• Basic Research •

Pathophysiology of sildenafil-induced ocular toxicity in rats and treatment

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Abstract

● **AIM:** To examine the ocular toxicity linked to sildenafil usage and the possible protective benefits of adenosine triphosphate (ATP) against this toxicity in rats.

● **METHODS:** Twenty-four male albino Wistar-type rats were divided into four equal groups (*n*=6/group) as follows: healthy group (HG), ATP-only group (ATPG), sildenafil-only group (SILG), and ATP+sildenafil group (ATP+SLD). ATPG and ATP+SLD groups were injected intraperitoneally with ATP (4 mg/kg), while SILG and HG groups were injected with saline (0.9% NaCl) by the same route as a solvent. One hour after the administration of ATP and solvent, sildenafil (10 mg/kg) was administered orally to the SILG and ATP+SLD groups. This procedure was repeated once a day for 4wk. The animals were then sacrificed, eyeballs were removed and oxidant and antioxidant parameters were measured biochemically. Additionally, the ocular tissues were evaluated histopathologically.

● **RESULTS:** Sildenafil increased oxidant (malondialdehyde) levels and decreased antioxidant levels (total glutathione, superoxide dismutase, catalase) in rat ocular tissues and caused severe oxidative stress. In addition, sildenafil has been shown histopathologically to cause oxidative damage in retinal layers. ATP treatment suppressed oxidative stress and attenuated histopathological damage in the retinal layers.

● **CONCLUSION:** ATP protects retinal tissue against sildenafil-induced ocular oxidative damage in rats and may contribute to the development of novel approaches to prevent or treat this damage.

● **KEYWORDS:** adenosine triphosphate; ocular toxicity; oxidative stress; rats; retina; sildenafil

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INTRODUCTION

Sildenafil is a phosphodiesterase-5 (PDE-5) inhibitor drug utilized in the management and treatment of erectile dysfunction and pulmonary arterial hypertension[1]. This pharmacological action inhibits cyclic guanosine monophosphate (cGMP)-specific phosphodiesterases (PDEs), thus preventing the degradation of cGMP and elevating cGMP levels. This process regulates multiple physiological functions in the body, including neuroprotection, antinociception, synaptic plasticity, calcium homeostasis, and vasodilation[2]. The interaction of sildenafil with both the PDE-5 isoform and other PDE isoforms elucidates the drug's diverse pharmacological effects and the constraints on its application[3]. The existence of PDE-5 in vascular smooth muscle tissue induces adverse effects including headaches, flushing, dyspepsia, and nasal congestion[4]. A comprehensive study has highlighted concerns that sildenafil usage may result in severe side effects, including myocardial ischemia, stroke, and mortality[5]. Furthermore, it has been observed that sildenafil induces psychosis *via* an unidentified mechanism[6]. A review of the literature reveals that numerous case reports have linked sildenafil to adverse effects including dyspepsia, visual abnormalities, and hepatotoxicity, despite its general tolerability[7-8]. However, certain research indicate that

sildenafil may possess hepatoprotective characteristics owing to its antioxidant actions[9]. A study on isolated rat hearts indicated that sildenafil's effects on coronary blood flow and oxidative stress were dose-dependent and that it may provide a cardioprotective effect[10]. The complex impact profile of sildenafil may result in unforeseen consequences in tissues with elevated energy demands, such as the retina and choroid. Visual adverse effects include photopsias, color perception problems, retinal toxicity, and optic neuropathy, with certain instances resulting in irreversible vision loss[11-14]. It is considered that elevated formation of reactive oxygen species (ROS) in the retina results in oxidative stress, mitochondrial failure, and disruptions in energy metabolism[14]. Furthermore, diminished retinal blood flow, dysregulation of cGMP pathways, and localized inflammation are critical contributors causing retinal toxicity[12,15].

