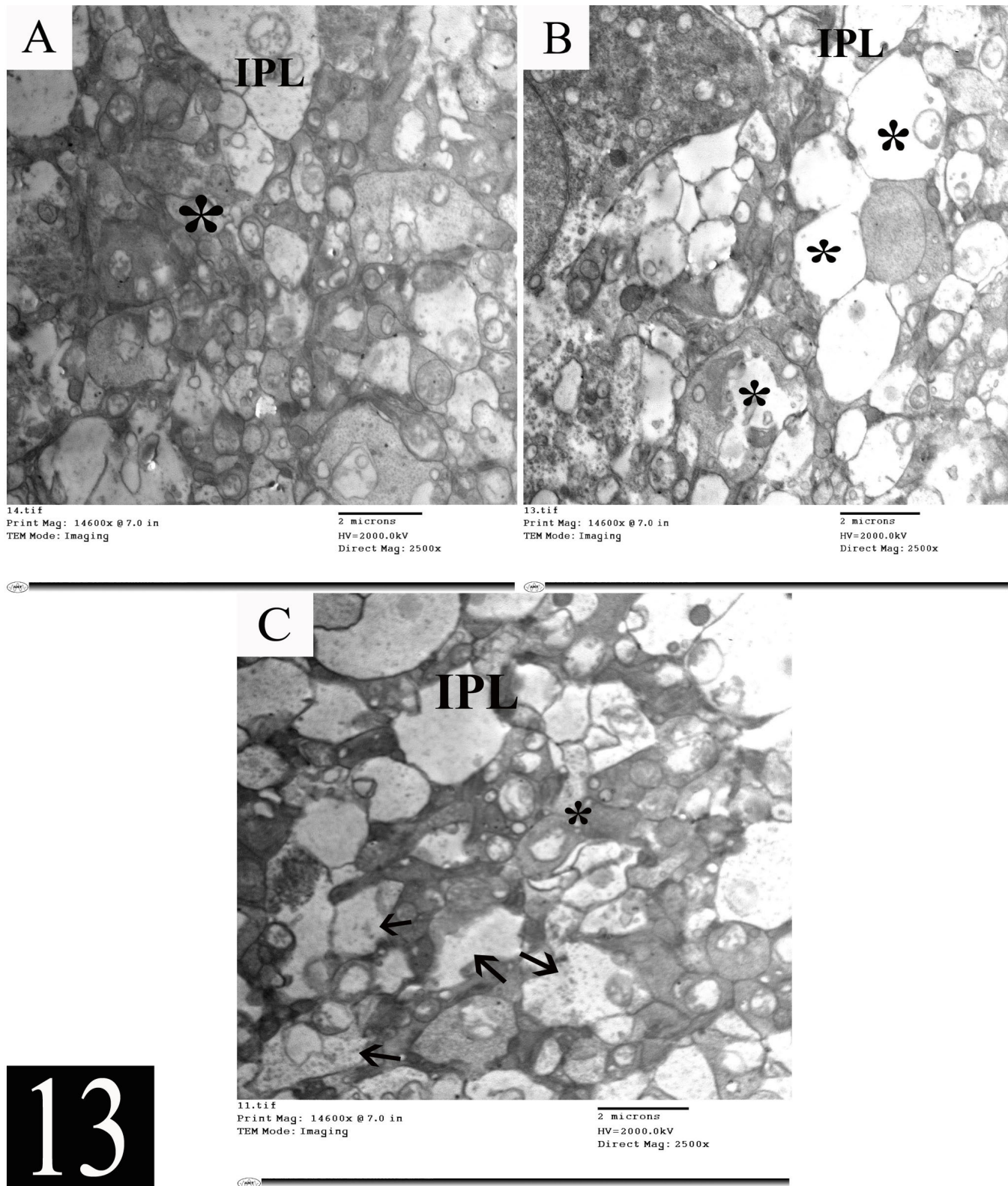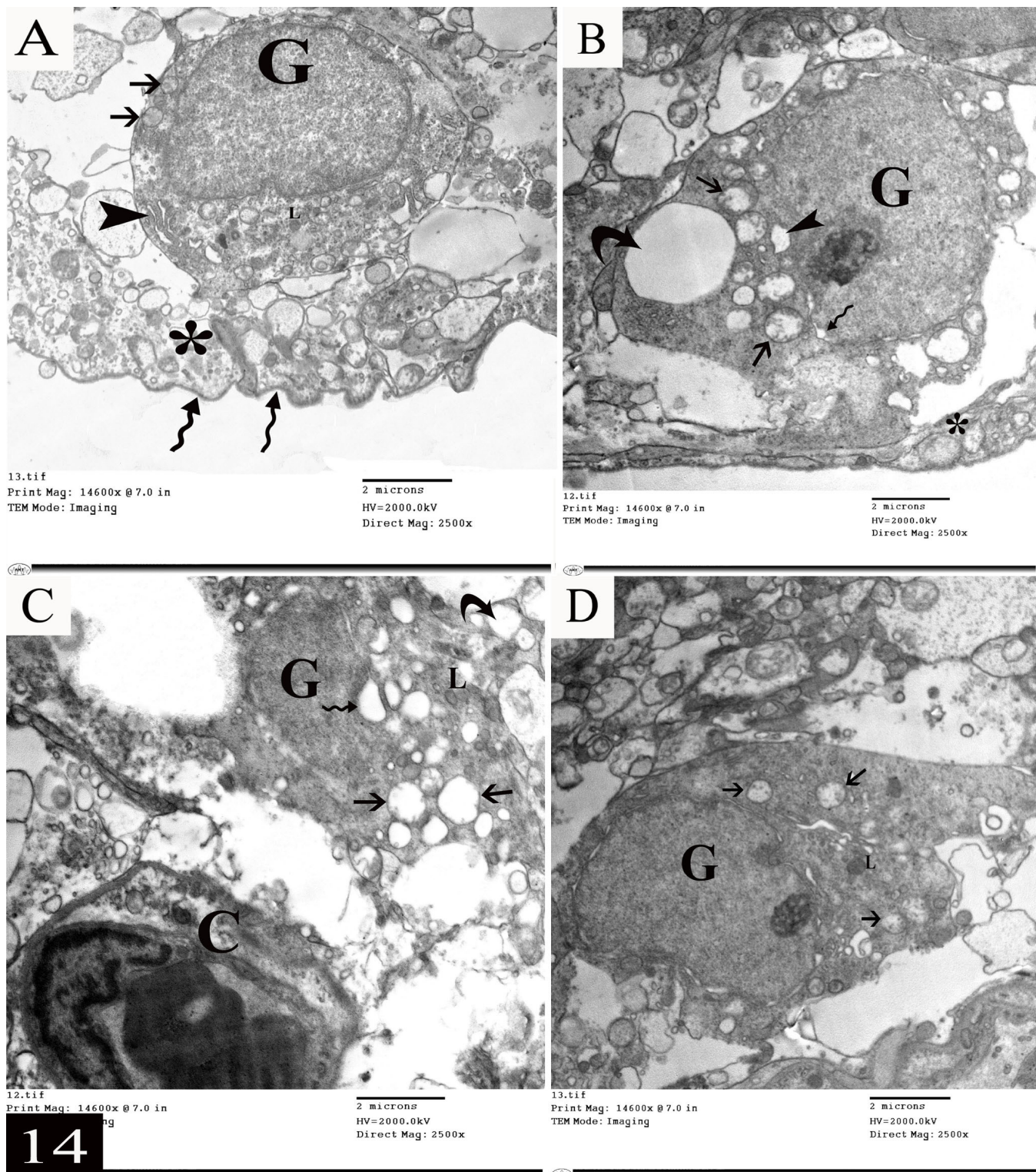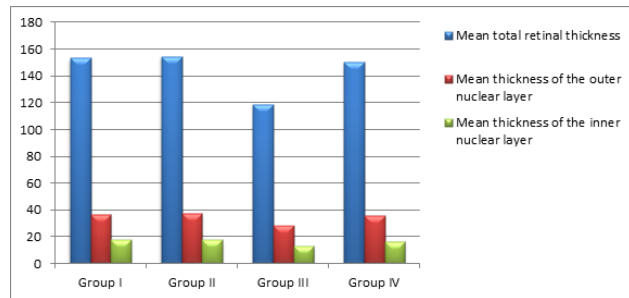2 microns
HV=2000.0kV
Direct Mag: 2500x

