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Ameliorating Effect of Azilsartan on Cisplatin -Induced Ocular Toxicity in Male Rats

Naza Mohammed Ali Mahmood*,1 and Noor Majid Raheem Kareem[1]

[1] Department Pharmacology and Toxicology, College of Pharmacy, University of Sulaimani, Sulaimani,Iraq

Abstract

The aim of the present study is to evaluate the protective effect of Azilsartan against Cisplatin-induced ocular damage by ameliorating the oxidative stress and inflammation status. Since Azilsartan has a pleiotropic activity by selectively blocking angiotensin II type 1 receptor.

Forty-eight Wister-albino male-rats, weighing 270±30 g were used in this study. The duration of the treatment protocol was for 14 successive days. The rats were assigned randomly into six groups, as follows: Group 1 (Healthy control) was given 0.5 ml/day of 0.5% carboxymethylcellulose orally. Group 2 was given a single injection of 7 mg/kg Cisplatin intraperitoneally and then 0.5 ml/day of 0.5% carboxymethylcellulose. Group 3 was given 3.5mg/kg/day Azilsartan orally. Group 4 was given 7mg/kg/day Azilsartan orally. Group 5 was given 3.5mg/kg/day Azilsartan orally with a single injection of 7 mg/kg Cisplatin intraperitoneally. Group 6 was given 7mg/kg/day Azilsartan orally with a single injection of 7 mg/kg Cisplatin intraperitoneally. Biochemical analysis of malondialdehyde, superoxide dismutase, and interleukin-1β levels in the eye tissue was estimated, and histological examination of the eye was performed.

The co-therapy of Azilsartan at a low dose (3.5mg/kg) with Cisplatin showed a significant ($p<0.05$) reduction in interleukin-1β pro-inflammatory levels in comparison with Cisplatin only group, but the co-therapy of two different doses of Azilsartan with Cisplatin showed no significant ($p>0.05$) effect on malondialdehyde and superoxide dismutase levels in comparison with Cisplatin only group. Additionally, there was no significant ($p>0.05$) difference between the Azilsartan only groups and the healthy control group. Histologically, co-administration of two different doses of Azilsartan with Cisplatin showed a significant ($p<0.05$) reduction in inflammatory exudates, edema, and inflammatory cells infiltration in ocular tissue particularly with the Azilsartan low dose which is recognized by the lesion scoring system compared to the Cisplatin group.

Our data validate that Azilsartan has a favorable anti-inflammatory effect in Cisplatin-induced ocular toxicity in rat model in dose-dependent manner, by suppressing interleukin-1β overexpression and through potently blocking angiotensin II type 1 receptors in the eye.

Keywords: Azilsartan, IL-1β, MDA, SOD, Cisplatin, Ocular Toxicity

التأثير التحسني لأزيلسارتان على السمية العينية المستحثة بالسيسبلاتين في ذكور الجرذان

نازا محمد علي محمود *[١] و نور ماجد رحيم كريم [١]

[١] قسم الادوية والسموم ، كلية الصيدلة ، جامعة السليمانية ، السليمانية ، العراق

الخلاصة

الغرض من هذه الدراسة هو تقييم التأثير الوقائي للأزلسارتان ضد السمية العين الناجم عن السيسبلاتين عن طريق تخفيف الإجهاد التأكسدي وحالة الالتهابية. نظرًا لأن أزلسارتان له نشاط متعدد المسارات عن طريق الحجب الانتقائي لمستقبلات الأنجيوتنسين ٢ من النوع ١.

ثمانية وأربعون من ذكور الجرذان البيضاء، وزنها ٢٧٠ ± ٣٠ جم استخدمت في هذه الدراسة. مدة بروتوكول العلاج كانت ١٤ يوم متتالي. تم تقسيم الجرذان عشوائياً إلى ست مجموعات، على النحو التالي: المجموعة ١ (التحكم الصحي) أعطيت ٠,٥ مل/يوم من ٠,٥٪ كربوكسيل ميثيل سلولوز عن طريق الفم. المجموعة ٢ أعطيت حقنة واحدة من ٧ ملجم / كجم سيسبلاتين داخل الصفاق ثم ٠,٥ مل/ يوم من ٠,٥٪ كربوكسيل ميثيل سلولوز. المجموعة ٣ أعطيت ٣,٥ ملجم / كجم/ يوم أزيلسارتان عن طريق الفم. المجموعة ٤ أعطيت ٧ ملجم / كجم/ يوم أزيلسارتان عن طريق الفم. المجموعة ٥ أعطيت ٣,٥ مجم / كجم/ يوم أزيلسارتان عن طريق الفم مع حقنة واحدة من ٧ مجم / كجم سيسبلاتين داخل الصفاق. المجموعة 6 أعطيت 7مجم /كجم/ يوم أزيلسارتان عن طريق الفم مع حقنة واحدة من ٧ مجم / كجم سيسبلاتين داخل الصفاق. تم تقدير التحليل الكيميائي الحيوي لمستويات المالون ثنائي الالدهايد وسوبر أوكسيد دسميوتيس والأنترليوكين ١ بيتا في أنسجة العين، كما تم إجراء الفحص النسيجي للعين. أظهر العلاج المشترك لأزيلسارتان بجرعة منخفضة (٣,٥ مجم / كجم) مع سيسبلاتين انخفاضًا احصائيا في مستويات الإنترليوكين ١-بيتا، لكن لم يكن هنالك تأثير احصائي على مستويات المالون ثنائي الالدهايد وسوبر أوكسيد دسميوتيس في انسجة العين بالمقارنة مع المجموعة السيسبلاتين فقط. بالإضافة لم يكن هنالك اختلاف أحصائي بين المجموعات اللاتي أعطيت أزيلسارتان فقط بجرعات مختلفة بالمقارنة مع مجموعة التحكم الصحي في مستويات التحليلات الكيميائية لمستويات المالون ثنائي الالدهايد وسوبر أوكسيد دسميوتيس والأنترليوكين ١ بيتا. اظهرت النتائج النسيجية انخفاضا كبيرا في الافرازات الالتهابية والوذمة والتسرب الخلايا الالتهابية في مجموعات العلاج المشترك لأزيلسارتان مقارنة مع مجموعة سيسبلاتين فقط. تؤكد بياناتنا ان أزيلسارتان له تأثير مضاد الالتهاب في نموذج الجرذان لسمية السيسبلاتين على العين بطريقة تعتمد على الجرعة، عن طريق قمع زيادة الإنتاج انترليوكين بيتا-١ ومن خلال منع مستقبلات الأنجيوتنسين الثاني من النوع الأول بشكل فعال في العين.

الكلمات المفتاحية : أزلسارتان ، والأنترليوكين ١ بيتا، MDA ، SOD ، السيسبلاتين ، السمية العين

[1]Corresponding author E-mail: naza.ali@univsul.edu.iq
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Chemotherapeutic drugs may result in a br oad range of ocular toxicities due to the unique anatomical, physiological, and biochemical charact eristics of the eye. If the symptoms are not detected early, these toxicities may become permanent [1]. Cisplatin is a chemotherapeutic alkylating drug that is very effective in the treatment of a variety of malignant tumors, including those of the head/neck, lung, bladder, cervical, ovarian, testicular, and gastrointestinal system [2,3]. However, Cisplatin has limited use in clinical practice due to various deleterious side effects. The use of high-dose cisplatin treatment regimens causes acute kidney injury, persistent diarrhea, neurological disorders, hearing loss, and vision loss, which become significant obstacles to cisplatin therapy [4,5]. Cisplatin-associated eye toxicity is linked to multiple mechanisms, including oxidative stress, DNA adducts, inflammation, mitochondrial dysfunction, and direct cytotoxicity to the retina and optic nerve [6]. Cisplatin causes an increase in the production of reactive oxygen species and depletion of the intrinsic antioxidant system, which results in cell membrane lipid peroxidation, activation of the p38 Mitogen-activated protein kinases (MAPKs) signaling pathway, and dysfunction of the DNA repair mechanism [7]. Accordingly, several studies were conducted to overcome Cisplatin ocular toxicity by using antioxidants such as selenium and resveratrol to minimize the oxidative stress and protect the eye from the toxic effects of Cisplatin [8,9].

The positive involvement of the angiotensin receptor blockers (ARBs) in ameliorating the diseases that are associated with the eye has been studied due to the local availability of angiotensin II type 1 (AT1) receptors in the ocular tissues [10,11]. Angiotensin receptor blockers have been investigated for their potential to reduce intraocular pressure and provide neuroprotection in the treatment of glaucoma [12]. Azilsartan is the latest approved angiotensin receptor blocker for the management of hypertension. It is a potent and highly selective antagonist to angiotensin II type 1 (AT1) receptor that tightly binds to and dissociates slowly from AT1 receptor than other angiotensin receptor blockers (ARBs) [13]. Furthermore, Azilsartan possesses a pleiotropic effect with anti-inflammatory and antioxidant activity [14]. It is believed that Azilsartan exerts its protective effect through diminishing the pathogenesis of the angiotensin converting enzyme (ACE)/ angiotensin (Ang) II/ angiotensin II type 1 (AT1) receptor axis and upregulating the activity of angiotensin converting enzyme 2 (ACE2)/ angiotensin (Ang1-7)/mas receptor axis [15,16]. This is the first study to look at the protective effect of Azilsartan in Cisplatin-induced ocular toxicity in terms of oxidative stress and inflammation. Moreover, an enzyme linked immunosorbent assay (ELISA) was carried out in this research to measure malondialdehyde (MDA), interleukin-1β (IL-1β), and superoxide dismutase (SOD) levels in the homogenates of eye tissue. Furthermore, an eye histological investigation was also carried out.

Materials and Methods

Drugs and chemicals

Cisplatin injection (50 mg/100 ml) was obtained from Kocak Pharmaceuticals /Turkey and Azilsartan Medoxomil powder was obtained from Apollo Pharmaceuticals/Malaysia. Azilsartan Medoxomil was prepared in a 0.5% carboxymethylcellulose (CMC) suspension. Phosphate buffer saline (PBS; pH 7.4) was obtained from DNA biotech Co./ Ireland. The doses of Cisplatin and Azilsartan that were used in this study were utilized based on previous researches [8,17,18].

Animals and experimental design

Forty-eight Wister-albino male-rats, weighing 270±30 g, were obtained from the University of Tikrit /College of Veterinary. They were housed in University of Sulaimani / College of Pharmacy, were kept in standard conditions (24 °C, 45% humidity, and a 12 h light/dark cycle) and acclimatized for seven days with standard chew and tap water. The treatment protocol for this study was for 14 successive days.

The rats were allocated randomly into six groups (n=8 rats per group), as follows:

- Group 1 (Healthy control) was given 0.5 ml of 0.5% CMC orally [19].
- Group 2 was given a single injection of 7mg/kg Cisplatin intraperitoneally and then 0.5ml/day of 0.5% CMC orally [20].
- Group 3 was given 3.5mg/kg/day Azilsartan orally [21].
- Group 4 was given 7mg/kg/day Azilsartan orally [21].
- Group 5 was given 3.5mg/kg/day Azilsartan orally with a single injection of 7mg/kg Cisplatin intraperitoneally.
- Group 6 was given 7mg/kg/day Azilsartan orally with a single injection of 7mg/kg Cisplatin intraperitoneally.

