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L-arginine ameliorate acute ocular toxicity induced by cisplatin administration in rats: role of inducible nitric oxide synthase

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ABSTRACT

Cisplatin (CP) is a chemotherapeutic agent employed as a therapy for different solid tumors. CP elicits many negative side effects including ocular toxicity. L-arginine able to increase the anti-inflammatory cytokine levels in the bloodstream. This study aimed to evaluate the possible protective impact of L-arginine against ocular toxicity of cisplatin through its anti-inflammatory action. Forty male rats were utilized in this experiment. The treatment protocol duration lasted for one week. The rats were divided randomly into four equal groups, as follows: the normal control group (G1) received a single intraperitoneal injection of normal saline, Cisplatin-administered group (G2) received single intraperitoneal injection of cisplatin in a dose (10 mg/kg body weight), Cisplatin + L-arginine-treated group (G3) received i.p. injection of cisplatin in a dose (10 mg/kg b.wt.) followed immediately by i.p. injection of L-arginine in a dose (10 mg/kg b.wt.), and L-arginine-treated group (G4) received intraperitoneal injection of 10 mg/kg b.wt. L-arginine. The protective effect of L-arginine was assessed through histopathology and immunohistochemical analysis of inducible nitric oxide synthase (iNOS) in ocular tissues. Cisplatin-administered group revealed corneal neovascularization, stromal inflammatory infiltration, corneal epithelial hyperplasia, and endothelial degeneration. Sclera revealed vascular congestion and inflammatory infiltrates. There were congested blood vessels, apoptosis, and a decrease the ganglionic cells number in the retinal tissue. Ciliary body had epithelial necrosis, congestion, and hemorrhage. These pathological changes were attenuated to be mild in the cisplatin + L-arginine-treated group. The intensity of the immunohistochemical expression of iNOS appeared weak and significantly decreased in G3 compared to G2. The obtained data validate that Co-treatment of cisplatin with L-arginine cause a reduction in histopathological ocular damage through its anti-inflammatory effect.

Introduction

Chemotherapeutic agents are powerful medications that have significant side effects on the eyes. It is crucial to recognize the symptoms of ocular toxicities at an early stage to prevent irreversible harm (Omoti and Omoti, 2006). Cisplatin, a chemotherapeutic alkylating drug, is highly effective in treating a range of malignant tumors in various regions of the body (Chirtes and Albu, 2014; Tada *et al*., 2022). Cisplatin is limited in clinical practice due to its negative side effects e.g., blurred vision, papilledema, retinal and optic neuropathy as well as acute renal disease, gastrointestinal disturbances, and neurological problems which present significant challenges to the effectiveness of cisplatin therapy (Oun *et al*., 2018). There are several processes were involved in cisplatin-induced ocular damage, including inflammatory processes, oxidative stress, formation of DNA adducts, and malfunctioning mitochondria (Polat *et al*., 2016). Oxidative stress and the raised levels of cell death cause progression of inflammation by liberating pro-inflammatory mediators, including tumor necrosis factor (TNF)-α, IL-6, and IL-8 (Kiss *et al*., 2020). Such mediators promote the induction of iNOS enzyme (Kumar *et al*., 2017; Patil *et al*., 2019). Therefore, employing anti-inflammatories and antioxidants has become a crucial approach to shield the eyes from the harmful effects of cisplatin (Liang *et al*., 2018; Trimarco *et al*., 2023). L-arginine (2-amino-5-guanidinovaleric acid) is a solid dry water-soluble white powder or crystals. It is a conditionally essential amino acid in protein biosynthesis. $C_6H_{14}N_4O_2$, the formula for l-arginine, presents the L-isomer of L-arginine (L-alpha-amino acid) (Ashwini and Radheep, 2018). L-arginine incorporates a nitrogenous guanidine group that binds to nitric oxide (NO) synthase, serving as a substrate to help this enzyme produce NO (Sadeghi *et al*., 2023). L-arginine is the primary precursor for the synthesis of nitric oxide (NO), which triggers many biological reactions like angiogenesis and epithelial regeneration helping in the healing process (Shiny *et al*., 2021).