Adenosine triphosphate (ATP) is pivotal in energy metabolism, facilitating cellular processes *via* the mitochondrial energy production cycle and significantly contributing to the reduction of ROS levels[16]. The impact of sildenafil on energy metabolism and ATP production has been examined in multiple biological systems. Moon *et al*[17] revealed that sildenafil diminishes ATP production efficiency by elevating cGMP levels in skeletal muscle. Sildenafil has also been demonstrated to inhibit ATP release in urothelial tissue[18]. In metabolically active tissues like the retina, the conservation of ATP and the strengthening of antioxidant defense mechanisms may be crucial to alleviate the impacts of oxidative stress induced by sildenafil[9,19]. Case reports, clinical investigations, and biochemical research in the literature establish a basis for comprehending the detrimental effects of sildenafil on the visual system and the modulation of these effects *via* ATP. In this context, assessing the capacity of ATP to maintain retinal health and its potential role in mitigating damage induced by sildenafil may offer a novel approach for future therapeutic methods. The objective of our study is to thoroughly examine the ocular toxicity linked to sildenafil usage and the possible protective benefits of ATP against this toxicity.

MATERIALS AND METHODS

Ethical Approval Experimental procedures were done in the laboratories of the Experimental Animal Application and Research Centre of Erzincan Binali Yıldırım University. All procedures were carried out with the approval of the local ethics committee for animal experiments (Erzincan Binali Yıldırım University, Erzincan, Turkey; Meeting Date: 30.01.2025; Meeting Number: 2025/01; Decision Number: 03). All experiments were performed in compliance with the European Parliament and Council Directive 2010/63/EU (Number of approvals: 2016-24-199) and the ARRIVE directives[20].

Animals The experiment utilized twenty-four male albino Wistar-type rats, weighing between 280 and 292 g each and aged 9-10wk. All animals originated from the Experimental Animal Application and Research Center (Erzincan Binali Yıldırım University, Erzincan, Türkiye). The animals were randomly divided into four groups, each of which had a similar average body weight. Prior to the experiment, the rats were housed in groups of six in conventional laboratory wire cages (height: 20 cm; width: 35 cm; length: 55 cm; floor area: 1925 cm²) for acclimatization to the laboratory environment, under a 12-hour light/12-hour dark cycle, at a temperature of 22°C, and a humidity level of 30%-70%. The animals were provided with ad libitum access to tap water and a regular diet of pelleted food (experimental animal feed; Bayramoglu Stock Company, Erzurum, Türkiye).

Chemical Substances The chemical substances used in the experiment; thiopental sodium (Pental Sodyum® 0.5 g vial) was obtained from IE Ulagay (Istanbul, Türkiye), ATP (10 mg/mL vial) was supplied by Zdorovye Narodu (Kharkiv, Ukraine), sildenafil (Degra® 100 mg tablet) was obtained from Deva Holding (Istanbul, Türkiye).

Experimental Design The sample size was determined to utilize the smallest number of animals permissible in accordance with the 4R requirements. Criteria including slumped posture, diminished mobility, and injuries inflicted by other animals were employed to remove subjects throughout the experiment and data points during analysis. No exclusions occurred throughout the experiment. The random number table was utilized to generate the randomization sequence. Cages and animals are assigned numbers to reduce potential confounding variables.

Experimental Groups The rats designated for the experiment were categorized into four groups: healthy group (HG), ATP alone group (ATPG), sildenafil alone group (SILG), and ATP+sildenafil group (ATP+SLD).

Experimental Procedure ATP was administered intraperitoneally at a dosage of 4 mg/kg to the ATPG (n=6) and ATP+SLD (n=6). Both SILG (n=6) and HG (n=6) were supplied saline (0.9% NaCl) as a solvent in the same way. One hour subsequent to the administration of ATP and the solvent, sildenafil was orally delivered *via* an oral gavage at a dosage of 10 mg/kg to the SILG and ATP+SLD. This procedure was conducted once a day for a duration of 4wk. At the conclusion of this period, the animals were euthanized using a substantial dosage of anesthetic (50 mg/kg thiopental sodium), and the eye globes were excised. Oxidant and antioxidant parameters were assessed in the removed eye tissues. The tissues were subjected to histopathologic examination. The experimental outcomes obtained from the entire animal cohort were assessed by comparative analysis.

Biochemical Analysis

Sample preparation Tissues were rinsed in physiological saline solution, pulverized in liquid nitrogen, and subsequently homogenized. Supernatants were utilized for the analysis of malondialdehyde (MDA), total glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and proteins.

