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The Effect of Sildenafil on Selenite-Induced Cataract in Rats

Hatice Tuba Atalay^a, Ahmet Yucel Ucgul^b, Ummuhani Ozel Turkcu^c, Mehmet Cuneyt Ozmen^a, Samet Yilmaz^d, and Ayse Bilgihan^d

^aDepartment of Ophthalmology, Gazi University Medical School, Ankara, Turkey; ^bDepartment of Ophthalmology, Izzet Baysal Training and Research Hospital, Abant Izzet Baysal University, Bolu, Turkey; ^cDepartment of Medical Biochemistry, Training and Research Hospital, Mugla Sitki Kocman University, Mugla, Turkey; ^dDepartment of Medical Biochemistry, Gazi University Medical School, Ankara, Turkey

ABSTRACT

Purpose: To investigate the effect of sildenafil on an experimental sodium selenite-induced cataract model in rats.

Materials and Methods: Twenty-six young Wistar rats were separated into four groups. On postpartum day 10, six rats received only selenite (group 1, selenite-induced cataract), seven rats received selenite and high dose oral sildenafil (group 2, high-dose sildenafil-treated), seven rats received selenite and low dose oral sildenafil (group 3, low-dose sildenafil-treated), and six rats received only saline (group 4, controls). On postpartum day 30, cataract formation was graded and recorded using an operating microscope. The rats were sacrificed, lens tissues were isolated, and serum samples were collected. Nitrite oxide metabolites (NOx), advanced oxidative protein products (AOPP), and total sulfhydryl (TSH) levels were assessed in both serum and lenticular samples.

Results: The rats treated with low-dose sildenafil showed lower levels of AOPP and NOx, and the higher levels of TSH than the rats in other experimental groups. Otherwise, the rats treated with high-dose sildenafil, similar to the selenite-induced cataract group, showed higher levels of AOPP and serum NOx than rats in the low-dose sildenafil-treated group. The rats treated with low-dose sildenafil also showed less cataract development than rats in the other experimental groups.

Conclusion: Low doses (0.7 mg/kg) of oral sildenafil might show a protective effect on cataract development by lowering oxidative stress.

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AOPP; cataract model; NOx; selenite; sildenafil; total sulfhydryl

Introduction

Lens opacification, otherwise known as cataract, is the most widely recognized reason for curable blindness in the world's population. It represents around 42% of all blindness.¹ Today, cataract treatment is provided only through surgery in which an artificial lens is implanted instead of the cataractous lens, which is removed to restore visual acuity. The rising cost of cataract surgery and surgical complications have prompted scientists to investigate ways to prevent cataract development. Therefore, in the last decades, preventive approaches for cataract formation have increasingly been presented.^{2,3}

The development of cataract is not yet fully understood. However, several studies have shown that oxidative stress plays a key role in the etiopathogenesis of cataracts.^{4,5} Based on this evidence, the importance of strengthening antioxidant activity is underlined to prevent or delay cataract development. Accordingly, many natural products with antioxidant properties have been tried for this purpose.⁶

Many agents have been used to create a cataract formation. However, the selenite induced cataract model is the most widely accepted method today. Selenium (Se) is an indispensable rare-earth element for humans, animals and a few microscopic organisms.⁷ It is present in the structure of selenocysteine, an essential amino acid with antioxidant properties.^{8,9} However, ironically, in 1978, Ostadalova et al

first demonstrated that overdose of selenite caused rapid cataract development by increasing oxidative stress.¹⁰ In the laboratory, the selenite cataract model could be induced by a single subcutaneous injection of excess sodium selenite (Na₂SeO₃) into the suckling rats on postpartum day 10. This model is usually presented with severe bilateral cataract development within 16 days after eye opening in rats, and could be used as a model for evaluating anti-cataract agents.