L-arginine enhanced the antioxidant enzyme activity and free radical reduction, which were on the same level with or even higher than those of vitamin C and other parameters (Liang *et al*., 2018). There are two mechanisms contributing to this antioxidant activity: dependent and independent of NO. In the direct (NO-independent) mechanism, L-arginine scavenges oxygen radicals, especially superoxide ions (Sadeghi *et al*., 2023). In the latter pathway, a NO-dependent mechanism, it acts through multiple mechanisms including attenuating the oxidants' chemistry, enhancing the antioxidants' potency, and inducing the expression of antioxidant enzymes and many genes related to antioxidant homeostasis (Abu-Serie *et al*., 2015).

Another beneficial effect of L-arginine exerts through a mechanism involving the reduction of the pro-inflammatory cytokines and increase anti-inflammatory cytokine (IL-10) (Trimarco *et al*., 2023). Also, exogenous L-arginine treatment has a powerful anti-inflammatory action through restoring circulating L-Arginine levels, correcting nitric oxide dysfunction, and directly regulating the immune response (Crowther *et al*., 2022.).

Despite the toxic effect of cisplatin on ocular tissues, it has not been widely reported probably because of the eyes' biological nature, where end-stage impaired vision may be irreversible if discovered lately (Omoti and Omoti, 2006). Reviewing the available literatures, research works studied the protective effect of L-arginine on eye toxicity of cisplatin are insufficient and deserve more investigations. Thus, the current study was designed to demonstrate cisplatin-associated ocular damage and evaluate the impact of concurrent administration of CP and L-arginine against cisplatin-induced ocular damage through histopathology and immuno-

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histochemical examination of iNOS.

Materials and methods

Ethical approval

The present experiment was conducted in accordance with national and international ethical guidelines for care and use of laboratory animals. The work was approved by the research ethical committee of the Faculty of Veterinary Medicine, Assiut University, Egypt.

Chemicals

Cisplatin (Unistin) vials (50mg/50ml): were obtained from El-Hikma Specialized Pharmaceuticals, Cairo, Egypt. L (+)-Arginine, 98+%: was obtained from Cornell lab company (Acros Organic (Thermo Scientific), Janssen pharmaceutical, B-2440 Geel, Belgium). The L-arginine solution was prepared by dissolving 50 mg of L-arginine powder in 12.5 mL of normal saline to obtain a concentration of 2 mg/0.5mL and given intraperitoneally in a single dose of 10 mg/kg b.wt.

Experimental animals

Forty male Albino rats were obtained from Al-Nahda University Animal House, Beni-Suef, Egypt. The rats appeared to be in good health, with an average weight of about 200 g. The rats were retained in cages with regulated humidity and temperature (25ºC). Before the experiment, the animals were kept in the laboratory for ten days to help them acclimate. They had access to tap water and laboratory food which they could consume at their will.

Experimental design

Rats were randomly assigned into 4 equal groups (n=10) as follows:
Group1 (G1) (Normal control group): Rats received single intraperitoneal (i.p.) injection of normal saline. They were sacrificed by cervical dislocation after one week from cisplatin administration.
Group 2 (G2) (cisplatin-treated group): Rats were administered a single intraperitoneal (i.p.) injection of cisplatin (50 mg/50 ml) in a dose (10 mg/kg body weight) (Okafor, 2021; Baker *et al*., 2022).
Group 3 (G3): cisplatin + L-arginine-treated group: Rats of this group were treated by i.p. injection of cisplatin in a dose (10 mg/kg b.wt.) followed immediately by i.p. injection of L-arginine (50mg/12.5 ml saline) in a dose (10mg/kg b.wt.) (Pennisi *et al*., 2005; Guo *et al*., 2014; Dong *et al*., 2020).
Group 4 (G4): L-arginine treated group: The rats were intraperitoneally injected with L-arginine (50mg/12.5 ml saline) in a dose of 10mg/kg b.wt.

After one week from the beginning of the experiment, all experimental rats were anesthetized with diethyl ether and then sacrificed by cervical dislocation. The eye tissues were gathered and fixed in neutral buffered formalin solution (10%) for histopathological and immunohistochemical examinations.