Quantification of MDA, GSH, SOD, and CAT The quantification of MDA, SOD, and GSH in tissues was conducted using the protocols provided by each respective rat enzyme-linked immunosorbent assay (ELISA) analysis kit (MDA product number: 10009055; tGSH product number: 703002; SOD product number: 706002; Cayman Chemical Co., Ann Arbor, Michigan, USA). The determination of CAT was conducted following the methodology established by Goth[21]. The protein concentration was quantified using spectroscopy at 595 nm utilizing the Bradford method[22].

Histopathological Analysis All of the ocular tissue samples were first soaked in a 10% formaldehyde solution for 72h for evaluation by light microscopy. Tissue samples were placed in cassettes and kept under running water for 24h, at the end of the period. The samples were then treated with conventional grades of alcohol (70%, 80%, 90%, and 100%) for removal of water from the tissues. The tissues were then processed through xylol and embedded in paraffin. From the obtained paraffin blocks, 4-5-micron thick sections were cut and stained with hematoxylin-eosin. Photographs were taken and evaluated using the Olympus DP2-SAL firmware software Ver. 3.3.1.198 program (Olympus® Inc., Tokyo, Japan) at 400× magnification. Inner plexiform layer (IPL), inner nuclear layer (INL), outer nuclear layer (ONL), and total retinal (TR) thickness measurements and ganglion cell numbers of ganglion cell layer (GCL) were calculated using measurement tools of the Image J program. Histopathological changes of retinal tissue were defined as the presence of retinal destruction (foamy appearance in the rod-cone layer, intracellular vacuolization, condensation of nuclei, and increased heterochromasia), edema (decrease in intercellular connections and increase in intercellular space), vascular congestion (increased density of the vasculature and erythrocyte accumulation on the vessel wall), and polymorphonuclear cell infiltration (accumulation of inflammatory cells). The histopathological scoring for each tissue sample, a tool used to obtain semi-quantitative data from tissues, was scored for each criterion as follows: 0, no damage; 1, mild damage; 2, moderate damage; 3, severe damage. A modified histopathologic evaluation of the retina was performed by a blinded histologist using the method of Gibson-Corley *et al*[23].

Statistical Analysis IBM SPSS Statistical Program for Windows (IBM Corp., V27.0, 2020 Release, Armonk, New York, USA) was used for all statistical analyses. Figures were produced with the GraphPad Prism software (GraphPad Software, V8.0.1, 2018 edition, San Diego, California, USA). All data are stated as mean±standard deviation or median (minimum-maximum). The normality assumption was tested using the Shapiro-Wilk test. In order to evaluate the mean differences between the groups, one-way analysis of variance (ANOVA) or Welch's ANOVA test was used if the normality assumption was met. If the assumption of normality was met, the assumption of homogeneity of variances was tested using Levene's test. If the assumption of homogeneity of variances was met, the Tukey's honestly significant difference (HSD) test was used; if not, the Games-Howell test was used. In cases where the normality assumption was not met, the Kruskal-Wallis test, a non-parametric method, was used to determine the disparity between groups in terms of median values. Dunn's test with Bonferroni correction was used for pairwise comparisons between groups and adjusted *P*-values were provided. Statistical significance was defined as a probability value of $P<0.05$.

RESULTS

Biochemical Findings

Outcomes of MDA and tGSH analysis in ocular tissue Figure 1 and Table 1 illustrated that the MDA level in the ocular tissues of rats treated with ATP alone was significantly lower ($P<0.001$) than that of the HG group. The MDA level in the eye tissue of animals administered sildenafil alone was significantly elevated compared to the healthy group ($P<0.001$) and the group treated with ATP alone ($P<0.001$). ATP markedly inhibited the elevation of MDA induced by sildenafil ($P<0.001$).

The administration of ATP has resulted in elevated tGSH levels in eye tissue ($P<0.001$). The disparity in tGSH levels between the HG group and the ATP-only group was significant ($P<0.001$). Furthermore, ATP markedly ($P<0.001$) inhibited the decrease of tGSH induced by sildenafil.

SOD and CAT analyses of ocular tissue Figure 2 and Table 1 illustrated that ATP elevated the activities of SOD and CAT in the ocular tissues of the ATP-treated group. In the ATP-only group, SOD and CAT activities were significantly ($P<0.001$) elevated compared to HG, while ATP also significantly ($P<0.001$) mitigated the reduction in SOD and CAT activities induced by sildenafil.