The selenite cataract is similar to the human senile cataract sharing some characteristics such as an increase in free cytosolic calcium and protein aggregates and a decrease in water-soluble protein and reduced glutathione (GSH). However, it is different from human senile cataract in some characteristics such as increased disulfide formation or the absence of high-molecular-weight covalent aggregates.¹¹ Additionally, rapid calpain-induced proteolytic deposition seems to predominate in the formation of selenite cataracts, whereas human senile cataracts are mainly caused by prolonged oxidative stress.¹²

Sildenafil citrate (Pfizer, Viagra 100 mg), a prototypical inhibitor phosphodiesterase type 5 (PDE-5), converts cyclic guanosine monophosphate (cGMP) to its inactive form in blood vessels.¹³ Sildenafil increases levels of cellular cGMP, resulting in smooth muscle relaxation and increased blood flow to the entire body. Previous studies have demonstrated that sildenafil may have anti-inflammatory effects by

activating antioxidant enzymes, reducing malondialdehyde (MDA) levels, and inhibiting reactive oxygen species (ROS).^{14,15} Furthermore, numerous studies have revealed that sildenafil might be protective against ischemic damage in organs, for example, the testes and kidneys.^{16,17}

In this study, we aimed to evaluate whether sildenafil had a protective effect on selenite cataract formation and the levels of oxidative stress markers, including advanced oxidative protein product (AOPP) and nitric oxide metabolites (NOx) in both the blood and the lens. Total sulfhydryl (TSH) levels were also assessed as a marker of an antioxidant system in lenses and serum samples.

Materials and methods

The present study was approved by the Animal Experiments Local Ethical Committee at the University of Gazi (Ankara, Turkey). The study was conducted in accordance with the Helsinki Declaration for the use of animals in ophthalmic and vision research in Gazi University Laboratory Animals Breeding and Experimental Researches Centre and Eye Diseases Department.

Animals

Twenty-six postnatal (10-day-old) Wistar rats were separated into four groups according to the amount of sildenafil and sodium-selenite they received. Group 1 (n = 6) received only subcutaneous sodium selenite (30 nmol/g), group 2 (n = 7) received subcutaneous sodium selenite and a single dose of high-dose (1.4 mg/kg) oral sildenafil citrate, group 3 (n = 7) received subcutaneous sodium selenite and a single dose of low-dose (0.7 mg/kg) oral sildenafil citrate, and group 4 (n = 6) received a subcutaneous balanced saline solution and served as the control group. Rats were housed with ad-libitum access to food and water under a 12:12 hour light–dark cycle. Euthanasia was performed by cardiac puncture under deep anesthesia at the postnatal 30th day. Intramuscular ketamine and xylazine were used for deep anesthesia. Blood and lens samples were collected following euthanasia.

Evaluation of lens

Both eyes of the rats were dilated with tropicamide 1%, and were then examined using a slit-lamp biomicroscope (TOPCON, Tokyo, Japan) and photographed under coaxial illumination of an operating microscope (Leica Microsystem, Morrisville, NC) just before the euthanasia. A single

researcher (HTA), who was blinded to the treatment type, evaluated and noted the cataract levels. Cataract levels in lenses were graded as follows: 0- absence of cataract (completely clear lens), 1- partially developed cataract, 2-mature cataract (Figure 1).

The extracted lens tissues were placed in liquid nitrogen and then kept in a freezer at -80°C degrees until required for analysis. The intracardiac blood samples were centrifuged and serum samples were separated and kept in a freezer at -80°C degrees until required for analysis. Advanced oxidative protein product (AOPP) levels, total sulfhydryl (TSH) levels and nitric oxide metabolites (NOx) levels were analyzed in both lens and serum samples.

NOx assay

NOx (nitrite-nitrate) of lens tissues and serum were measured using the spectrophotometric method of Miranda et al.¹⁸ Lens tissues were homogenized in phosphate buffer (pH: 7.4) and deproteinized with 96% ethanol at 1:2 (v/v). Homogenates were vortexed for 5 minutes and then mixed samples were centrifuged at 10,000 g at 4°C . Then, the collected supernatants were analyzed to detect NOx levels.