Histopathological examination

After fixation, eyes underwent routine processing for conventional histopathological examination. Briefly, the tissue samples were cleaned with tap water and then kept in 70% ethyl alcohol for an entire night. To dehydrate the specimens, they were submerged in rising concentrations of ethyl alcohol (70%, 80%, 90%, and 100%). Tissue samples were clarified with xylene then embedded in the paraffin wax. After that, they were blocked with freshly melted paraffin. Hematoxylin and eosin staining was applied to 5-µm serial tissue slices (Suvarna *et al*., 2019) for light microscopy (Olympus, CX31; Tokyo Japan) evaluation. The stained sections were then photographed with a digital camera (Toup view, LCMos10000KPA, China).

Semiquantitative scoring of histopathological lesions

Histopathological lesions of different eye regions including cornea, sclera, choroid, retina, and ciliary body of rats in all groups were scored semiquantitatively in ten random high power fields/section/rat as follows: -: No lesion; + : Slight lesion; ++: Moderate lesion: +++: Severe lesion. The lesions for all groups were depicted in a table to clarify their types, incidence, and score of severity (Mahmood and Kareem, 2023).

Immunohistochemical analysis

Immunohistochemistry staining was performed on six rats from each group using anti-iNOS Rabbit polyclonal antibody (Diluted 1:20, Cat.NO. # PA1-036, Thermo Fisher Scientific, 168 Third Avenue Waltham, MA USA 02451) according to method des cribbed by Hamad *et al*. (2022). Briefly, paraffin-embedded block sections were first subjected to deparaffinization as well as rehydration steps. To avoid non-specific staining, due to the action of endogenous peroxidase, hydrogen peroxide was applied to the slides for 10 minutes. To unmask the antigenic epitope, an antigen retrieval step had occurred by using 300 ml of 10 mM citrate buffer (pH 6.0) into the container of the slides and then incubating them at 95-100°C for 10 minutes. The primary anti-iNOS antibody was diluted according to the manufacturer's instructions and then laid on the slides then incubated. Then, the tissue sections were incubated with horseradish peroxidase (HRP) for about 15 minutes at room temperature. In all previous steps, the slides were washed three times with phosphate buffer. Then, a mixture of 40 µl of 3,3' Diaminobenzidine (DAB) Plus Chromogen and 2 ml of DAB Plus Substrate was applied to the tissue sections and left for 5 minutes. The tissue sections were rinsed four times with distilled water. The sections were dehydrated, counter stained with Mayer's hematoxylin, and ventually covered with coverslips. Negative control of eye tissue sections was raised beneath the equivalent conditions without primary antibody. The stained slides were inspected under microscope and photographed.

Evaluation of the intensity of the immunostaining

The intensity of brown-colored cells was scored in five high-power fields/slide in 6 slides, representing the ocular tissue sections of 6 rats of each group. The intensity of iNOS immunreaction was scored from 0 to 3 as follows: 0 (none), 1 (weak), 2 (moderate), and 3 (strong). Thereafter, the final scores of the study groups were presented as means, which were statistically compared (Fisher *et al*., 2005).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the collected data. To demonstrate the differences among the experimental groups, one-way analysis of variance (ANOVA) was carried out, followed by Tukey-Kramer post-hoc multiple comparison test. Every data point was presented as means ± standard error of mean (SEM). When $P < 0.05$, statistically significant differences were considered acceptable.

Results

Histopathological findings

Histopathological evaluation of H&E stained rat corneal tissue in the normal control group (G1) revealed the normal microscopic structure of corneal epithelium which formed by few layers of non-keratinized stratified squamous epithelial cells with basal columnar cells lining the Bow-

man's membrane. Moreover, corneal stroma formed of collagen bundles parallel to each other with several scattered flattened keratocytes and endothelial cell layer adjacent to the Descemet's membrane (Figure 1a). In contrast, corneal tissue in cisplatin treated group (G2) revealed hyperplasia of corneal epithelium with increasing EP layer thickness and neovascularization in corneal stroma, The stroma showed disrupted and disorganized and widely separated thick collagen fibers with inflammatory cell infiltrates. Furthermore, vacuolar degeneration of the corneal endothelium was seen in most examined sections of this group (Figure 1b, c, d, e). Cisplatin and L-arginine-treated group (G3) revealed slight corneal epithelial vacuolation and weak separation between stromal collagen fibers (Figure 1f). L-arginine-treated group (G4) revealed the normal structure of corneal epithelium, stroma, and endothelium (Figure 1g).