The present study was approved by the Ethical Committee on Animal Research of University of Sulaimani, College of Pharmacy (Certificate no. PH35-21 on 14th November 2021) and carried out in accordance with the Guidelines for Animal Research of the National Institutes of Health. All efforts were made to minimize the suffering of the animals.

Sample collection

The animals were sacrificed on day 15 after being fasting for 24 hours with free access to water, and the eye samples were processed as follow:

- The right eyes were enucleated, washed with ice-cold phosphate buffered saline (PBS) then dried and weighed using electronic balance. As such, the

whole eye dissected by adding 9-fold ice-cold PBS per g of the eye used in preparing tissue homogenate. The ocular tissue homogenate was made by homogenization of eyes in PBS and centrifugation at 5000 rpm for 20 min at 4°C, and then the supernatant was frozen at -80°C for further biochemical investigation [22].

- The left eyes were fixed in an accepted volume of neutral buffered formalin 10% solution for further histological investigation.

Biochemical parameters

The MDA, IL-1β, and SOD levels in the eye tissue homogenates of all experimental groups were estimated according to the manufacturer's procedure by utilizing commercial ELISA kits (Bioassay technology, UK).

Histotechnique procedure

The left eyes were collected and fixed in neutral buffered formalin 10% solution for 48-72 hours [23]. Next, the eyes were dissected at the horizontal meridian line through the optic nerve and embedded in paraffin blocks. Further Routine paraffin wax embedding procedures were used for histopathologic evaluation, and in short, the tissue samples were fixed in 10% neutral buffered formalin, alcohol dehydrated, and paraffinized. Thereafter, Paraffin embedded specimens were cut into 5 µm thick sections, mounted on slides and stained with Hematoxylin-Eosin (H-E).

Semi-quantitative histopathological evaluation

In general, as a semi-quantitative morphometric measure, the ocular tissues were examined using light microscope image analyzer under a power of 100X magnifications. The whole histological architecture of the eye, such as the cornea, sclera, iris, ciliary body, and retina, as well as the optic nerve sections, were investigated histopathologically for the existence of numerous abnormalities; congested blood vessels, edema, and inflammatory exudates. Also, exploring the degeneration, vacuolation, and hemorrhage as well as the proliferating capillaries within glial cells (gliosis) and associated ischemic necrosis of the optic nerve. The ocular tissue was evaluated and measured in µm and statistically calculated as mean percentage. Whereas, inflammatory cells together with ocular degenerative cells (Cellular swelling) were counted in a total of ten fields randomly chosen under high power magnification (1000X), then the mean average was calculated statistically in percentage. Overall, tissue samples were analyzed under the light microscope (Olympus BX51, Japan) using an image analyzer (Am Scope 3.7, for digital camera, MU300, 2019). Finally, the morphometric semi-quantitative mean percentage calculation was estimated using the following lesion score-grade system (score 0-10% as no lesions; score 10-25% as mild; score 25-50% as moderate; score 50-75% as severe; and score 75-100% as critical lesions). Overall, the eye sections were analyzed under the light microscope (Olympus BX51, Japan) using an image analyzer (Am scope 3.7, for digital camera, MU300, 2019).

Statistical analysis

The statistical analysis was accomplished using GraphPad Prism 8. The values of the measured parameters were expressed as mean ±standard deviation (std) One-way analysis of variance (ANOVA) was used for the comparisons between different groups, followed by Tukey multiple comparison tests. Unpaired t-tests were used to compare each group with the Cisplatin group. The results were considered statistically significant when the p-value was less than 0.05.

Results

The effect of two different doses of azilsartan on the mda MDA levels in the ocular tissue

Table 1 showed a significant reduction in the MDA levels in the ocular tissue of the healthy control group compared to the Cisplatin group (group 1 vs group2; p-value=0.0271). Similarly, the MDA levels were significantly lowered in the group of rats that received only a low dose of Azilsartan compared to the Cisplatin group (group 3 vs group 2; p-value=0.0271). While, the MDA levels showed a non-significant change in the group of rats that received only a high dose of the Azilsartan compared to the Cisplatin group (group 4 vs group 2; p-value=0.179).

In addition, there was a non-significant reduction in the MDA levels in the combination groups compared to the Cisplatin group [(group 5 vs group 2; p-value=0.438), (group 6 vs group 2; p-value=0.774), respectively]. Furthermore, there was a non-significant difference in the MDA levels between the groups that received only a low and a high dose of the Azilsartan (group 3 vs group 4; p-value=0.9554). Moreover, the MDA levels showed a non-significant change between the combination groups that received two different doses of the Azilsartan and a single injection of the Cisplatin (group 5 vs group 6; p-value=0.9944), as shown in Figure 1.

The effect of two different doses of azilsartan on the antioxidant sod enzyme activity in ocular tissue

Table 1 showed a non-significant difference in the SOD levels among the experimental groups. There was a non-significant elevation in the SOD levels in the healthy control group compared to the Cisplatin group (group1 vs group 2; p-value=0.7156). Additionally, the SOD levels were insignificantly different in the groups of rats that received only two different doses of the Azilsartan compared to the Cisplatin group [(group 3 vs group 2; p-value=0.1728), (group 4 vs group 2; p-value=0.2945), respectively]. Likewise, the SOD levels showed a non-significant change in the combination groups compared to the Cisplatin group

[(group 5 vs group 2; p-value=0.9966), (group 6 vs group 2; p-value=0.9995), respectively].
Furthermore, the SOD levels showed a non-significant alteration between the groups of rats that received only two different doses of the Azilsartan (group 3 vs group 4; p-value=0.9996). Besides, the SOD levels showed a non-significant difference between the groups that received a combination of treatments (group 5 vs group 6; p-value=0.9946), as shown in Figure 2.

The effect of two different doses of azilsartan on the pro-inflammatory IL-1β levels in the ocular tissue

Table 1 showed a significant decreased in the IL-1β Levels in the healthy control group compared to the Cisplatin group (group 1 vs group 2; p-value=0.001). Also, the IL-1β Levels were significantly lowered in the group of rats that received only a low dose of the Azilsartan compared to the Cisplatin group (group 3 vs group 2; p-value=0.009). Similarly, the IL-1β levels showed a significant reduction in the group of rats that received only a high dose of the Azilsartan compared to the Cisplatin group (group 4 vs group 2; p-value=0.0244). Furthermore, the IL-1β levels were significantly reduced in the combination group of the low dose of the Azilsartan with the Cisplatin compared to the group of rats that received only Cisplatin injection (group 5 vs group 2; p-value=0.0427). However, the IL-1β levels showed a non-significant reduction in the combination group that received a high dose of the Azilsartan with the Cisplatin compared to the group of rats that received Cisplatin injection (group 6 vs group 2; p-value=0.5185). Furthermore, the IL-1β levels showed a non-significant difference between the groups of rats that received only different doses of the Azilsartan (group 3 vs group 4; p-value=0.9959). Moreover, there was a non-significant difference in the IL-1β levels between the combination groups that received two different doses of the Azilsartan with the Cisplatin injection (group 5 vs group 6; p-value=0.6613), as shown in Figure 3.

Table 1. Shows the effect of two different doses of the Azilsartan on the MDA, SOD, and IL-1β levels in the ocular tissue.

Variables	G1 Healthy control	G2 7mg/kg CIS	G3 3.5mg/kg Azil	G4 7mg/kg Azil	G5 3.5mg/kg Azil+ 7mg/kg CIS	G6 7mg/kg Azil+ 7mg/kg CIS
MDA (nmol/ml)	2.20 ±0.28 **a**	2.82 ±0.13**b**	2.20 ±0.28 **a**	2.36 ±0.27 **a, b**	2.46 ±0.32 **a, b**	2.56 ±0.42 **a, b**
SOD (ng/ml)	3.64 ±0.54**a**	3.10 ±0.48**a**	4.04 ±0.29 **a**	3.92 ±0.97**a**	3.42 ±0.54 **a**	3.22 ±0.54 **a**
IL-1β (ng/ml)	11.46 ±1.43 **a**	16.60 ±1.96 **b**	13.04 ±0.80 **a**	13.40 ±0.59 **a**	13.62 ±1.21**a**	14.98 ±2.13 **a, b**

The values of the biomarkers of six experimental groups (n=8 rats) are expressed as mean± standard deviation. Mean values with different letters (a, b) among the groups represent a significant difference at (P < 0.05), while identical letters among the groups represent a non-significant difference at (p>0.05). MDA: malondialdehyde; SOD: super oxide dismutase; IL-1β: interleukin-1beta; G: Group, CIS: Cisplatin; Azil: Azilsartan.

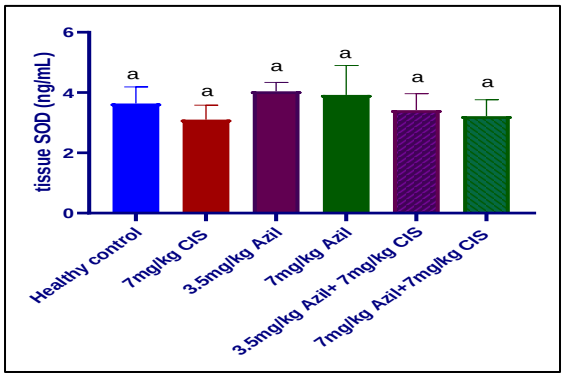

Figure 1. Shows the effect of two different doses of Azilsartan on the MDA levels in the ocular tissue. The values of six experimental groups (n=8 rats) are expressed as mean± standard deviation. The mean values with different letters (a, b) among the groups represent a significant difference at (P < 0.05), while identical letters among the groups represent a non-significant difference at (p>0.05).

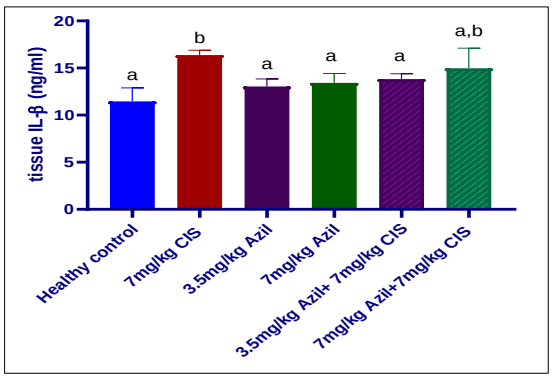

Figure 2. Shows the effect of two different doses of Azilsartan on the SOD levels in the ocular tissue. The values of six experimental groups (n=8 rats) are expressed as mean± standard deviation. The mean value with identical letter (a) among the groups represents a non-significant difference at (p>0.05).