Histopathological Findings In the histopathological examination were evaluated as histopathological grading data, the thickness of retinal layers and the number of ganglion cells in the ganglion cell layer and are presented in Table 2.

Upon assessment of retina specimens from the HG (Figure 3A) and ATPG (Figure 3B) animal cohorts, it was noted that GCL, IPL, INL, outer plexiform layer (OPL), ONL, and photoreceptor layer (PRL) exhibited normal morphology and thickness. Cell nuclei in the INL and ONL layers were identified as basophilic and the cells had a regular morphology.

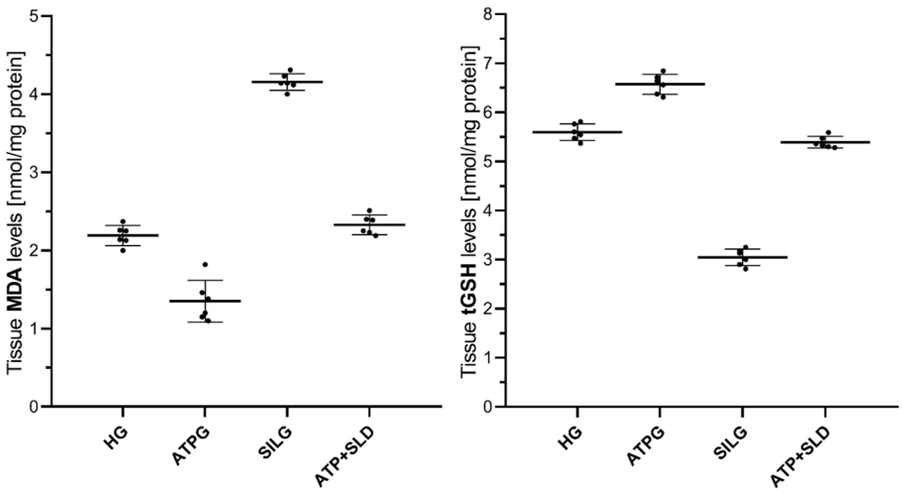

Figure 1 Effects of sildenafil and ATP on MDA and tGSH levels in rat ocular tissues Data were expressed as mean±standard deviation. Statistical analyses were performed using the one-way ANOVA test followed by Tukey's honestly significant difference (HSD) test as posthoc. ATP: Adenosine triphosphate; HG: Healthy group; ATPG: ATP-only group; SILG: Sildenafil-only group; ATP+SLD: ATP+sildenafil group; MDA: Malondialdehyde; tGSH: Total glutathione.

Table 1 Sildenafil and ATP effects on rat ocular tissue oxidants and antioxidants

Group comparisons	*P*-values of biochemical variables			
	MDA (nmol/mg protein)	tGSH (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
HG *vs* ATPG	<0.001	<0.001	<0.001	<0.001
HG *vs* SILG	<0.001	<0.001	<0.001	<0.001
HG *vs* ATP+SLD	0.516	0.187	0.755	0.106
ATPG *vs* SILG	<0.001	<0.001	<0.001	<0.001
ATPG *vs* ATP+SLD	<0.001	<0.001	<0.001	<0.001
SILG *vs* ATP+SLD	<0.001	<0.001	<0.001	<0.001
F value	291.722	477.669	205.130	579.696
df1/*df2*	3/20	3/20	3/20	3/20
P	<0.001	<0.001	<0.001	<0.001

Statistical analyses were performed using one-way ANOVA test followed by Tukey's honestly significant difference (HSD) test as a post-hoc test. For all groups *n*=6. ATP: Adenosine triphosphate; SLD: Sildenafil; HG: Healthy group; ATPG: ATP-only group; SILG: Sildenafil-only group; ATP+SLD: ATP+sildenafil group; MDA: Malondialdehyde; tGSH: Total glutathione; SOD: Superoxide dismutase; CAT: Catalase; df: Degree of freedom.