The sclera of G1 showed normal stroma consisted of collagen fibers running in different random directions and normally appeared choroidal blood vessels (Figure 1h). Sclera of G2 revealed markedly congested blood vessels surrounded by inflammatory infiltrates with disrupted scleral stromal fibers (Figure 1i). Sclera of G3 revealed mild scleral stromal disruption and mild vascular congestion in choroid (Figure 1j). G4 showed normal scleral stroma and choroidal blood vessel (Figure 1k).

Histopathological evaluation of H&E-stained rat retinal tissue in the normal control group (G1) revealed normal microscopic structure of retinal layers including ganglionic cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL) and photoreceptor layer which consisted of inner segment (IS) and outer segment (OS) (Figure 2a). Retinal tissue of G2 revealed vacuolation, necrosis of GCL as well as apoptotic cells (eosinophilic cytoplasm and hyperchromatic nuclei). Also, there were some congested blood vessels in the nerve fiber layer and GCL (Figure 2b, c, d). G3 revealed slight degenerative changes in GCL and vascular congestion (Figure 2e). G4 showed normal histomorphology of retinal layers (Figure 2f).

Ciliary body of rats in G1 revealed normal microscopic structure of ciliary body as well as pigmented and non-pigmented epithelial layers of ciliary process (Figure 3a). Ciliary process epithelial cells of G2 revealed degenerative changes, necrosis and focal losing with cellular infiltration. Vascular congestion and hemorrhage were also seen (Figure 3b, c). G3 revealed normal appearance of ciliary body and processes with slight loss in epithelial cells of ciliary process and mild inflammatory infiltration (Figure 3d). G4 showed normal structure of ciliary process and body with slight congestion in few examined sections (Figure 3e).

Semiquantitative scoring of histopathological changes in different eye regions (cornea, sclera, retina, and ciliary body) of normal control and other groups were presented in Table 1, which clarify type, incidence, and the severity score of each lesion.

Immunohistochemical results

The immunostaining of iNOS was accomplished to evaluate the anti-inflammatory effect of L-arginine and was assessed regarding the intensity of cells with positive immunreactions in the ocular tissues including cornea (Figure 4), retina (Figure 5), and ciliary body (Figure 6) of various groups. The immunexpression of iNOS in the G1 has been shown a weak reaction in the corneal tissue, retinal layers, and ciliary body. In the G2, the corneal epithelium, corneal stroma, retinal layers especially the ganglionic cell layer, inner plexiform layer, inner nuclear layer, and photoreceptor layer as well as ciliary body showed a strong iNOS staining expression. Moreover, its intensity significantly ($p < 0.05$) increased compared to G1. In contrast, it revealed a weak immunoreaction in the G3 and G4 with significantly ($p < 0.05$) decreased densities compared to G2.

Discussion

Cisplatin has been known as a widely used chemotherapy drug against different types of malignancies. However, its regimen may be threatened by retinal, corneal, and ocular neurotoxicities, which pose a challenge to its benefits (Dulz *et al*., 2017; Karakurt *et al*., 2018; Fındık *et al*., 2019; Polat *et al*., 2023). It has been demonstrated that oxidative stress and inflammation play a crucial part in the toxic course of cisplatin (Sarı *et al*., 2014).

Histopathological findings of this study revealed that cisplatin causes corneal neovascularization, stromal inflammatory infiltration, corneal epithelial hyperplasia, and endothelial degeneration. Sclera revealed vascular congestion and inflammatory infiltrates. Okkay *et al*. (2021) reported that in cisplatin administration, the Bowman's membrane of the cornea was found to have a severe loss of integrity with irregular dislocation and some focal desquamation of the corneal epithelial cells and more eosinophilic corneal cells. As well as edema, degeneration in the corneal

Table 1. Scoring of histopathological ocular changes in normal control and other groups.