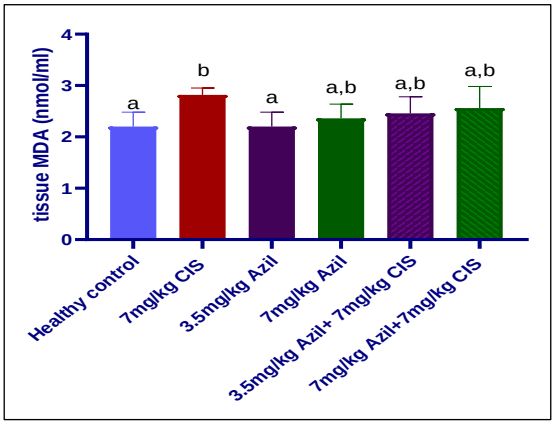

Figure 3. Shows the effect of two different doses of Azilsartan on the IL-1β levels in the ocular tissue. The values of six experimental groups (n=8 rats) are expressed as mean± standard deviation. The mean values with different letters (a, b) among the groups represent a significant difference at (P < 0.05), while identical letters among the groups represent a non-significant difference at (p>0.05).

Histopathology Findings

Table 2 showed histopathological morphometric semi-quantitative evaluation and lesion scoring for ocular tissue from different treatment groups. Animals were sacrificed and eye samples were collected for histopathology at the experiment end point. Microscopic structures of the eye in healthy control group revealed well organized histological layers and intact features of all tunics; fibrous tunic including cornea and sclera with vascularized tunic such as; iris, ciliary body, and process, also neural tunic-like retina, with the optic nerve (Figure 4 a, b). The microscopic section of sclera showed tough fibrous connective tissue layers that compose of a thin, loose collagenous connective tissue and the stroma is a thick layer of dense collagenous connective tissue made of intertwining collagen fibers alternating with networks of elastic fibers, also limbus had a normal structure (sclera-corneal junction) in **G1**(Figure 4 d, e). **G1** showed a normal histological arrangement and cell distribution of retina tissue, ganglionic cell layer, normal glial cellularity, and astrocytes in the optic nerve (Figure 5a, b, and c). Histopathologically, 7mg/kg Cisplatin administration led to a severe-critical disruption in the eye morphology and layer organization. In **G2** (Figure 6 a,b, and c) marked degeneration of lens capsule with sloughing of lining epithelium was seen, also accumulation of water in the lens (cataract or lens opacity) in comparison to **G1**. Cornea histological alterations were very severe in **G2** vs. **G1** and showed proliferation in the stromal layer with sloughing of lining epithelium and endothelium, with marked infiltration of the neutrophil inflammatory cell as seen in figure 7 c. The marked proliferation of ciliary process, vascular congestion, and infiltration of inflammatory cells in **G2** (Figure 7 a, b) in comparison to **G1**. Cytoplasmic vacuolation of episcleral epithelium, thickening of sclera with a significant increment of inflammatory exudates and cells, together with severe vascular congestion and critical degenerative changes within the retinal photoreceptor cells were seen in **G2** as shown (Figure 8 a). Also marked ischemic optic nerves had a lot of inflammatory cells in the form of cell clusters, multi vascular congestion with a critical reduction in glial cells, As shown in Figure 8 (b, c, and d). Histological structure of the eye in **G3** revealed the normal arrangement of each eye tissue with normal cell distribution. In general, sample scoring in **G3** and **G4** respectively, show mild to no significant morphological changes in comparison to **G1** group, in which the lesions are much milder in **G3** than **G4**, as shown in table 2. In **G3**, there were well-organized histological layers structures of the lens (inset), cornea, eye chambers, and iris (Figure 9 a, b, and c), as well as Intact features of sclera with ciliary body and process (Figure 9 d, e). Also, the retina revealed normal cellularity and mild vascular congestion in optic nerves of **G3** (Figure 10 a, b). Similarly, the histological structure in **G4** showed an intact histological layer of the lens (inset), cornea, eye chambers, and iris (Figure 11 a, b, and c). **G4** shows a normal morphological appearance of sclera with mild congestion of ciliary process and iris (Figure 11 d, e). **G4** showed (Figure 12 a, b) mild degeneration of photoreceptor cells, and exhibited a normal distribution of cells in retinal layers, as well as presented a mild vascular congestion of optic nerves with a mild reduction in glial cell number vs **G1**. Likewise, Microscopic evaluation of the eye in **G5** revealed a mild-moderate disruption in eye layers arrangement particularly the histological structures of lens layers and iris (Figure 13 a, b), such group also showed thinning of a capsule with moderate degeneration of capsular epithelium, and vascular congestion of iris while moderate degeneration and vacuolation of a stromal layer of the cornea were seen in (Figure 13 a, b, and c). Also, **G5** showed a proliferation of ciliary processes with mild vascular congestion as well as mild thinning and degeneration of sclera (Figure 13 d, e). Histologically, the retina in **G5** revealed a mild vacuolation of the photoreceptor cells, mild gliosis, and mild degeneration of ganglionic cells (Figure 14 a). There was thinning and hemorrhage of optic nerves with a mild reduction in glial cells in **G5** (Figure 14 b, c, and d). Whereas histological examination of **G6** revealed a severe lesion in fibrous and vascularized tunic structures. Additionally, there were a thickening of ciliary process, moderate vascular congestion with severe thinning and degeneration of sclera in **G6** (Figure 15 a, b, and c), as well as a moderate-severe vascular congestion in the ciliary process (Figure 15 c-e) moderate-severe proliferation and congestion of

ciliary process (Figure 15 d). The retina of **G6** revealed moderate degeneration of photoreceptor cells, moderate gliosis, and severe degeneration of ganglionic cells (Figure 16 a). Moreover, there was a moderate ischemic optic nerve in **G6** which had inflammatory cells in the form of cell clusters, vascular congestion with a moderate reduction in glial cells, as shown in (Figure 16 b, c). Moreover, histopathological evaluation showed a significant reduction in lesion severity as well as scoring system in **G5** and **G6** in comparison to **G2** for 14 days, in which it was much more effective in **G5** in comparison to **G6**, wherein the lesion intensity been reduced from marked-critical in **G2** to moderate-severe in **G6** then after to a mild-moderate in **G5**, as demonstrated in table 1. Prophylactic trials with Azilsartan showed significant anti-inflammatory efficacy in reducing morphometric inflammatory biomarkers, in which it was much more effective in a lower dose regimen pilot.

Table 2. Histological quantitative evaluation of eye lesions with different treatment values

Experimental Groups N=8	Edema* (Mean%) **	Inflammatory Exudates* (Mean%) **	Inflammatory Cells Infiltration* (Mean %) **	Vascular Congestion* (Mean %) **	Cellular Swelling* (Mean %) **	Lesion Scoring (0 - 100%)	Lesion Grading
G1(HC†)	7.92 % ^{A}	4.87 % ^{A}	5.26 % ^{A}	8.71 % ^{A}	5.65 % ^{A}	0-10 %	No lesions
G2(7mg CIS)	87.32 % ^{E}	79.54 % ^{E}	76.39 % ^{E}	86.21 % ^{E}	92.75 % ^{E}	75-100 %	Marked-Critical
G3 (3.5mg Azil)	12.48 % ^{B}	11.28 % ^{B}	9.89 % ^{A}	16.32 % ^{B}	11.42 % ^{B}	10-25 %	Mild
G4(7mg Azil)	14.62 % ^{B}	12.83 % ^{B}	13.34 % ^{B}	18.74 % ^{B}	15.96 % ^{B}	10-25 %	Mild
G5(3.5mg Azil+7mg CIS)	57.91 % ^{D}	47.83 % ^{C}	49.32 % ^{C}	49.67 % ^{C}	50.46 % ^{D}	25-50 %	Mild-Moderate
G6(7mg Azil+7 mg CIS)	74.33 % ^{D}	68.74 % ^{D}	71.52 % ^{D}	59.86 % ^{D}	65.24 % ^{D}	50-75 %	Moderate-Severe

Notes: *Area of edema, Inflammatory exudates, vascular congestion were estimated in (µm), inflammatory cells were calculated as mean percentage from different fields in ocular tissue and optic nerve. **Each value represents mean percentage of (n=8). Statistical comparison among groups: Mean values with different capital letters (A, B, C, D, E) have significant differences at ($P < 0.05$). †: **Hc:** healthy control, **Azil:** Azilsartan, **CIS:** Cisplatin, **G:** Group.

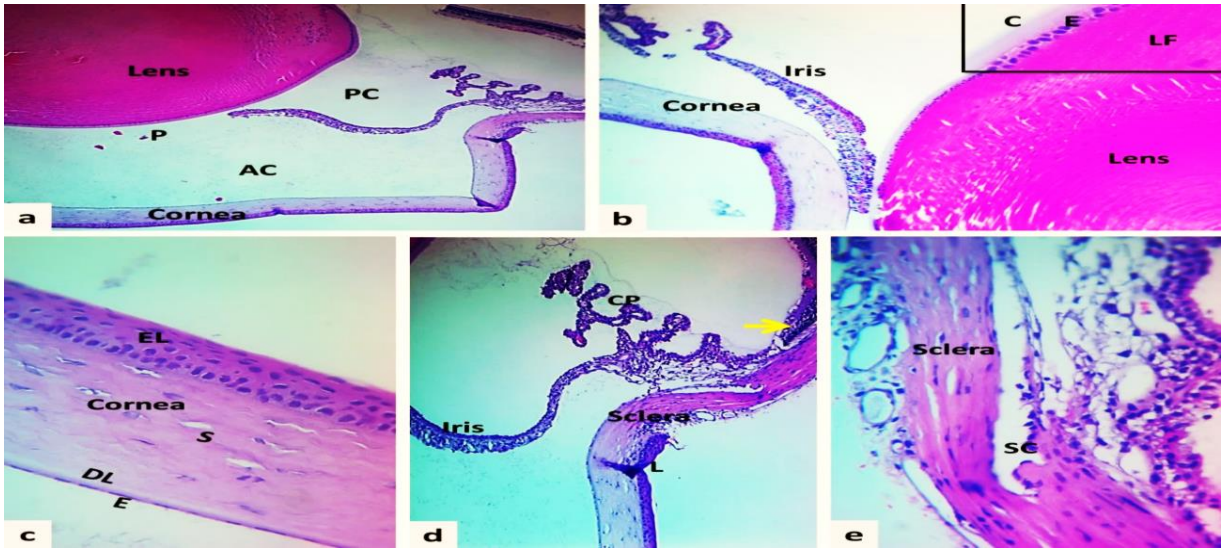

Figure 4. Microscopic section of normal eye histological appearance in healthy control group (G1). a and b: Normal tissue sections of the cornea, lens, pupil (P), iris, anterior and posterior chamber (AC and PC), the inset section showed structures of a lens; capsule (C), the simple cuboidal subcapsular epithelium (E), and LF which is lens fibers, (H &E stain, 100X, 200X). c: Intact corneal morphology; Epithelium layer (EL), Bowman's layer or membrane (BM), Stroma (S), Descemet's layer (DL), and Endothelium (E), (H &E stain, 400X). d and e: Normal histological organization of fibrous tunic (cornea and sclera), and intact vascular tunic (iris, ciliary body indicated by yellow arrow, and process (CP)), limbus (L), and Schlemm canal (SC), (H &E stain, 100X, 400X).