Table 2 Evaluation of histopathological parameters in rat retinal tissue

Histopathological parameters	HG (*n*=36)	ATPG (*n*=36)	SILG (*n*=36)	ATP+SLD (*n*=36)	*F* or *H*	*P*
Histopathological grading data						
Destruction	0 (0-0)	0 (0-1)	3 (2-3)[a,b]	0 (0-1)[c]	113.564[e]	<0.001[f]
Edema	0 (0-0)	0 (0-1)	3 (2-3)[a,b]	0 (0-2)[c]	115.086[e]	<0.001[f]
Vascular congestion	0 (0-0)	0 (0-1)	3 (2-3)[a,b]	0 (0-1)[c]	117.544[e]	<0.001[f]
PNL cell infiltration	0 (0-0)	0 (0-1)	2 (1-3)[a,b]	0 (0-1)[c]	112.846[e]	<0.001[f]
Retina thickness of layers (µm)						
Inner plexiform layer	40.64±1.37	40.78±1.43	64.77±0.80[a,b]	41.28±1.01[c]	5886.846[d]	<0.001[g]
Inner nuclear layer	22.30 (21.30-23.60)	22.40 (21.30-24.30)	33.50 (31.30-36.20)[a,b]	22.90 (21.40-25.10)[a,b,c]	95.205[e]	<0.001[f]
Outer nuclear layer	45.90 (44.60-48.30)	46.10 (44.60-48.30)	56.75 (53.20-59.10)[a,b]	47.80 (45.20-49.60)[a,b,c]	98.469[e]	<0.001[f]
Total retina	152.85 (150.90-154.90)	152.10 (148.80-153.90)	206.75 (200.20-209.60)[a,b]	154.35 (148.80-156.90)[b,c]	99.160[e]	<0.001[f]
Number of ganglion cells in the ganglion cell layer	8 (7-9)	8 (7-9)	5 (3-6)[a,b]	8 (7-9)[c]	87.839[e]	<0.001[f]

[a]*P*<0.05 *vs* HG; [b]*P*<0.05 *vs* ATPG; [c]*P*<0.05 *vs* SILG; [d]Welch ANOVA; [e]The test statistic is adjusted for ties; [f]Kruskal-Wallis test, Dunn's test with Bonferroni correction, median (maximum-minimum); [g]Welch ANOVA, Games-Howell test, mean±standard deviation. ATP: Adenosine triphosphate; SLD: Sildenafil; HG: Healthy group; ATPG: ATP-only group; SILG: Sildenafil-only group; ATP+SLD: ATP+sildenafil group; PNL: Polymorphonuclear leukocyte.

Blood vessels were infrequently detected in the GCL and IPL layers.

In the study group administered sildenafil monotherapy, significant degenerative alterations were noted in the retinal

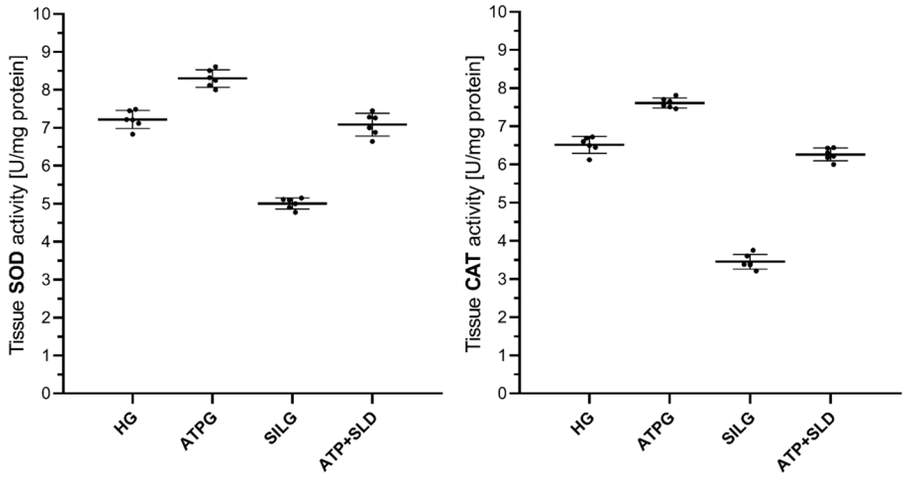

Figure 2 Effects of sildenafil and ATP on SOD and CAT activities in rat ocular tissues Data were expressed as mean±standard deviation. Statistical analyses were performed using the one-way ANOVA test followed by Tukey's honestly significant difference (HSD) test as posthoc. ATP: Adenosine triphosphate; HG: Healthy group; ATPG: ATP-only group; SILG: Sildenafil-only group; ATP+SLD: ATP+sildenafil group; SOD: Superoxide dismutase; CAT: Catalase.