Organ	Lesion	Normal control	CP	CP + L- arginine	L-arginine
Cornea	Epithelial hyperplasia	0	0.4	0.3	-
	Neovascularization	0	0.8	0.1	-
	Inflammatory cells	0	1	-	-
	Endothelial degeneration	0	0.8	0.1	-
	Epithelial degeneration	0	0.6	0.2	-
	Stromal fiber widening	0	1	0.2	0.2
Sclera	Congestion	0	1	0.2	-
	Inflammatory cells	0	1	-	-
	Stromal disruption	0	0.8	0.2	-
Choroid	Congestion	0	1	0.3	0.1
Retina	Vacuolation	0	1	0.1	-
	Apoptosis	0	0.7	0.1	-
	Nuclear loss	0	0.8	-	-
	Congestion	0	0.9	-	-
	Degenerative changes	0	0.9	0.2	-
Ciliary body	Epithelial loss	0	1	0.3	-
	Vascular congestion	0	1	-	0.2
	Hemorrhage	0	0.8	-	-
	Inflammatory cells infiltration	0	1	0.2	-

Severity scale: Severe: +++; Moderate: ++; Slight: +; No change: - . Percentages represent the no. of affected rats in each group. CP: Cisplatin.

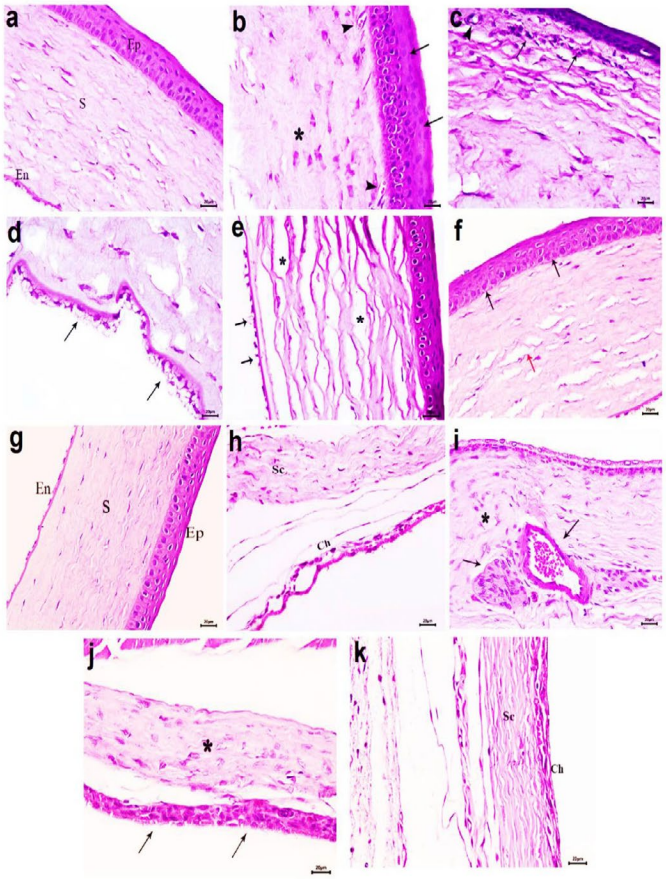

Fig. 1. Histopathological section of H&E-stained rat corneal and scleral tissues of all groups. a: Normal control group (G1) revealed the normal microscopic structure of corneal epithelium (Ep), corneal stroma (S), and endothelial cell layer (En) adjacent to Descemet's membrane. Cisplatin-treated group (G2) showing, b: hyperplasia of corneal epithelium (arrow), neovascularization (arrowhead), disrupted and disorganized corneal stroma (asterisk). C: inflammatory cells infiltrate the corneal stroma (arrow) and neovascularization (arrowhead). d: vacuolar degeneration in the endothelial cell layer (arrow). e: disruption and wide separation between thick stromal collagen fibers (asterisk) and degenerative changes in the endothelial cell layer (arrow). f: Cisplatin + L-arginine-treated group (G3) showing mild corneal epithelial degenerative changes (black arrow) and weak separation between stromal collagen fibers (red arrow). g: L-arginine-treated group showing the normal structure of corneal epithelium (Ep), stroma (S), and endothelium (En). h: The control group (G1) showed normal scleral stroma (S) and normal choroidal blood vessels (Ch). i: sclera of G2 showing congested blood vessels surrounded by inflammatory infiltrates with disrupted scleral stromal fibers. j: sclera of G3 showing mild scleral stromal disruption and mild vascular congestion. k: G4 showing normal scleral stroma (S) and choroidal blood vessel structure (Ch). bar= 20