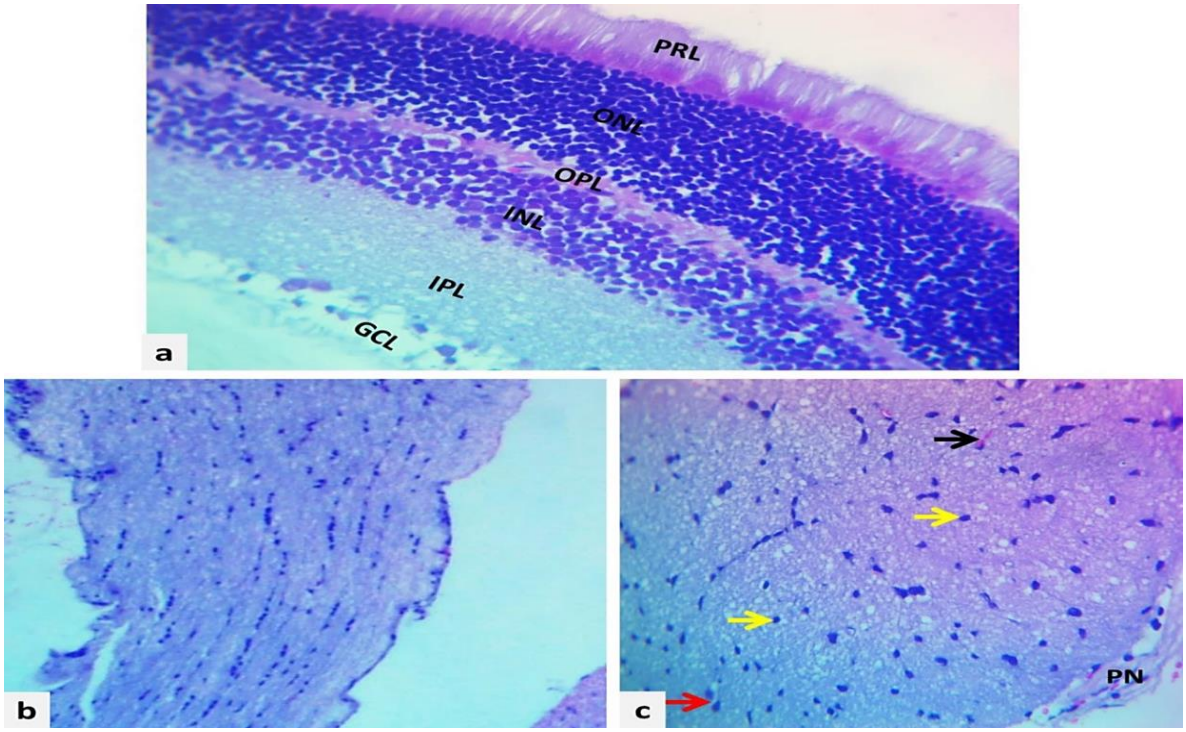

Figure 5. Microscopic section of normal eye histological appearance in healthy control group (G1). a: Normal histological arrangement and cell distribution of retina tissue, ganglionic cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and photoreceptor layer (PRL), (H &E stain, 400X). b and c: Well-arranged nerve fiber, normal glial cellularity; Oligodendrocytes (yellow arrows), astrocytes (red arrows), vascular structures (black arrow), and perineurium as indicated by PN, (H &E stain, 400X).

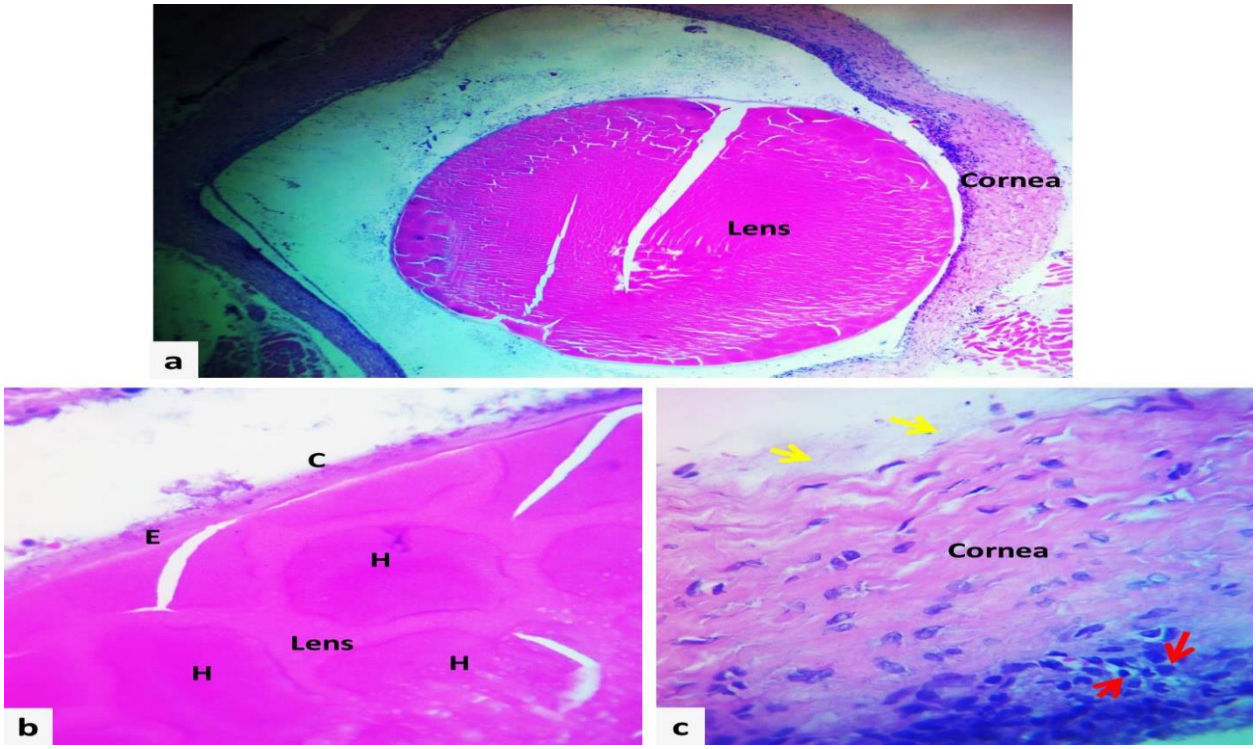

Figure 6. Microscopic section of an eye in the 7mg/kg Cisplatin group (G2). a and b: Disturbed eye morphology, degeneration of lens capsule (C) and sloughing of the epithelium (E), accumulation of water in the lens (H), (H &E stain, 100X and 400X). c: Thickening of the cornea with sloughing of the epithelium (yellow arrows), infiltration of an inflammatory cell as indicated by red arrows, (H &E stain, 400X).

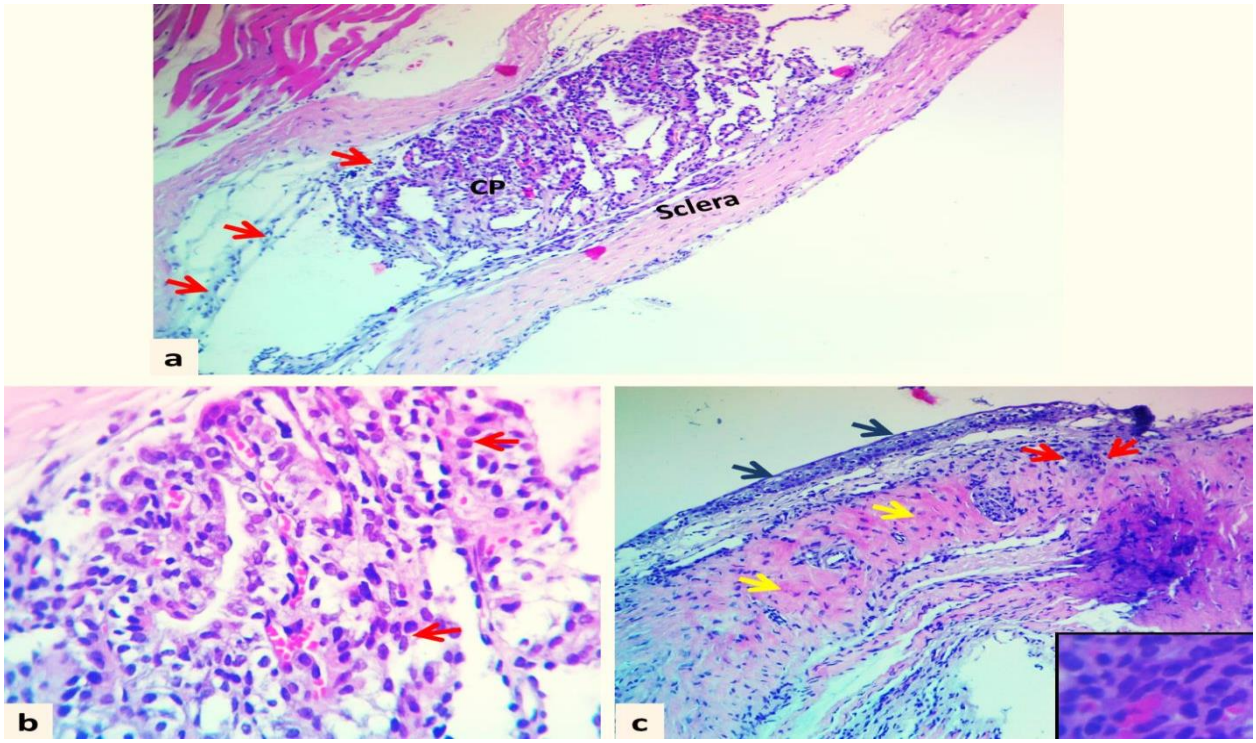

Figure 7. Microscopic section of an eye in the 7mg/kg Cisplatin group (G2). a-c: Thickening or proliferation of ciliary process with vascular congestion and inflammatory reaction (red arrows). Thickening of the sclera (yellow arrows) with infiltration of inflammatory cells (red arrows and inset), cytoplasmic vacuolation of the scleral epithelium (black arrows), (H &E stain, 100X and 400X).