For NOx analysis in tissues, 100 μL of supernatant, 100 μL of vanadium (III) chloride and the following Griess reagents, 50 μL of sulfanilamide and 50 μL of N-(1-naphthyl) ethylenediamine dihydrochloride (NEED) were pipette into each microplate well and then incubated for 30 minutes at 37°C . The absorbance of NOx was read at 540 nm. NOx levels were calculated from the linear standard curves prepared by sodium nitrate (10–100 μM range). The results of NOx for lens tissue and serum were expressed as mM/g and Mmol/L, respectively.

TSH levels

Serum and lens tissues were homogenized in the phosphate buffer at pH 7.4 and then homogenates were centrifuged at 10,000 g for 10 minutes. Supernatants were mixed with an equal volume of 6% sodium dodecyl sulphate (SDS). Then, 200 μL of 0.25 M Tris-HCl, pH 8.2, containing 20 mM EDTA, 25 μL of the samples with SDS or serum, and 10 μL of Ellman's reagent were pipette into microplate wells respectively, and incubated for 15 minutes at room temperature. After incubation, the plate was read at 412 nm using a microplate reader. The TSH levels of serum and lens tissue were calculated from a linear standard curve constructed using



Figure 1. Grading of the rat lenses. A-Clear lens (grade 0), B-Partially developed cataract (grade 1), C-Mature cataract (grade 2).

reduced glutathione. The results of TSH for lens tissue and serum were expressed as $\mu\text{M}/\text{mg}$ and $\mu\text{mol}/\text{L}$, respectively.

AOPP assay

AOPP levels in serum were measured according to the spectrophotometric method of Witko-Sarsat et al.¹⁹ To measure the levels of AOPP of serum, 200 μL of serum was reconstituted with phosphate-buffered saline (PBS). After that, 10 μL of potassium iodide and 20 μL of acetic acid were added. The samples were vortexed, and a spectrophotometric measurement was obtained with PBS as a blank at 340 nm absorbance. Dilutions of the chloramine T standard (0–100 μM) were used for calculation of sample concentration. The results of AOPP for lens tissue and serum were expressed as $\mu\text{M}/\text{mg}$ and $\mu\text{mol}/\text{L}$, respectively.

Statistical analysis

The SPSS software for Windows version 22.0 (Chicago, IL) were used for statistical analysis. The results were shown as median (minimum-maximum). Shapiro-Wilk test was used to determine whether the continuous variables were parametric or non-parametric. Kruskal-Wallis variance analysis was performed to determine whether there were significant differences of markers of the serum and lens samples among the four groups. Tamhane's T2 post hoc test was also used for pairwise comparisons. Fisher's exact test was performed for the pairwise comparison of cataract development between the groups. A p value less than 0.05 was accepted as statistically significant.

Results

Morphologic evaluation of cataract formation

In the selenite-induced cataract group, cataracts developed in all lenses, with the majority of them being mature; this

indicates success in the creation of the selenite-induced cataract model in the present study. In the low-dose sildenafil-treated group, cataracts developed partially in 8 eyes, and 6 eyes were completely clear. In the high-dose sildenafil-treated group, cataracts developed partially in 10 eyes and 4 eyes experienced mature cataracts. In the control group, all lenses were clear. The results of cataract development in the rats are shown in Figure 2.

Evaluation of indicators of protein oxidation

The collected data of AOPP, TSH and NOx levels in serum samples are shown in detail in Figure 3. Serum AOPP, TSH and NOx levels were significantly different between the four groups ($p < .001$, $p < .001$, and $p < .001$, respectively). The rats in group 3 had lower serum AOPP levels than those in groups 1 and 2 ($p = .002$ and $p = .004$, respectively). The rats in groups 1 and 2 had similar serum AOPP levels ($p = .421$). The rats in group 3 had higher serum TSH levels than those in groups 1 and 2 ($p = .005$ and $p = .004$, respectively). The rats in groups 1 and 2 had similar serum TSH levels ($p = .672$). Serum NOx levels in group 2 were significantly higher than those in groups 1 and 3, ($p = .002$ and $p = .004$, respectively). The rats in groups 1 and 3 had similar serum NOx levels ($p = .364$). Group 4, the control group, had the highest serum TSH levels and the lowest serum AOPP and serum NOx levels among the four groups.