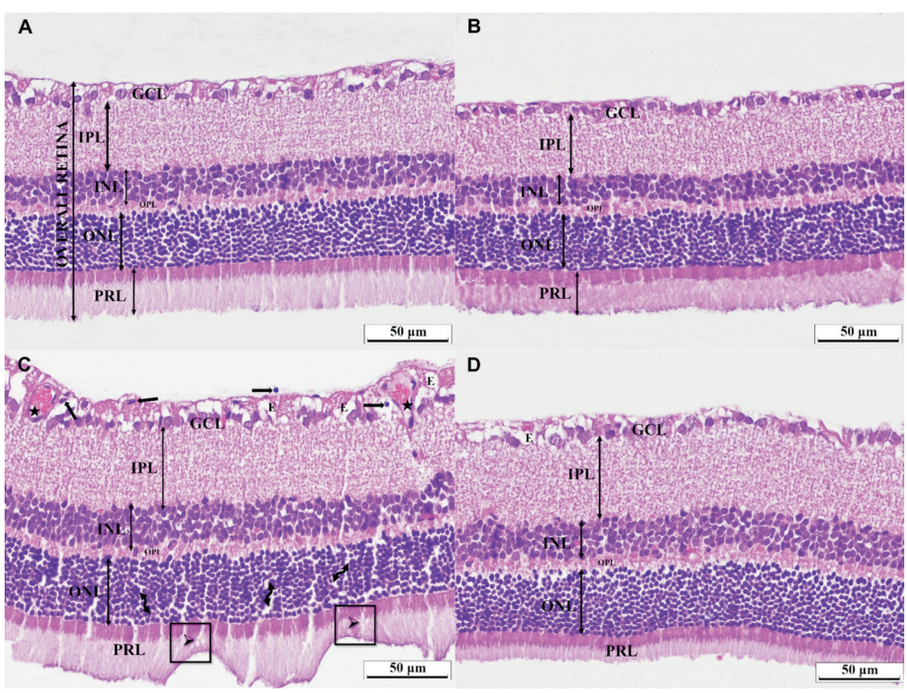

Figure 3 Histopathological appearance of retinal tissue in each experimental group A: Retinal tissue of HG group (H&E ×400); B: Retinal tissue of ATPG group (H&E ×400); C: Retinal tissue of SILG group; Black arrow: Polymorphonuclear cell; E: Edema; Black star: Dilated and congested blood capillaries; Wavy arrow: Dissociations in cells; Arrowhead: Vacuolization areas (H&E ×400); D: Retinal tissue of ATP+SLD group; E: Mild edema, (H&E ×400). GCL: Ganglion cell layer; IPL: Inner plexiform layer; INL: Inner nuclear layer; OPL: Outer plexiform layer; ONL: Outer nuclear layer; PRL: Photoreceptor layer; ATP: Adenosine triphosphate; HG: Healthy group; ATPG: ATP-only group; SILG: Sildenafil-only group; ATP+SLD: ATP+sildenafil group; H&E: Hematoxylin and eosin.

tissue. Marked edema and considerable thickening were noted in all retinal layers. The cells of the GCL layer were observed to be markedly irregular and considerably diminished in quantity. Severe dilatation and congestion were observed in the blood vessels of this layer, with a notable presence of polymorphonuclear cells around the vessels. Irregularities were noted in the interconnections between the cells of the inner nuclear layer (INL) and outer nuclear layer (ONL). The photoreceptor layer exhibited a dense foamy appearance along with regions of vacuolization (Figure 3C).

The retinal tissue samples from the sildenafil group treated with ATP (ATP+SLD) exhibited mild and relatively uncommon edema. All retinal layers were found to be comparable to those of the HG, with ganglion cells and blood vessels in the GCL layer exhibiting a typical configuration. The cells in the INL and ONL layers were found to be better ordered and exhibited

basophilic staining. No foamy appearance or vacuolation was noted in the photoreceptor layer, and this appearance was similar to that of the HG (Figure 3D).