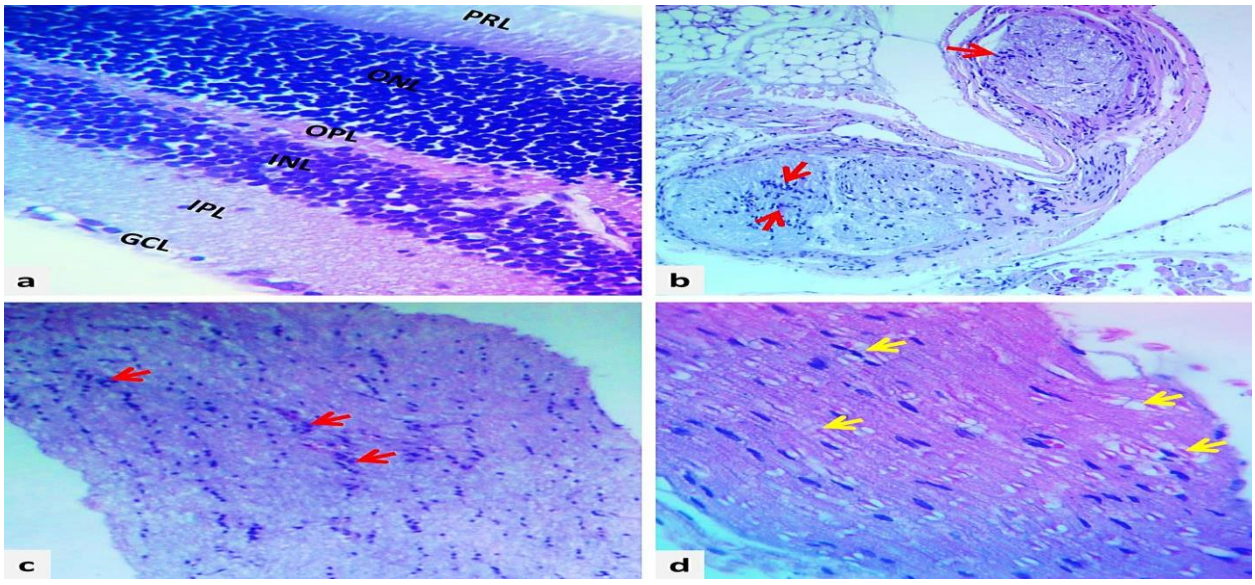

Figure 8. Microscopic section of an eye in the 7mg/kg Cisplatin group (G2). a: The retina revealed severe degeneration of the photoreceptor layer (PRL), marked gliosis in the inner and outer nuclear layer (INL and ONL), (H &E stain, 400X). b-d: marked ischemic optic nerves had a lot of inflammatory cells in the form of cell clusters (red arrows), multi vascular congestion (yellow arrows) with a moderate-marked reduction in the glial cells, (H &E stain, 100X and 400X).

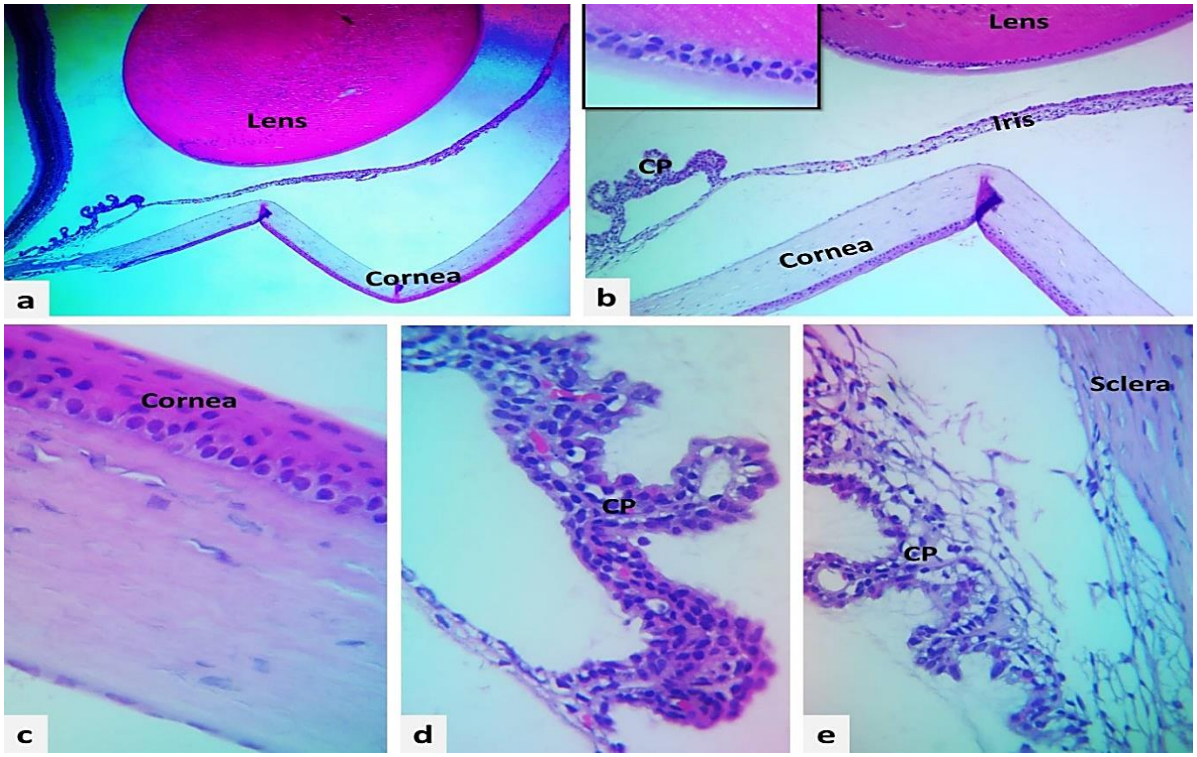

Figure 9. Microscopic section of an eye in 3.5 mg /kg Azil group (G3). a-c: Well-organized histological layers structures of the lens (inset), cornea, eye chambers, and iris, (H &E stain, 100X, 200X, and 400X). d and e: Intact features of sclera with ciliary body and process, (H &E stain, 400X).

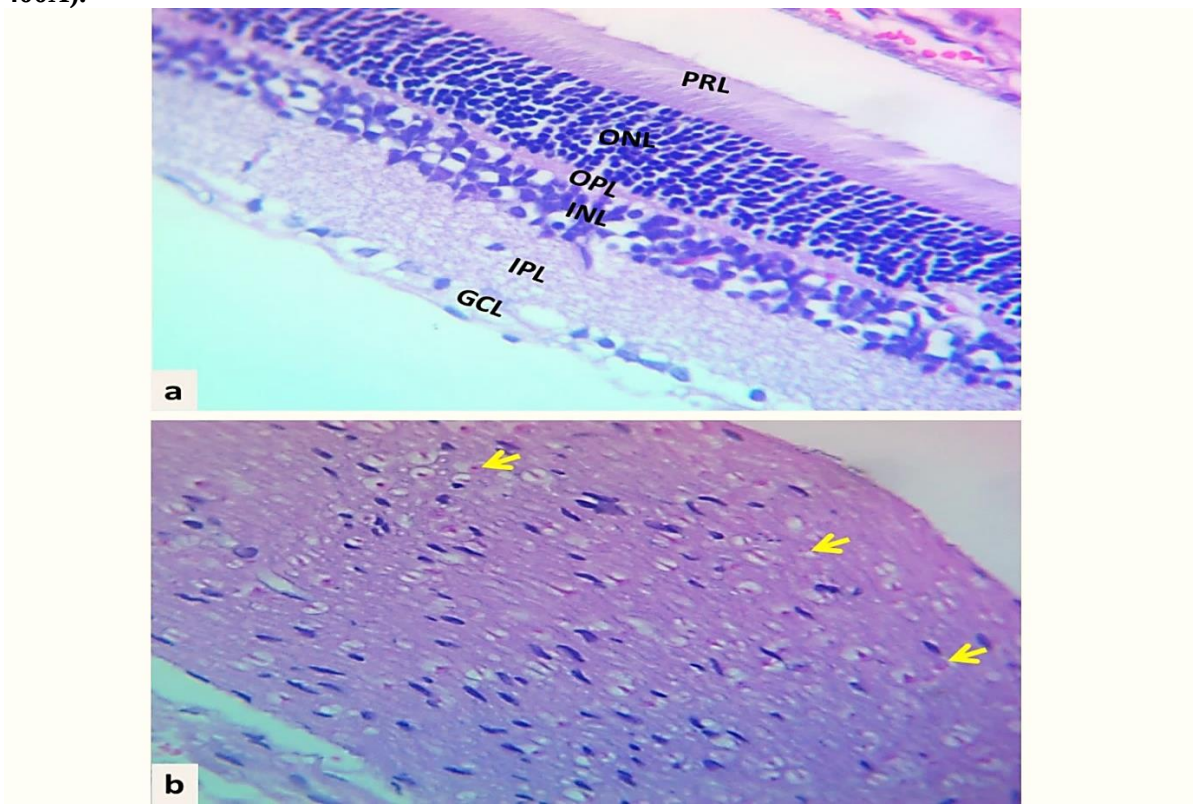

Figure 10. Microscopic section of the eye in 3.5mg/kg Azil group (G3). a: The retina revealed normal cellularity, (H &E stain, 400X). b: Mild vascular congestion (yellow arrows) in optic nerves, (H &E stain, 400X).

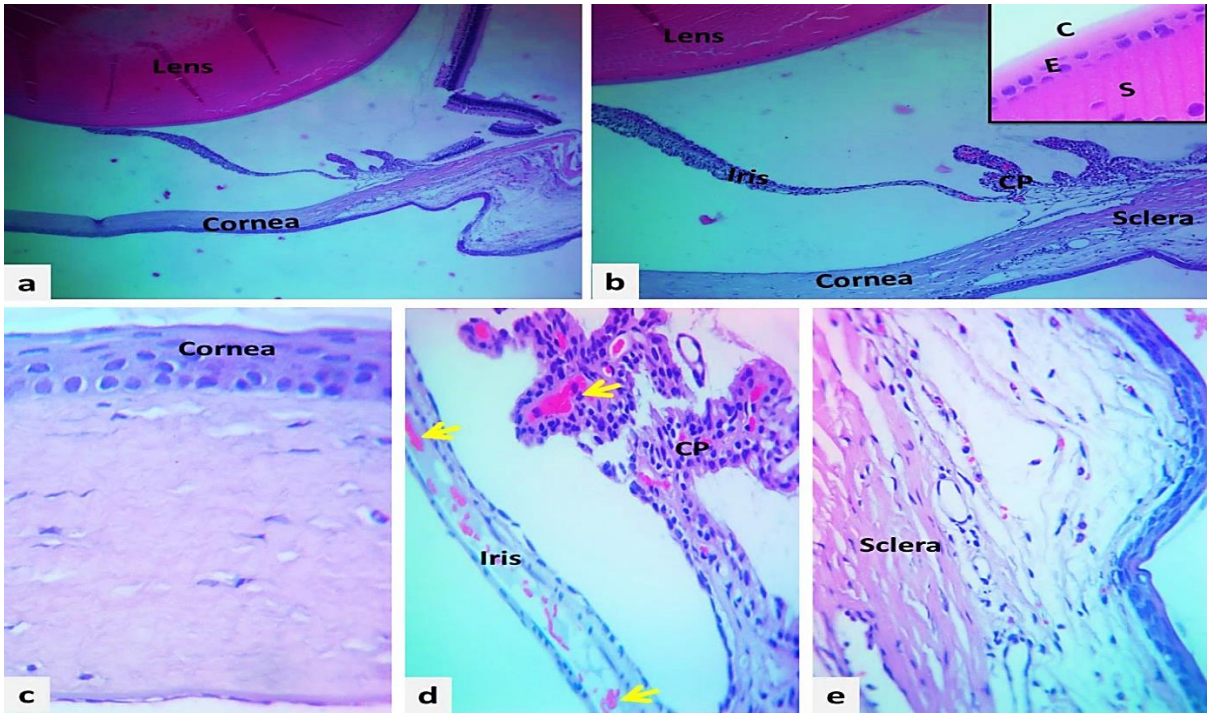

Figure 11. Microscopic section of the eye in 7 mg/kg of Azil group (G4). a-c: Intact histological layers of the lens (inset), cornea, eye chambers, and iris, (H &E stain, 100X, 200X, and 400X). d and e: Normal morphological appearance of sclera with mild congestion of ciliary process and iris as indicated by yellow arrows, (H &E stain, 400X).