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Fig. 14: Electron micrographs of the ganglion cell layer (G) of rat retina from (A, control group; B&C, tadalafil-treated group; D, tadalafil & KFE-treated group).

- A: showing a ganglion cell with a large euchromatic nucleus (G), mitochondria (arrow), rER (arrow head) and lysosomes (L). Notice the nerve fiber layer (asterisk) and the inner limiting membrane (wavy arrow).
- B: showing a ganglion cell (G) with cytoplasmic vacuoles (curved arrow), many swollen mitochondria with cristolysis (arrow) and dilated perinuclear space (wavy arrow) and rER (arrow head). Notice marked thinning of the nerve fiber layer (asterisk).
- C: showing a shrunken ganglion cell with dark shrunken irregular nuclei (G), cytoplasmic vacuoles (curved arrow), many swollen mitochondria with cristolysis (arrow), many lysosomes in its cytoplasm (L) and wide perinuclear spaces (wavy arrow). Notice congested blood vessels (C) in the ganglion cell layer.
- D: showing a ganglion cell with a large euchromatic nucleus (G), a few swollen mitochondria with disrupted cristae (arrow) and lysosomes (L).
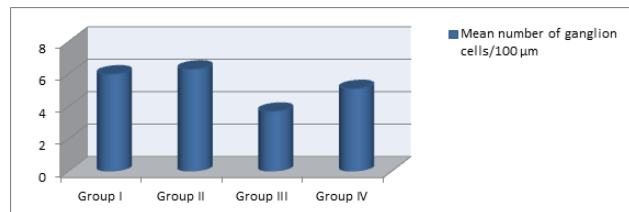
(TEM X 14600).

Table 1: Morphometric analysis of the retinal specimens in all groups. Data were expressed as mean ± SD

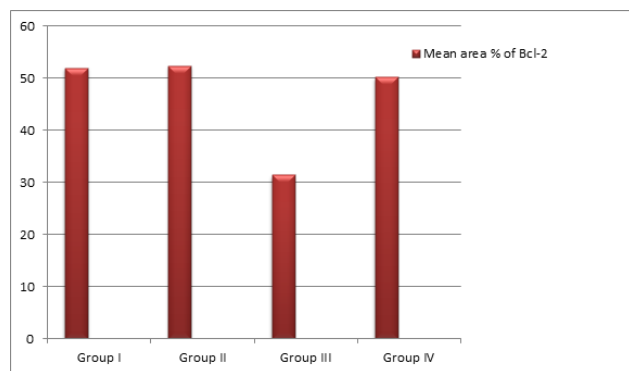| Groups Parameters	Group I	Group II	Group III	Group IV
Mean total retinal thickness (μm)	153.09±3.03	154.63±3.75	118.71±6.82*	150.33±2.89
Mean thickness of the outer nuclear layer (μm)	36.80±1.43	37.08±1.75	28.17±2.07*	35.46±1.43
Mean thickness of the inner nuclear layer (μm)	17.83±1.66	17.79±1.65	13.25±2.27*	16.49±1.24
Mean number of ganglion cells/100 μm	6±1.05	6.3±1.06	3.7±0.949*	5.1±0.876
Mean area percentage of Bcl2 immunoreaction	51.98±2.31	52.35±3.06	31.47±2.1*	50.25±1.03

*$P < 0.05$ is significant versus group I (control)

Histogram 1: The mean total retinal thickness and the mean thickness of the outer and inner nuclear layers in all groups

Histogram 2: The mean number of the ganglion cells in all groups

Histogram 3: The mean area percentage of Bcl-2 positive immunoreaction in all groups

DISCUSSION

Tadalafil is a phosphodiesterase type 5 (PDE-5) enzyme inhibitor that is widely used for the treatment of erectile dysfunction[29]. A little information exists about the effects of chronic use of PDE-5 enzyme inhibitors on different body organs[30]. However, many studies have recorded their associated ophthalmic complications such as electroretinographic abnormalities[31,32,33] and central choroidopathy[34].

There are increasing trends and growing requests towards the use of safe and natural antioxidants extracted from natural plants in the treatment of different diseases[35]. Therefore, this study was performed to evaluate the possible role of kiwifruit extract in attenuating tadalafil induced retinal injury in rats by using different histological and immunohistochemical methods.