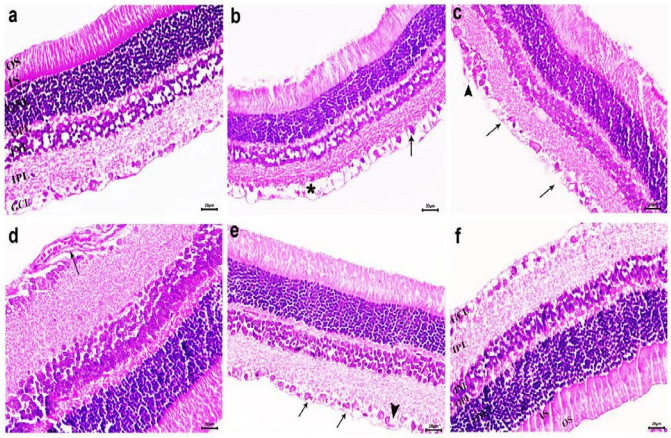

Fig. 2. Histopathological section of H&E-stained rat retinal tissue of all groups. a: Normal control group (G1) revealed the normal microscopic structure of retinal layers. G2 showing b: Vacuolation, decreasing in number of nuclei of GCL (asterisk) and apoptotic cells in GCL (eosinophilic cytoplasm and hyperchromatic nuclei) (arrow). c: Congested blood vessels (arrowhead) and degenerative changes in the GCL (arrows). d: congested blood vessel in the nerve fiber layer (arrow). e: G3 showing mild degenerative changes in GCL (arrows) and mild vascular congestion (arrowhead). f: G4 showing normal retinal layers' structure. bar=20.

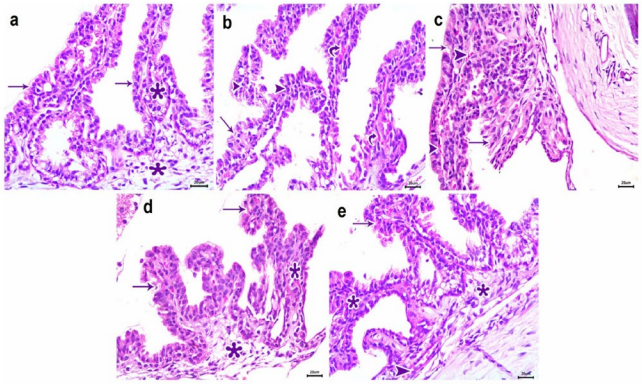

Fig. 3. Histopathological section of H&E stained rat ciliary body. a: normal control group (G1) showing the normal microscopic structure of the ciliary body (asterisk) and normal proliferation of epithelial cells (pigmented and non-pigmented) of ciliary process (arrow). G2 showing b: epithelial degeneration and focal loss (arrow), increasing in the inflammatory infiltration (arrowhead), and vascular congestion (curved arrow). c: epithelial degeneration and focal loss (arrow) and hemorrhage (arrowhead). d: G3 showing slight epithelial loss of ciliary process (arrow) and mild inflammatory infiltration (asterisk). e: G4 showing normal structure of ciliary process (arrow), normal ciliary body cell proliferation (asterisk) and slight congestion of blood vessel (arrowhead). bar =20