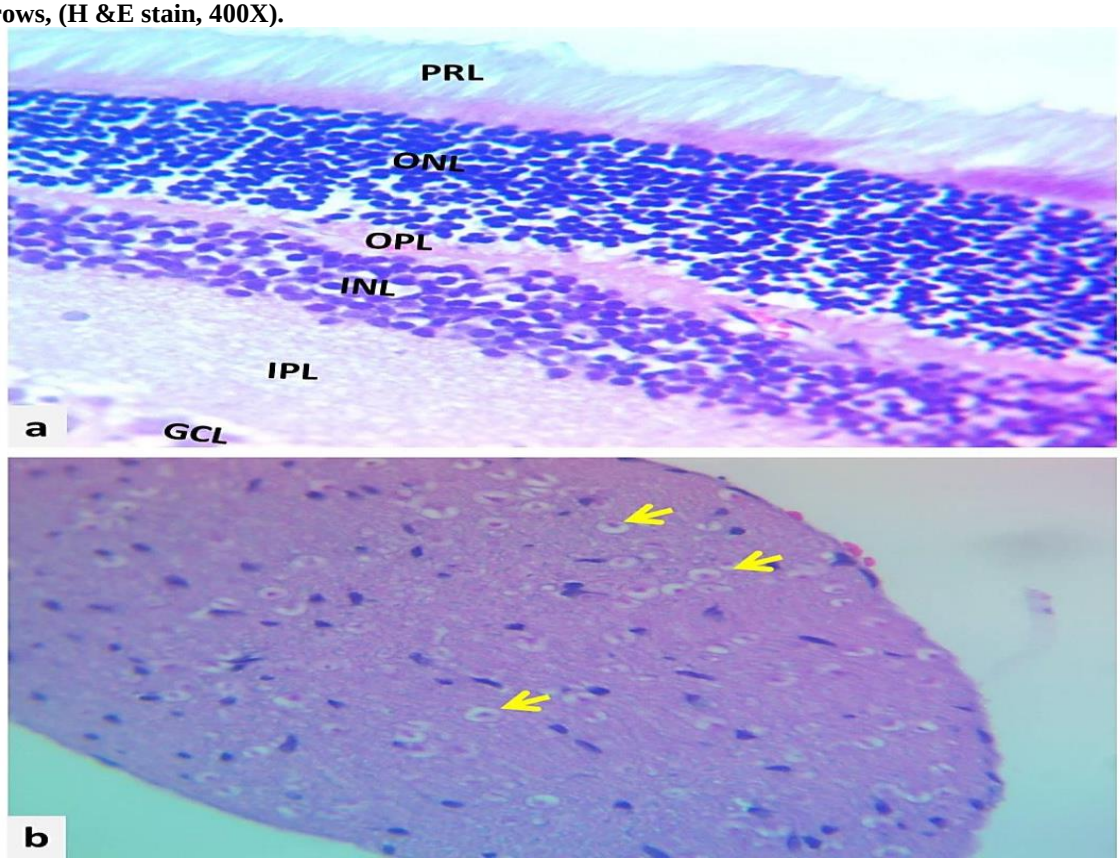

Figure 12. Microscopic section of the eye in 7mg/kg Azil group (G4). a: Mild degeneration of photoreceptor cells, normal distribution of cells in other layers, (H &E stain, 400X). b: Mild vascular congestion (yellow arrows) of optic nerves with a mild reduction in glial cell number as indicated by yellow arrows, (H &E stain, 400X).

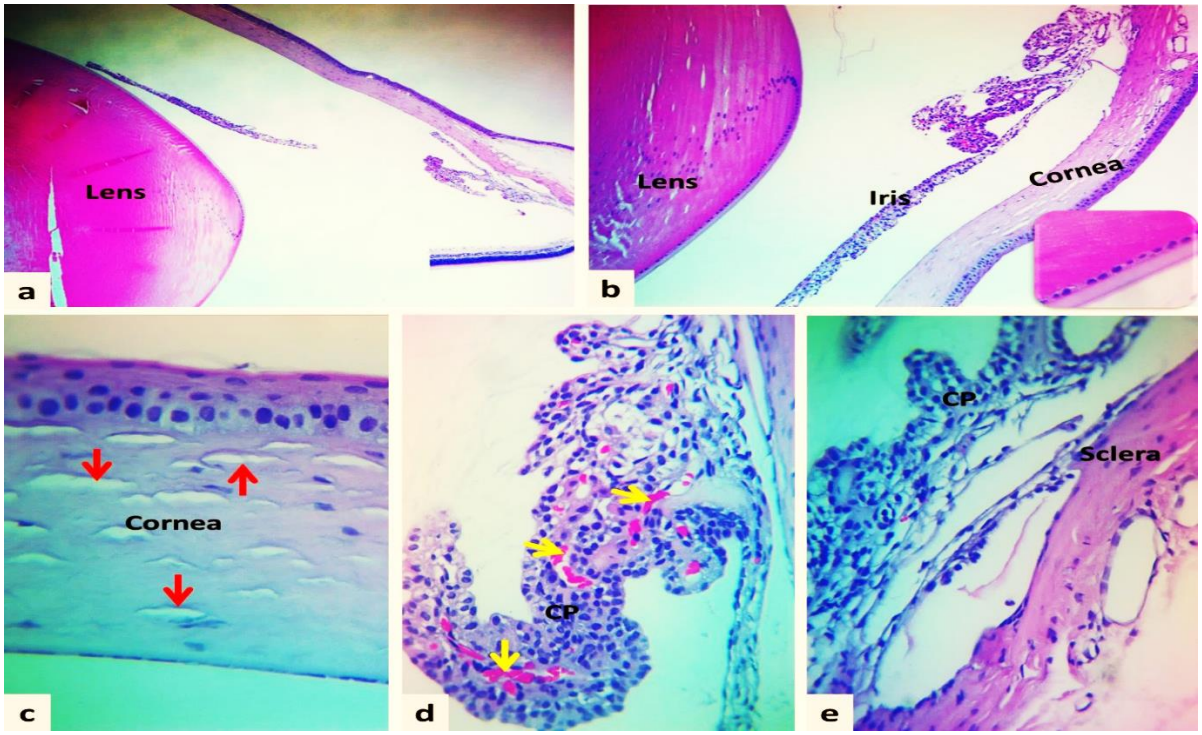

Figure 13. Microscopic section of the eye in 3.5 mg/kg of Azil + CIS group (G5). a and b: Normal histological structures of lens layers (inset) and iris, (H &E stain, 100X and 200X). c: Mild-moderate degeneration and vacuolation of a stromal layer of the cornea, (H &E stain, 400X). d: Proliferation of ciliary process with mild vascular congestion as indicated by yellow arrows, (H &E stain, 400X). e: Thinning and degeneration of sclera, (H &E stain, 400X).

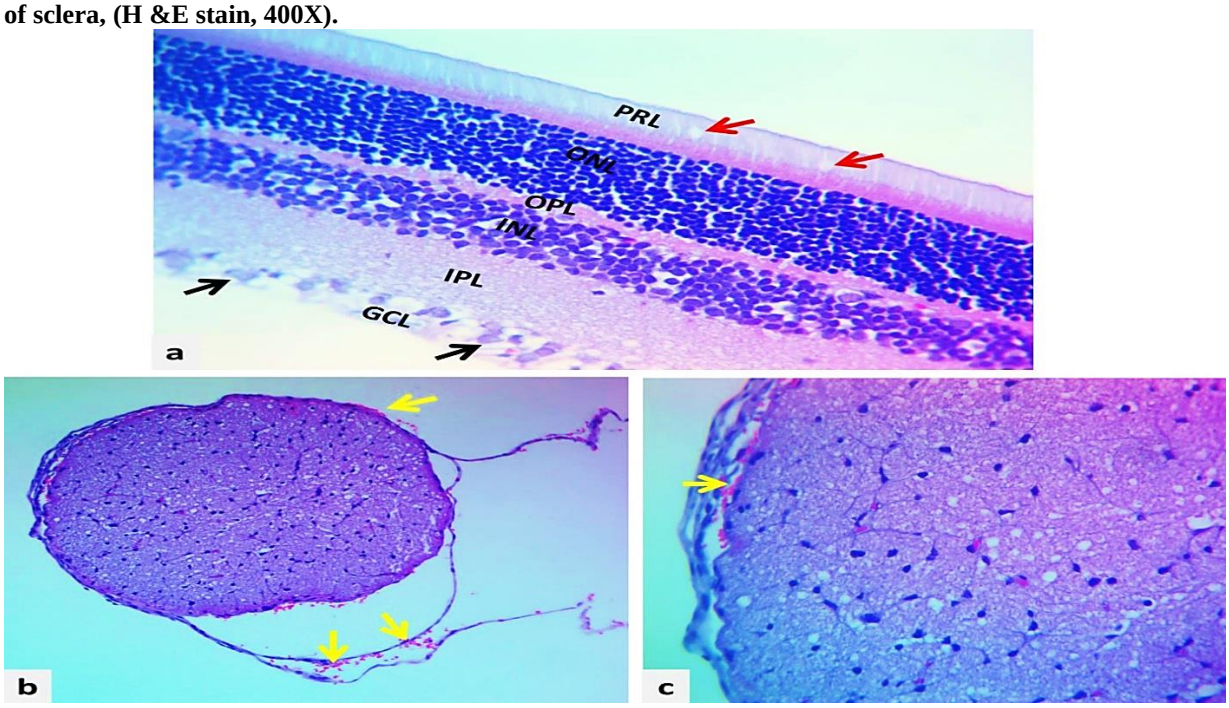

Figure 14.Microscopic section of an eye in 3.5 mg/kg of Azil + CIS group (G5). a: The retina revealed mild vacuolation of photoreceptor cells (PRL), mild gliosis in the inner and outer nuclear layer (INL and ONL), and mild degeneration of ganglionic cells as indicated by black arrows, (H &E stain, 400X). b and c: mild Thinning of epineurium (yellow arrows) of optic nerves with a mild reduction in glial cells, (H &E stain, 100X and 400X).