DISCUSSION

This study investigates the impact of sildenafil on ocular toxicity and the potential protective function of ATP in this toxicity. The results demonstrate that sildenafil elevates oxidative stress in ocular tissues and suppresses the antioxidant defense system. The notable elevation in MDA levels, along with the reduction in SOD, CAT, and tGSH levels, indicates that sildenafil induces oxidative stress in ocular tissue. Research on the retinal toxicity of sildenafil indicates that visual adverse effects mostly result from the suppression of PDE-5 and, to a lesser degree, PDE-6[24-26]. PDE-5 inhibition elevates intracellular cGMP levels, which modulates vasodilation by inducing relaxation of smooth muscles in arterioles[27]. The PDE-5 enzyme is present in multiple organs and tissues[28]. Furthermore, it has been demonstrated that PDE-5 affects the retina and choroidal arteries, regulating the expansion of the ganglion and bipolar cell layers, which are essential for the processing of visual information[24,29]. The PDE-6 enzyme is present in cone and rod cells and is crucial for the phototransduction of retinal light signals[30]. A dose-controlled clinical investigation has indicated that partial inhibition of PDE-6 is linked to adverse effects, including compromised color discrimination and diminished scotopic responses, associated with sildenafil[31]. In addition to this pharmacological action profile, the adverse effects of sildenafil on mitochondrial functioning are thought to cause energy shortage and oxidative stress in metabolically active organs, including the retina[32-33]. Research regarding the impact of sildenafil on oxidative stress yields inconclusive results. Certain studies highlight the antioxidant properties of sildenafil in diverse tissues[34-37], but others indicate that it induces an oxidative stress environment, particularly at elevated doses[33,38]. Mitochondrial dysfunction induced by sildenafil leads to disruption of the electron transport chain, resulting in the accumulation of ROS and an environment of oxidative stress[38]. MDA, generated by the oxidation of polyunsaturated lipids by ROS, is a highly toxic substance[39]. MDA has served as a biomarker for oxidative stress in ocular tissue[40]. Present study observed that sildenafil administration significantly increased MDA levels in the ocular tissues of rats. Literature on the damage caused by sildenafil in ocular tissue are generally limited to case reports, and there is no direct evidence that it increases MDA levels in animal experiments[8,11,13]. However, our findings are consistent with those of studies conducted on different tissues. Hafez and El-Kazaz[33] reported that a high dose of sildenafil (10 mg/kg) increased MDA levels in hippocampus tissue. In a similar vein, Laila *et al*[38] demonstrated that administering 10 mg/kg of sildenafil to rats significantly increased MDA levels in liver and testicular tissues.

In addition, the findings of our study show that sildenafil suppresses antioxidant tGSH levels and decreases the activities of ROS scavenging enzymes SOD and CAT in ocular tissue. GSH is a non-protein intracellular thiol and one of the major non-enzymatic antioxidants that can detoxify OH- radicals by giving electrons[33]. GSH, SOD and CAT are the main components of the antioxidant defense system developed against the harmful effects of ROS in ocular tissues[19]. When the present literature was reviewed, no direct experimental finding was found that sildenafil suppresses the antioxidant defense system in ocular tissue. However, the presence of this condition in different tissues has been shown in various studies[33,38]. In this context, our findings indicate that the oxidant/antioxidant balance in the retinal tissue of sildenafil-treated animals was altered in favor of oxidants, suggesting possible oxidative damage. However, some studies have reported results that contradict our findings. Various studies have highlighted that sildenafil mitigates endothelial dysfunction in rats by decreasing MDA levels in endothelial tissue and enhancing SOD activity[35]. Likewise, Sheweita *et al*[36] indicated that sildenafil stimulated SOD and CAT activities in hepatic and testicular tissues, leading to a reduction in MDA levels. A separate investigation assessing the impact of sildenafil on ocular tissues demonstrated that oxidative stress in photoreceptors, mostly rod cells resulting from sildenafil, did not correlate with the deterioration of cone-mediated visual function in wild-type mice[41]. In the light of all this literature, it is clear that oxidative stress indicators alone cannot fully reflect retinal toxicity and further studies supported by functional tests [*e.g.*, electroretinography (ERG)], imaging techniques [*e.g.*, optical coherence tomography (OCT)] and molecular analysis are planned.