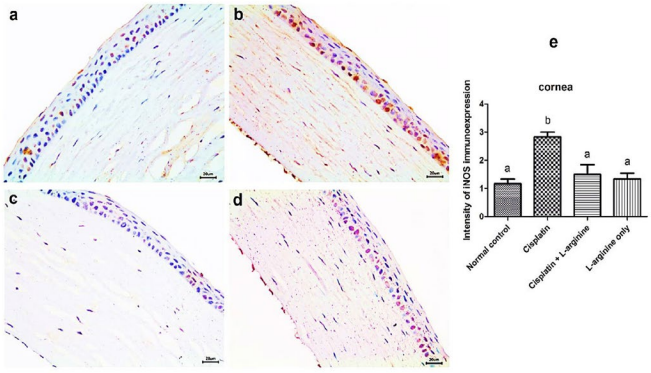

Fig. 4. Immunohistochemical iNOS staining expression in the corneal tissue. a: The G1 normal control group showing weak reaction. b: G2 showing a strong iNOS staining expression. c and d: iNOS revealed a weak immunoreaction in the G3 and G4, respectively. e: Statistical analysis of the intensity of the immunostaining of iNOS ocular sections of all groups. values are presented as mean ± SEM (n =6). $P < 0.05$ indicates a significant difference. Columns with different letters mean significant difference.

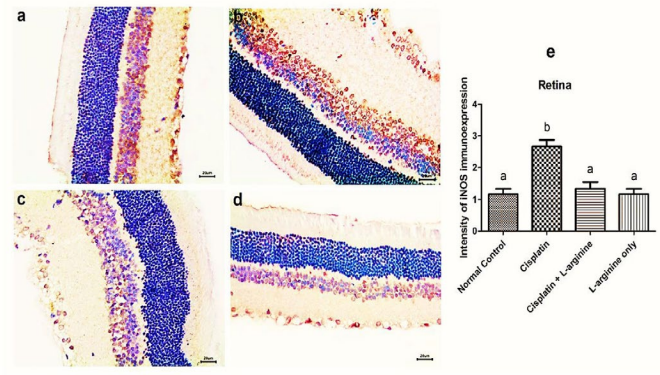

Fig. 5. Immunohistochemical iNOS staining expression in the retinal tissue. a: The G1 showing a weak reaction. b: G2 showing a strong iNOS staining expression. c and d: iNOS revealed a weak immunoreaction in the G3 and G4, respectively. e: Statistical analysis of the intensity of the immunostaining of iNOS ocular sections of all groups. Values are presented as mean ± SEM (n =6). $P < 0.05$ indicates a significant difference. Columns with different letters mean significant difference.

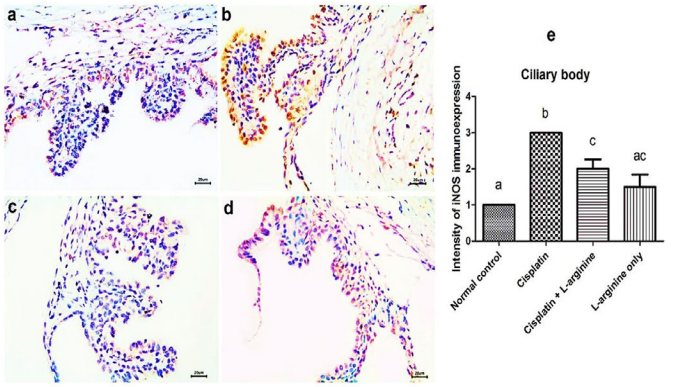

Fig. 6. Immunohistochemical iNOS staining expression in the ciliary body. a: The G1 showing a weak reaction. b: G2 showing a strong iNOS staining expression. c and d: iNOS revealed a weak immunoreaction in the G3 and G4, respectively. e: Statistical analysis of the intensity of the immunostaining of iNOS ocular sections of all groups. Values are presented as mean ± SEM (n =6). P < 0.05 indicates a significant difference. Columns with different letters mean significant difference.

stroma and polymorph nuclear inflammatory cell infiltration were reported to be found. This result is compatible with our results about corneal lesions. Our results are also established by the same corneal and scleral lesions found in previous research after i.p. injection of cisplatin (Baker *et al*., 2022).