The light and electron microscopic findings of the present study clearly revealed that the oral administration of tadalafil for six weeks has altered the structure of retina leading to disorganized retinal layers in addition to evident degenerative changes such as detached retinal pigmented epithelium, cytoplasmic vacuolations and nuclear pyknosis of some photoreceptors as well as many cells of inner nuclear layer and ganglion cell layer. Similar results were recorded by some authors[36] who studied the effect of another PDE-5 inhibitor; sildenafil citrate on the rat retina. Moreover, other physiological studies stated that PDE-5 enzyme inhibitors decrease retinal ganglion cells light response and reversibly inhibit the photo-transduction in retina of monkeys[37,38]. Therefore, tadalafil adverse effects on retinal functions were suggested to be due to PDE-5 enzyme inhibition[39]. On the other side, other study denied the negative effect of this drug on the structure of retinal layers[40].

The histological findings of our study coincide with the morphometric results that revealed a highly significant decrease in total retinal thickness as well as thickness of outer and inner nuclear layers in tadalafil treated group as compared with controls. Moreover, this study demonstrated diminished cell populations in both outer and inner nuclear layers, and this might explain the reduction in thickness of these layers.

Oxidative stress is thought to be a possible mechanism through which tadalafil and other PDE-5 enzyme inhibitors could induce retinal degeneration. An in-vitro study on murine-derived photoreceptor cell line treated with a PDE enzyme inhibitor showed an activation of cyclic guanosine monophosphate (cGMP) with subsequent increase in the intracellular calcium ions (Ca2+) levels in the retinal photoreceptors leading to oxidative stress[41,42,43]. These events together disturb retinal cells viability leading to retinal degeneration and loss of photoreceptors[44].

In the present study, tadalafil administration caused nuclear pyknosis of the affected retinal cells, and this is thought to be one of the apoptotic signs as reported by some authors[45]. Consistently, the immunohistochemical results revealed a significant down-regulation of Bcl-2 immune-expression in the retinal cells of tadalafil treated group as compared to control group. Bcl-2 protein is well known as an anti-apoptotic marker[46]. Another in-vitro study showed that the inhibition of phosphodiesterase enzymes could induce oxidative stress and apoptotic cell death in cultures of porcine retinas[47]. Therefore, apoptosis is suggested to be one of the mechanisms of tadalafil induced retinal injury.

The results of the present study illustrated many cytoplasmic vacuoles in the inner segments of the retinal photoreceptor layer of tadalafil group. These vacuoles are proposed to be autophagic vacuoles, as increasing evidence has reported the role of autophagy in the process of photoreceptors degeneration and its implication in elimination of dysfunctional cellular organelles and protein aggregates in diseased retina[48,49].

In this study, the mean number of ganglion cells showed a statistically significant decrease in the tadalafil-treated group as compared to control one, and this might be contributed to the increased intraocular pressure caused by tadalafil treatment. This elevated intraocular pressure might cause retinal ganglion cells apoptosis[50,51]. It was reported that PDE-5 enzyme inhibitors cause dilatation of the choroidal fenestrated capillaries and leakage of plasma rich fluid into the anterior chamber with subsequent increase in the intraocular pressure[52,53,54]. In addition, the increased intraocular pressure as well as the changes in ocular blood flow diminishes the power of retinal pigment epithelium to pump fluid from retina to choroid[55], and this negatively affect the retinal metabolism and might contribute to the pathogenesis of retinal pigment epithelium detachment,

outer limiting membrane disruption and wide separation of photoreceptors outer segments that were observed in tadalafil group of this study.

In this study, many dilated capillaries were observed in the retina of tadalafil group, and this was in accordance with other clinical studies that revealed a remarkable vasodilatation effect of PDE5 enzyme inhibitors on the arterioles and venules of retina, and they attributed this effect to the increased nitric oxide production that induces vascular smooth muscle relaxation and vasodilation[56,57,58].

The current study noticeably illustrated that administration of kiwifruit extract caused an evident improvement in the different histological and immunohistochemical findings. This retino-protective effect of kiwifruit could be attributed to its evident antioxidant property as reported by some authors[59,60].

Kiwifruit contains high level of bioactive compounds that possess evident antioxidant properties such as polyphenols, carotenoids (lutein, zeaxanthin and β-carotene) and flavonoids including flavones and flavonones[61,62,63]. In addition, this fruit is rich in many vitamins particularly vitamin C that has a strong antioxidant capacity[64]. Several studies declared the antioxidant effect of kiwi fruit both in *vivo*[65] and in *vitro*[66]. Furthermore, the antioxidants in kiwi fruit have been reported to be absorbed more effectively by the body than the antioxidants of other fruits[67,68].