The collected data of AOPP, TSH and NOx levels in lens tissues are shown in detail in Figure 4. Lens AOPP, TSH and NOx levels were significantly different between the four groups ($p = .001$, $p = .001$, and $p < .001$, respectively). The rats in group 2 had the higher lens AOPP levels than those in groups 3 and 4 ($p = .003$ and $p = .002$, respectively). The rats in groups 3 and 4 had similar lens AOPP levels ($p = .978$). The rats in group 3 had higher lens TSH levels than those in groups 1 and 2 ($p = .003$ and $p = .050$, respectively). The

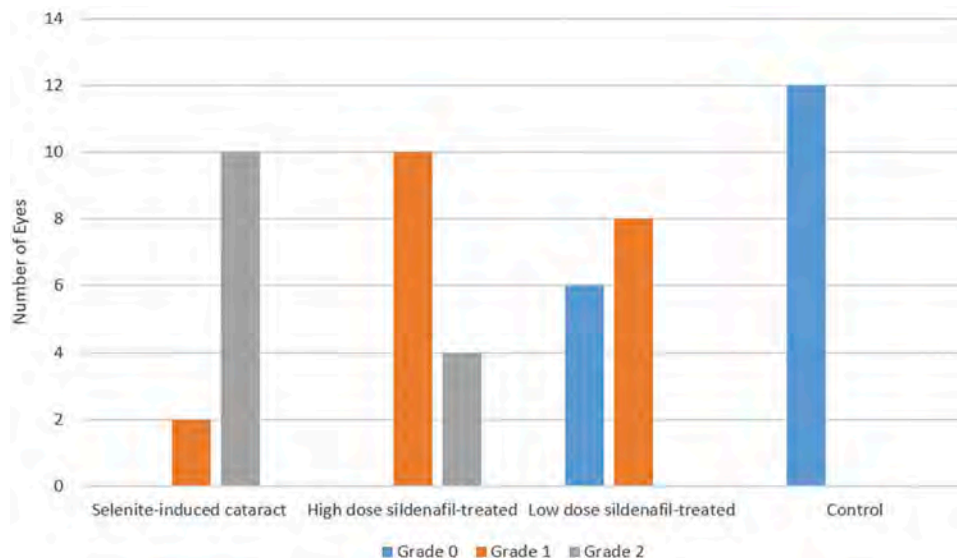


Figure 2. Morphological evaluation of cataract formation of isolated lenses in each group.

Fisher Exact Test was used for pairwise comparison.(Group 1 versus Group 3, $p < .001$. Group 2 versus Group 3, $p = .005$. Group 2 versus Group 4, $p < .001$. Group 1 versus Group 2, $p = .008$. Group 3 versus Group 4, $p = .002$. Group 1 versus Group 4, $p < .001$).