Previous research has shown that cisplatin has adverse neuro-ocular toxic effects such as blepharitis and conjunctivitis; periorbital edema, retinal toxicity with maculopathy characterized by pigmentary changes resulting from localized retinal pigment disturbances, altered color perception attributable to cone dysfunction, or mild retinal ischemic changes such as cotton-wool spots and posterior pole intraretinal hemorrhage (Li *et al*., 2014), retinal ischemia with neovascularization and occlusion of retinal vasculature (Kwan *et al*., 2006). The present study showed the presence of congested blood vessels in the nerve fiber layer, vacuolation, and apoptosis in the ganglionic cell layer of the retina with loss of nuclei. These retinal findings are in accordance with that demonstrated by Katz *et al*. (2003) which revealed the presence of mild attenuation of the ganglionic cell layer and outer nuclear layer in retinal tissue after intravenous cisplatin administration. Also, the findings reported by Ibrahim *et al*. (2019) which revealed that the retinal pigment epithelial layer (RPE) was edematous and vacuolated, marked fragmentation of outer and inner photoreceptor segments, swelling nuclei of inner nuclear layer, pyknosis was seen in some nuclei of the outer nuclear layer while ganglion cell nuclei showed no abnormalities.

It is hypothesized that cisplatin causes inflammation by upregulating pro-inflammatory cytokines, such as IL-1β. This occurs due to oxidative stress stimulation, which activates the p38 MAPK signaling pathway. The pathway ultimately results in mitochondrial damage and the liberation of extra free radicals, which cause cellular injury (Jing *et al*., 2019). This explains the mechanism of cisplatin's devastating effect on ocular tissue.

Oxidative stress activates a series of signaling pathways, for example, the inflammatory pathway nuclear Factor-B (NF-B) (Sahu *et al*., 2014). This promotes proinflammatory cytokines and enzymes, including cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and tumor necrosis factor-alpha (TNF-α) (Kumar *et al*., 2017).

The ROS induced by cisplatin resulted in an increase in the values of the neurotoxic iNOS and a decrease in the values of the neuroprotective nNOS (Dawson and Dawson, 2004). This interprets our study's immunohistochemical results about the strong expression of iNOS in G2 in cornea, retina, and ciliary body. This explains that iNOS may have an important role in oxidative stress and inflammatory status produced by cisplatin. Some immunohistochemical studies of cisplatin-treated animals, demonstrated elevated levels of iNOS in the liver, kidney (Srivastava *et al*., 1996), testicles associated with some pro-inflammatory parameters' elevation (Famurewa *et al*., 2020). Lee and Park (2023) found that iNOS was induced in the retinal layers, ciliary body, and iris.

Previous research has demonstrated an iNOS expression elevation in animals' retinal tissue suffering from retinal ischemia or elevated intraocular pressure (Neufeld *et al*., 1999). Locally elevated levels of NO can exacerbate the loss of retinal ganglion cells because of the generation of large amounts of peroxynitrite (Flammer and Mozaffarieh, 2007) and this established our histopathological result in the retina of G2 which showed vacuolation and a decrease in the number of GCL nuclei.

L-arginine is the primary precursor for the synthesis of nitric oxide (NO) through NOS enzyme isoforms action, which triggers many biological reactions (Shiny *et al*., 2021). Administering L-arginine has been related to enhanced antioxidant enzyme activity and free radical reduction, which were on the same level with or even higher than those of vitamin C and other parameters (Liang *et al*., 2018). L-arginine has anti-inflammatory characteristics which appeared through the reduction of inflammatory cytokines by its use (Trimarco *et al*., 2023). L-arginine exhibited nephroprotective (Saleh and El-Demerdash, 2005) and hepatoprotective properties against cisplatin by attenuation the expression of iNOS in hepatocytes and decreasing the oxidative stress (El-Sayed *et al*., 2019). Cornea epithelial healing was also promoted with exogenous NO supply by activating the MAPK pathway (Park *et al*., 2017).

In this experimental work, most of the histopathological lesions appeared to be slight or disappeared. On the other hand, immunohistochemical expression of iNOS in different ocular regions was weak and its intensity significantly decreased compared to the cisplatin-administered group.

The current histopathological findings confirmed the protective ability L-arginine against cisplatin ocular damage. This is parallel with immunohistochemical results which clarify the weak immunohistochemical iNOS expression

Conclusion

Results of this study clarified the promising oculo-protective effect of L-arginine against cisplatin-provoked toxicity through attenuating the inflammatory and oxidative damage. Moreover, iNOS is a crucial biomarker for inflammation and ocular damage in case of cisplatin toxicity and helpful in evaluation of the anti-inflammatory effect of L-arginine.

Conflict of interest

Regarding the publication of this manuscript, all authors declare that there are no conflicts of interest.

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