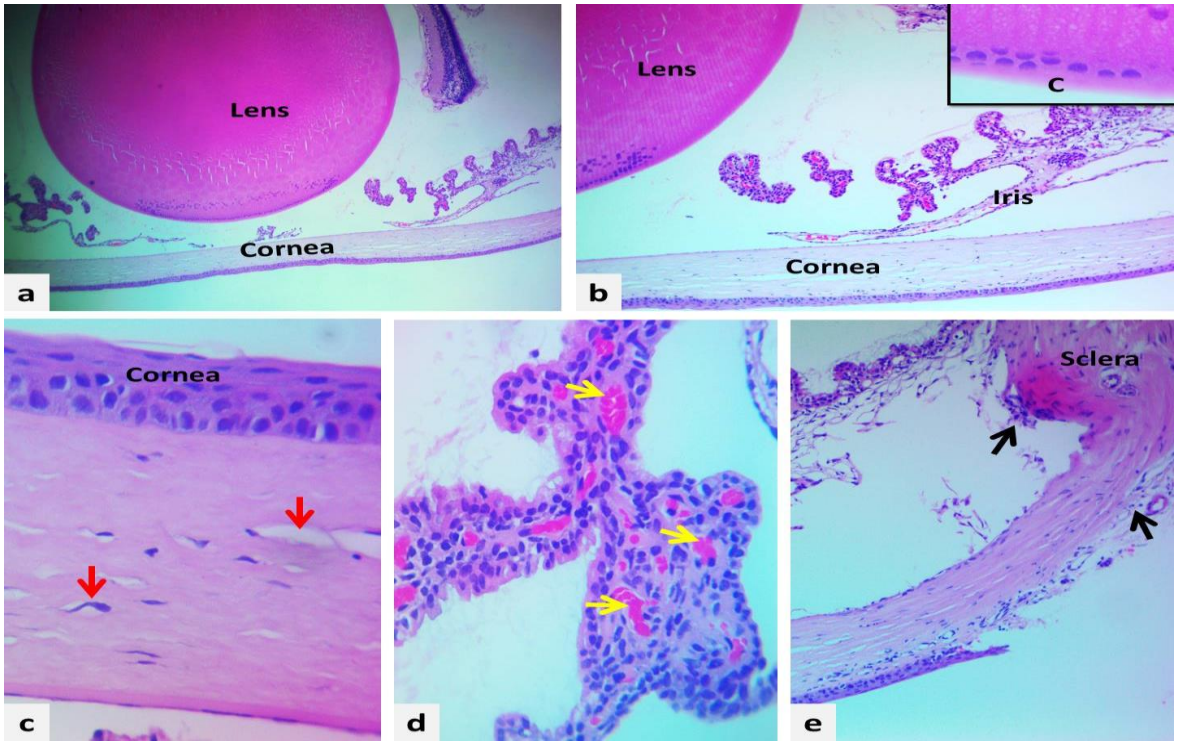

Figure 15. Microscopic section of an eye in 7 mg/kg of Azil + CIS group (G6). a and b: Normal organization of lens layers (inset) with thinning of the capsule (C) with mild degeneration of capsular epithelium (E), and vascular congestion of iris, (H &E stain, 100X and 200X). c: Mild-moderate degeneration and vacuolation of a stromal layer of the cornea, (H &E stain, 400X). d: Thickening of the ciliary process with moderate vascular congestion as indicated by yellow arrows, (H &E stain, 400X). e: Focal inflammatory reaction of the sclera as indicated by black arrows, (H &E stain, 400X).

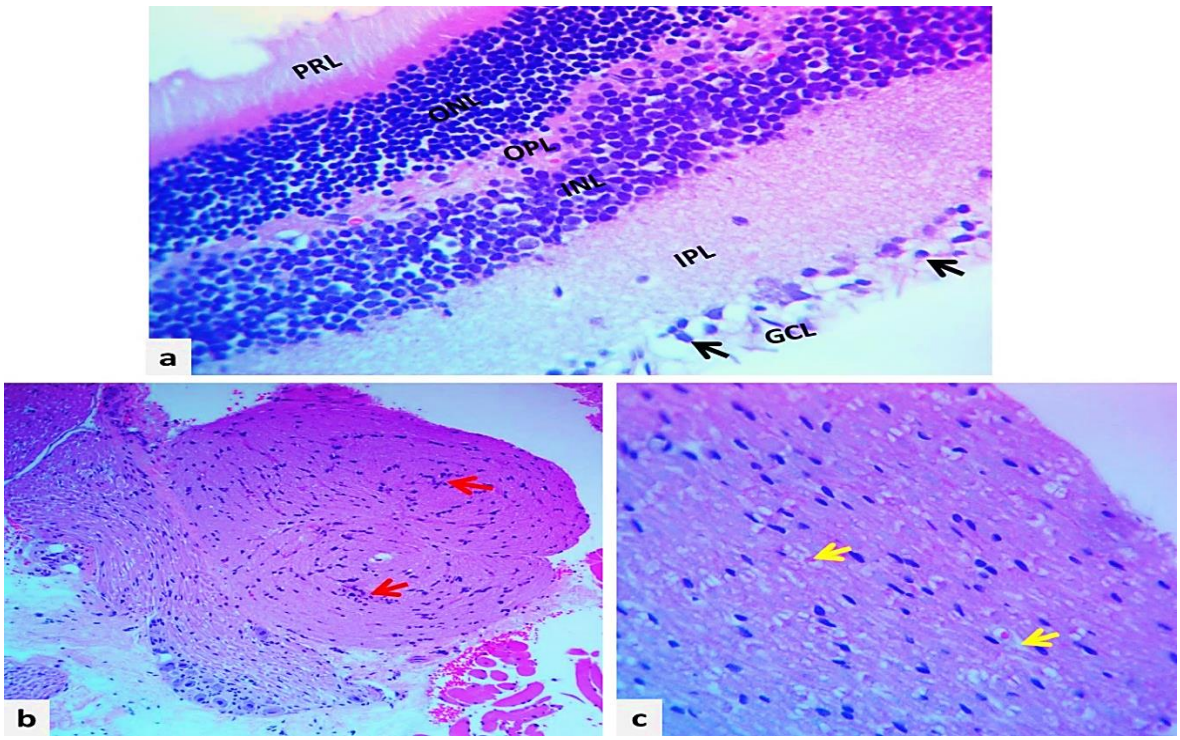

Figure 16. Microscopic section of the eye in 7 mg/kg of Azil + CIS group (G6). a: The retina revealed moderate degeneration of photoreceptor cells (PRL), moderate gliosis, and vacuolation in the inner and outer nuclear layer (INL and ONL), and severe degeneration of ganglionic cells as indicated by black arrows, (H &E stain, 400X). b and c: moderate ischemic optic nerves had inflammatory cells in the form of cell clusters (red arrows), vascular congestion (yellow arrows) with a moderate reduction in glial cells, (H &E stain, 100X and 400X).

Discussion

The nature of the eye functioning, in which end stage visual damage can be irreparable if detected later, contributes to the underreporting of ocular injury associated with the Cisplatin treatment [24]. Since Cisplatin is widely used in chemotherapy protocols of different types of malignancies, the retinal and ocular neuro-toxicities might be challenging the benefit of the drug regimen [5]. This research examined the protective effect of various doses of Azilsartan against the ocular toxicity generated by Cisplatin in rat models. Oxidative damage is an important factor in the pathophysiology of Cisplatin toxicity, and it has been shown that Cisplatin has a harmful impact on tissues by increasing free radical formation and depletion of antioxidant enzymes [25]. Previous studies demonstrated that cisplatin enhanced the generation of MDA, the final outcome of lipid peroxidation of cellular biomembranes in the ocular tissue and optic nerve, which indicates the damage caused by free radicals in such sites [22,26]. In vivo study showed that Cisplatin induced-neurotoxicity as a result of DNA-platinum binding and generation of DNA adducts, which was much higher than that seen in several other tissues [27]. A hypothesized mechanism of how Cisplatin causes inflammation includes the upregulation of pro-inflammatory cytokines like IL-1β as a result of oxidative stress stimulation and activation of the p38 MAPK signaling pathway, which is ultimately responsible for mitochondrial damage and release of additional free radicals as well as causing cellular injury [28]. The results of this study are parallel with aforementioned researches that conducted on Cisplatin toxicity on various tissues. The MDA levels were significantly higher in the rats that received Cisplatin as a single dose only than the healthy control group, as illustrated in Figure 1. Likewise, in the Cisplatin group the IL-1β levels were significantly higher than the healthy control group, this finding is consistent with several studies attributed to Cisplatin induced retinal toxicity, hepatic toxicity, and cognitive dysfunction respectively in rat models [29–31]. In this study, the histological examination of the retina of the rat that received a Cisplatin injection showed a degeneration of retinal layers with the formation of gliosis as shown in Figure 8a, such finding also occurred after injecting IL-1β into the retina and induced a retinopathy as a result of oxidative stress and depletion of the anti-oxidant enzymes which end with accelerating the apoptosis process in the retinal microvasculature as mentioned in the previous research [32].

Furthermore, the SOD level in the Cisplatin group was non-significantly lower than that of the other groups in this study. This finding is supported by a previous study showing that overwhelming the antioxidant enzymes with extra free radical genesis results in retinal toxicity [33]. Reactive oxygen species perturb the balance between the SOD and glutathione peroxidase (GPx) balance, which results in further damage to the integrity of the cells. The increased SOD to GPx ratio led to the accumulation of hydrogen peroxide (H2O2) along with the generation of hydroxyl radical, which is a very noxious radical with a very short half-life by Fenton reaction [34]. Overexpression of Ang II is associated with the upregulation of NADPH oxidase, which mediates the ROS production and causes diabetic retinopathy as reported by Chen et al [35].

In this investigation, co-treatment of Azilsartan with Cisplatin reduced the MDA level minimally and marginally normalized the SOD level, and this outcome was assured by multiple studies on different pathological rat models [36–39]. The key mechanism that attenuates the harmful impact of Ang II-induced ROS generation and lipid peroxidation is the pharmacological action of Azilsartan, which works by potently inhibiting the AT1 receptor and increasing the activity of the ACE2/(1-7) Ang/mas receptor axis more than Candesartan [40,41]. Additionally, a modest dose of Azilsartan (3.5 mg/kg) effectively suppresses inflammation in the ocular tissue brought on by Cisplatin in this trial, by significantly lowering the level of IL-1β. Several studies have reported Azilsartan anti-inflammatory effect at low doses in various experimental models [42–44], which is in agreement with our findings. Azilsartan blocks Ang II activity, which in turn inhibits the p38 MAPK signaling pathway, which in turn decreases the expression of pro-inflammatory cytokines [45]. While Azilsartan inhibited mitochondrial dysfunction and pro-inflammatory marker activity in an in vitro study, it had minimal to no effect on the antioxidant system [46]. Remarkably, our data reveals that Azilsartan has a negligible effect on the SOD levels in the group of rats that received a toxic dose of Cisplatin, which was associated with the induction of ocular injury. Moreover, histological examination of this study demonstrated the damaging effect of the Cisplatin on the eye tissue as whole and the optic nerve in particular, causing a significant disruption of eye morphology, degeneration of lens capsule, sloughing of the retinal epithelium, and accumulation of cataract in the lens, as shown in Figure 5. While co-administering Azilsartan with Cisplatin, particularly 3.5mg/ kg dose drastically protected the ocular tissue by reducing the congestion and inflammatory infiltration, as well as restoring the overall morphology of the eye layers, as shown in Figures 13 and 14.

Conclusion

Azilsartan shows a promising anti-inflammatory effect in ocular tissue in the current study by significantly reducing IL-1β overexpression and diminishing the features of inflammation in the eye tissue in a dose-dependent manner, mediated through its potent AT1 receptor blocking effect. However, Azilsartan shows a slight effect on the MDA levels and marginal effect on the SOD levels in the Cisplatin-induced ocular toxicity in the rat model.

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Ethics Statements

The present study was approved by the Ethical Committee on Animal Research of University of Sulaimani, College of Pharmacy (Certificate no. PH35-21 on 14th November 2021).