Retinal ganglion cells are neurons that require substantial energy owing to their considerable size and the frequent transmission of visual signals. This circumstance requires effective energy generation *via* ATP[42-43]. Furthermore, ATP is recognized for its role in energy generation as well as in facilitating ROS clearance and the synthesis of antioxidants[44]. Previous investigations have also highlighted the protective effects of ATP on the retina[45-46]. ATP is shown to support mitochondrial processes, sustain energy balance, and mitigate the adverse effects of oxidative stress in ocular tissue[47]. Prior research has demonstrated that sildenafil impedes mitochondrial ATP generation, resulting in oxidative stress and a disruption of cellular energy equilibrium[48-49]. The literature presents varying perspectives on the adverse effects of sildenafil on the retina. Certain studies indicate that PDE-5

inhibition may provide positive effects by enhancing retinal blood flow[12,15], whilst others imply that prolonged use elevates the risk of retinal toxicity and could result in irreversible vision loss[8,11,13]. Our experimental findings demonstrate that elevated oxidant levels and reduced antioxidant levels in the ocular tissues of rats treated with sildenafil were suppressed by ATP, and alleviate the retinal damage.

Histopathological investigations indicate that sildenafil induces substantial degenerative alterations in retinal tissue. In the sildenafil-treated group, retinal edema, cellular disarray, and vascular alterations were observed to have dramatically increased. Notable cell loss and vascular dilatation in the ganglion cell layer underscore the severity of sildenafil-induced retinal damage. The presence of foamy formations and vacuolization in the photoreceptor layer is regarded as a pathogenic alteration linked to cellular death and oxidative stress[50]. The fact that sildenafil causes hypoxia by disrupting blood flow in the retinal vasculature and its effects on cGMP signaling pathways may play a critical role in this toxic process[51]. Literature comprises case studies demonstrating that particularly high-dose and prolonged administration of sildenafil results in retinal toxicity, including photoreceptor malfunction, increased choroidal vascular permeability, and increased thickness[8,24,52-53]. Furthermore, Shams and Hashish[54] revealed that prolonged, daily administration of sildenafil in male rats resulted in pathological alterations in the superior colliculus, potentially leading to visual impairment. Eltony and Abdelhameed[26] similarly reported that prolonged sildenafil administration is marked by vacuolization, congested blood capillaries, and a thicker basal lamina in the retina and optic nerve. The findings, which coincide with our study, also demonstrate a decrease in the cell population within the GCL. Zahavi *et al*[55] observed retinal vascular dilatation, heightened choroidal effusion, and loss of retinal ganglion cells in mice subsequent to sildenafil administration. Our histopathology findings indicate that ATP administration markedly mitigated sildenafil-induced retinal damage. The externally administered ATP mitigated the histopathological damage associated with sildenafil. Prior research has demonstrated that ATP facilitates mitochondrial functioning in retinal cells and diminishes oxidative stress levels[45-46].

This study has limitations. Our study evaluated a single administration dose of sildenafil, which does not yield adequate data regarding potential retinal alterations that may arise from prolonged usage of varying levels. The protective effect of ATP against sildenafil-induced ocular damage has been examined in a single dose. Furthermore, enhanced imaging modalities and immunohistochemistry evaluations may be required to comprehensively determine the correlation between histopathological analyses and biochemical data. Additionally, this study only evaluated biochemical and histopathological parameters. In future studies, supporting the ocular toxicity of sildenafil with functional and structural tests such as electrophysiologic methods (*e.g.* ERG) and OCT will increase the validity of the findings. Furthermore, *in vitro* cellular toxicity analyses and the elaboration of ATP's pharmacodynamic properties will contribute to a better understanding of this compound's therapeutic potential.

In conclusion, our experimental findings indicate that ATP protects retinal tissue from oxidative damage induced by sildenafil. While the literature presents varying findings on the impact of sildenafil on the retina, our investigation reveals that sildenafil induces oxidative damage in retinal tissue and inhibits the antioxidant defense mechanism. The acquired data may aid in the formulation of novel strategies for the prevention or treatment of retinal damage linked to sildenafil usage. These findings may establish a crucial basis for comprehending the potential risks of sildenafil on the visual system and for formulating novel therapeutic techniques. Future extensive biochemical and molecular investigations may yield more robust evidence for clinical applications by explaining the protective mechanisms of ATP in retinal cells more extensively.

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Data Availability: All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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