In the present study, kiwifruit extract could ameliorate the degree of apoptosis of retinal cells. This anti-apoptotic effect could be probably mediated through up-regulation of the anti-apoptotic Bcl-2 protein as denoted in the immunohistochemical results of this study. Several in vitro studies have shown that extracts of kiwi fruits provide cell protection against apoptotic cell death and oxidative DNA damage[69]. Other researchers reported that the anti-apoptotic effect of kiwifruit extract is attributed to its content of lutein carotenoid that has also an ability to scavenge oxygen free radicals during oxidative stress[70,71,72].

CONCLUSION

kiwifruit extract looks to be beneficial in ameliorating tadalafil-induced retinal injury in rats, and this positive effect may be through its anti-oxidant and anti-apoptotic properties. Therefore, kiwifruit extract could be a promising protective agent recommended for patients receiving tadalafil drug to minimize its retinal complications.

Further studies are recommended to assess the effect of tadalafil on optic nerve and also to test the role of kiwifruit extract.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

مستخلص فاكهة الكيوي يخفف من إصابة شبكية العين التي يسببها التادالافيل فى ذكور الجرذان البيضاء البالغة: دراسة هستولوجية وهستوكيميائية مناعية

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مقدمة: يعد عقار التادالافيل من الأدوية الشائعة الموصوفة لعلاج ضعف الانتصاب. و قد تم الإبلاغ عن العديد من الآثار الجانبية للعين المصاحبة للعلاج بالتادالافيل .إن فاكهة الكيوي هي فاكهة شهيرة لها مجموعة واسعة من الأنشطة الدوائية ودور مقترح في مكافحة المضاعفات الخطيرة للعين.

الهدف من العمل: تقييم الدور الوقائي المحتمل لمستخلص فاكهة الكيوي ضد إصابة شبكية العين الناجم عن التادالافيل فى ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: تم تقسيم ثلاثين من ذكور الجرذان البيضاء البالغة إلى أربع مجموعات ؛ المجموعة الضابطة ، المجموعة المعالجة بمستخلص فاكهة الكيوي(٥٠٠ مجم / كجم) ، المجموعة المعالجة بالتادالافيل (١,٨ مجم / كجم) ، والمجموعة المعالجة بكل من التادالافيل ومستخلص فاكهة الكيوي . تم إعطاء الحيوانات هذه الجرعات بالفم مرة واحدة يوميا لمدة ستة أسابيع. وقد تم تجهيز عينات من شبكية العين للفحص بالمجهر الضوئى والإلكتروني. كما تم إجراء دراسة هستوكيميائية مناعية باستخدام أجسام ضد. Bcl٢

النتائج: أظهرت عينات من الحيوانات المعالجة بعقارالتادالافيل انخفاضاً ذو دلالة إحصائيةً كبيرة في السمك الكلى للشبكية وعدد الخلايا العقدية. كما انفصلت الخلايا الشبكية الصبغية. ظهر الجزء الداخلي من مستقبِلات الضوء غير منتظم مع انقطاع غشاء الحد الخارجي. وقد لوحظت أنوية داكنة في الطبقات النووية الخارجية والداخلية وطبقة الخلايا العقدية. على مستوى التركيب الدقيق ، لوحظت أقراص غشائية غير منظمة مع زيادة فى المسافات بين الأقراص في الجزء الخارجي ، وفراغات بالسيتوبلازم وتورم بالميتوكوندريا في الجزء الداخلي من طبقة مستقبلات الضوء .كما لوحظت أجسام وأنوية خلايا الطبقات النووية الخارجية والداخلية وكذلك خلايا الطبقة العقدية منكمشة وغير منتظمة. وقد أظهرت الدراسة الهستوكيميائية المناعية انخفاضًا ذو دلالة إحصائية في التفاعل Bcl-٢. . في المقابل ، لوحظت تغييرات طفيفة في الجرذان التي عولجت بالتزامن بكل من عقار التادالافيل ومستخلص فاكهة الكيوي مع وجود انخفاض بدون دلالة إحصائية فى التفاعل المناعي.

الاستنتاج: عقار التادالافيل تسبب فى حدوث تغيرات تركيبية فى شبكية الجرذ الأبيض التي يمكن أن تتحسن بالعلاج المصاحب بمستخلص فاكهة الكيوي.