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. Omoti AE, Omoti CE. Ocular toxicity of systemic anticancer chemotherapy. Pharm Pract. 2006;4(2):55.
2. Chirtes F, Albu S. Prevention and restoration of hearing loss associated with the use of cisplatin. BioMed Res Int. 2014;2014.
3. Tada H, Mitsudomi T, Misumi T, Sugio K, Tsuboi M, Okamoto I, et al. Randomized phase III study of gefitinib versus cisplatin plus vinorelbine for patients with resected stage II-IIIA non–small-cell lung cancer with EGFR mutation (IMPACT). J Clin Oncol. 2022;40(3):231–41.
4. Oun R, Moussa YE, Wheate NJ. The side effects of platinum-based chemotherapy drugs: a review for chemists. Dalton Trans Camb Engl 2003. 2018 ;47(19):6645–53.
5. Gonzalez F, Menendez D, Gomez-Ulla F. Monocular visual loss in a patient undergoing cisplatin chemotherapy. Int Ophthalmol. 2001;24(6):301–4.
6. Polat N, Ciftci O, Cetin A, Yılmaz T. Toxic effects of systemic cisplatin on rat eyes and the protective effect of hesperidin against this toxicity. Cutan Ocul Toxicol. 2016;35(1):1–7.
7. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxins. 2010;2(11):2490–518.
8. Agcayazi SB, Ugurlu A, Ucak T, Tasli NG, Karakurt Y, Icel E, et al. Protection against experimental cisplatin-induced optic nerve toxicity using resveratrol: A rat model study. Cutan Ocul Toxicol. 2021;40(3):263–7.
9. Yazici A, Sogutlu-Sari E, Yay A, Aksit H, Kilic A, Aksit D, et al. The protective effect of selenium in cisplatin-related retinotoxicity. Cutan Ocul Toxicol. 2014;33(4):327–32.
10. Hazlewood RJ, Kuchtey J, Wu HJ, Kuchtey RW. Telmisartan reduces axon degeneration in mice with experimental glaucoma. Invest Ophthalmol Vis Sci. 2020;61(5):51–51.
11. Hussein MQ, Kadim HM, Abdulsahib WK. Effect of telmisartan on intra-ocular pressure in induced open angle glaucoma in rabbits. Int J Sci Res . 2017;6(10):1565–661.
12. Hazlewood RJ, Chen Q, Clark FK, Kuchtey J, Kuchtey RW. Differential effects of angiotensin II type I receptor blockers on reducing intraocular pressure and TGFβ signaling in the mouse retina. PLoS One. 2018;13(8):e0201719.
13. Ojima M, Igata H, Tanaka M, Sakamoto H, Kuroita T, Kohara Y, et al. In vitro antagonistic properties of a new angiotensin type 1 receptor blocker, azilsartan, in receptor binding and function studies. J Pharmacol Exp Ther. 2011;336(3):801–8.
14. Mahmood NMA, Hussain SA, Mirza RR. Azilsartan improves the effects of etanercept in patients with active rheumatoid arthritis: a pilot study. Ther Clin Risk Manag. 2018;14:1379–85.
15. Iwanami J, Mogi M, Tsukuda K, Wang XL, Nakaoka H, Ohshima K, et al. Role of angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas axis in the hypotensive effect of azilsartan. Hypertens Res. 2014;37(7):616–20.
16. Senanayake P deS, Bonilha VL, W Peterson J, Yamada Y, Karnik SS, Daneshgari F, et al. Retinal angiotensin II and angiotensin-(1-7) response to hyperglycemia and an intervention with captopril. J Renin Angiotensin Aldosterone Syst. 2018; 19(3) :14703 203187 89323 .
17. Alzahrani YM, Sattar MAAA, Kamel FO, Ramadan WS, Alzahrani YA. Possible combined effect of perindopril and Azilsartan in an experimental model of dementia in rats. Saudi Pharm J. 2020;28(5):574–81.
18. Ferah Okkay I, Okkay U, Bayram C, Cicek B, Sezen S, Aydin IC, et al. Bromelain protects against cisplatin-induced ocular toxicity through mitigating oxidative stress and inflammation. Drug Chem Toxicol. 2021;1–8.
19. Sekar S, Mani S, Rajamani B, Manivasagam T, Thenmozhi AJ, Bhat A, et al. Telmisartan ameliorates astroglial and dopaminergic functions in a mouse model of chronic

Parkinsonism. Neurotox Res. 2018;34(3):597–612.

20. Okkay U, Ferah Okkay I, Aydin IC, Bayram C, Ertugrul MS, Gezer A, et al. Effects of Achillea millefolium on cisplatin induced ocular toxicity: an experimental study. Cutan Ocul Toxicol. 2021;40(3):214–20.

21. Alzahrani YM, Sattar MAAA, Kamel FO, Ramadan WS, Alzahrani YA. Possible combined effect of perindopril and Azilsartan in an experimental model of dementia in rats. Saudi Pharm J. 2020;28(5):574–81.

22. Okkay U, Ferah Okkay I, Aydin IC, Bayram C, Ertugrul MS, Gezer A, et al. Effects of Achillea millefolium on cisplatin induced ocular toxicity: an experimental study. Cutan Ocul Toxicol. 2021;40(3):214–20.

23. Taşlı NG, Uçak T, Karakurt Y, Keskin Çimen F, Özbek Bilgin A, Kurt N, et al. The effects of rutin on cisplatin induced oxidative retinal and optic nerve injury: an experimental study. Cutan Ocul Toxicol. 2018;37(3):252–7.

24. Omoti AE, Omoti CE. Ocular toxicity of systemic anticancer chemotherapy. Pharm Pract. 2006;4(2):55.

25. Ramkumar V, Mukherjea D, Dhukhwa A, Rybak LP. Oxidative Stress and Inflammation Caused by Cisplatin Ototoxicity. Antioxidants. 2021;10(12):1919.

26. Taşlı NG, Uçak T, Karakurt Y, Keskin Çimen F, Özbek Bilgin A, Kurt N, et al. The effects of rutin on cisplatin induced oxidative retinal and optic nerve injury: an experimental study. Cutan Ocul Toxicol. 2018;37(3):252–7.

27. McDonald ES, Randon KR, Knight A, Windebank AJ. Cisplatin preferentially binds to DNA in dorsal root ganglion neurons in vitro and in vivo: a potential mechanism for neurotoxicity. Neurobiol Dis. 2005;18(2):305–13.

28. Jing T, Liao J, Shen K, Chen X, Xu Z, Tian W, et al. Protective effect of urolithin a on cisplatin-induced nephrotoxicity in mice via modulation of inflammation and oxidative stress. Food Chem Toxicol. 2019;129:108–14.

29. Omar HA, Mohamed WR, Arafa ESA, Shehata BA, El Sherbiny GA, Arab HH, et al. Hesperidin alleviates cisplatin-induced hepatotoxicity in rats without inhibiting its antitumor activity. Pharmacol Rep. 2016;68(2):349–56.

30. Saral S, Topçu A, Alkanat M, Mercantepe T, Akyıldız K, Yıldız L, et al. Apelin-13 activates the hippocampal BDNF/TrkB signaling pathway and suppresses neuroinflammation in male rats with cisplatin-induced cognitive dysfunction. Behav Brain Res. 2021;408:113290.

31. Polat N, Ciftci O, Cetin A, Yılmaz T. Toxic effects of systemic cisplatin on rat eyes and the protective effect of hesperidin against this toxicity. Cutan Ocul Toxicol. 2016;35(1):1–7.

32. Kowluru RA, Odenbach S. Role of interleukin-1β in the development of retinopathy in rats: effect of antioxidants. Invest Ophthalmol Vis Sci. 2004;45(11):4161–6.

33. Polat N, Ciftci O, Cetin A, Yılmaz T. Toxic effects of systemic cisplatin on rat eyes and the protective effect of hesperidin against this toxicity. Cutan Ocul Toxicol. 2016;35(1):1–7.

34. De Haan JB, Cristiano F, Iannello R, Bladier C, Kelner MJ, Kola I. Elevation in the ratio of Cu/Zn-superoxide dismutase to glutathione peroxidase activity induces features of cellular senescence and this effect is mediated by hydrogen peroxide. Hum Mol Genet. 1996;5(2):283–92.

35. Chen P, Guo A, Edwards P, Trick G, Scicli A. Role of NADPH oxidase and ANG II in diabetes-induced retinal leukostasis. Am J Physiol Regul Integr Comp Physiol. 2007 Nov 1;293:R1619-29.

36. de Araujo AA, Varela H, de Medeiros CACX, de Castro Brito GA, de Lima KC, de Moura LM, et al. Azilsartan reduced TNF-α and IL-1β levels, increased IL-10 levels and upregulated VEGF, FGF, KGF, and TGF-α in an oral mucositis model. PloS One. 2015;10(2):e0116799.

37. Khan MAH, Neckář J, Haines J, Imig JD. Azilsartan improves glycemic status and reduces kidney damage in zucker diabetic fatty rats. Am J Hypertens. 2014;27(8):1087–95.

38. Zhang C, Zhao Y, Yang X. Azilsartan attenuates lipopolysaccharide-induced acute lung injury via the Nrf2/HO-1 signaling pathway. Immunol Res. 2022;70(1):97–105.

39. Alzahrani YM, Sattar MAAA, Kamel FO, Ramadan WS, Alzahrani YA. Possible combined effect of perindopril and Azilsartan in an experimental model of dementia in rats. Saudi Pharm J. 2020;28(5):574–81.

40. Tiwari V, Singh J, Tiwari P, Chaturvedi S, Gupta S, Mishra A, et al. ACE2/ANG-(1-7)/Mas Receptor Axis Activation Prevents Inflammation, Glial Activation And Cognitive Decline In a Rat Model of STZ Induced Impairment of Learning and Memory. 2021;

41. Dargad RR, Parekh JD, Dargad RR, Kukrety S. Azilsartan: novel angiotensin receptor blocker. J Assoc Physicians India. 2016;64(3):96–8.

42. Mahmood NMA, Hussain SA, Khan HAEK. Azilsartan as "Add-On" Treatment with Methotrexate Improves the Disease Activity of Rheumatoid Arthritis. BioMed Res Int. 2018; 14: 1379–1385.

43. Mustafa WW, Shukor S, Husain SA, Mahmood NMA. The anti-inflammatory activity of Azilsartan in animal models of experimentally-induced chronic and granulomatous

inflammations. International Journal of Research in Pharmaceutical Sciences. 2018; 9(4):1162-1168 .

44. Mahmood NMA, Hussain SA, Mirza RR. Azilsartan improves the effects of etanercept in patients with active rheumatoid arthritis: a pilot study. Ther Clin Risk Manag. 2018;14:1379.

45. Kajiya T, Ho C, Wang J, Vilardi R, Kurtz T. Molecular and cellular effects of azilsartan: A new generation angiotensin II receptor blocker. J Hypertens. 2011; 29:2476–83.

46. Liu H, Mao P, Wang J, Wang T, Xie CH. Azilsartan, an angiotensin II type 1 receptor blocker, attenuates tert-butyl hydroperoxide-induced endothelial cell injury through inhibition of mitochondrial dysfunction and anti-inflammatory activity. Neurochem Int. 2016; 94:48–56.