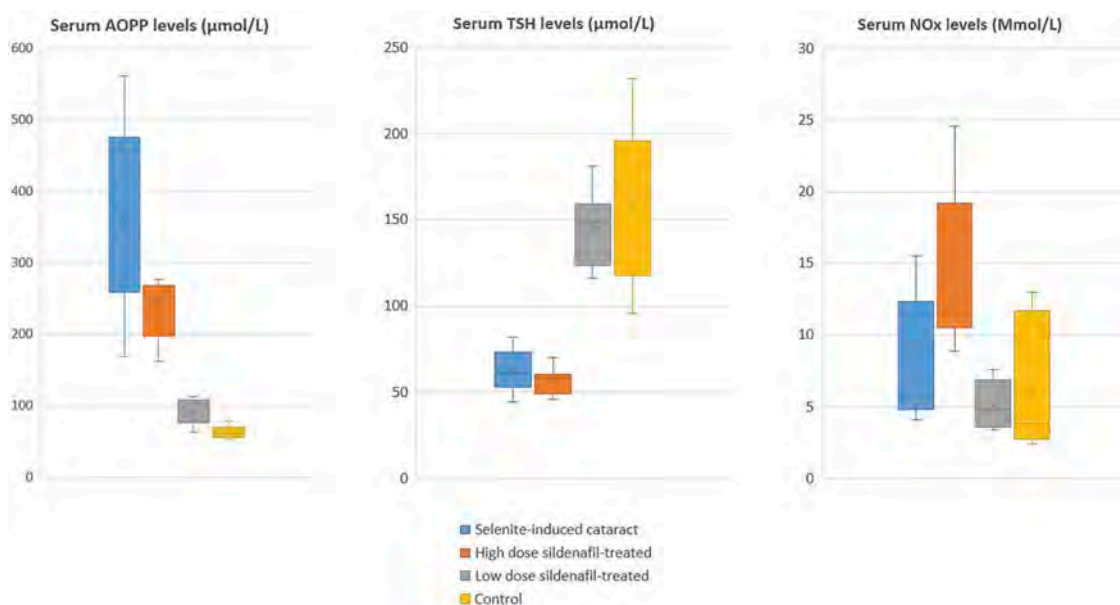


Figure 3. Levels of AOPP, TSH and NOx in serum samples.

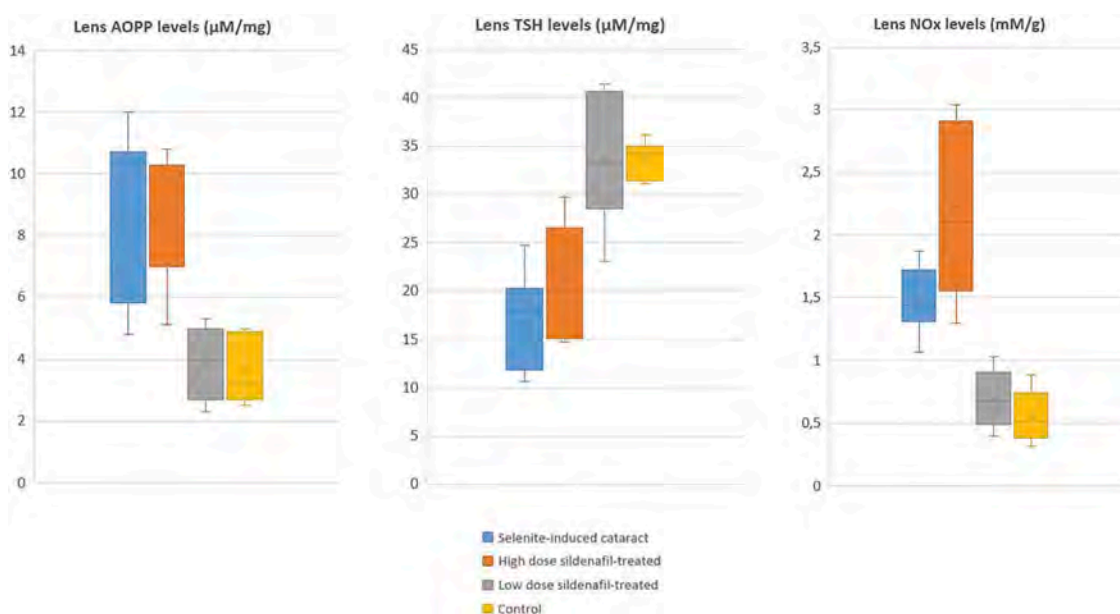


Figure 4. Levels of AOPP, TSH and NOx in lens samples.

rats in groups 1 and 2 had similar lens TSH levels ($p = .442$). The rats in group 3 had lower lens NOx levels than those in groups 1 and 2 ($p = .003$ and $p = .003$, respectively). The rats in groups 3 and 4 showed similar lens NOx levels ($p = .964$).

Discussion

Although selenium is an important trace element, it is well-known that high concentrations of selenium might be toxic. The potential mechanism of selenium toxicity is not fully understood; however, it is often ascribed to its capability to induce oxidative stress. Developing nuclear cataracts following administration of sodium selenite is proposed to be

a consequence of lens glutathione (GSH) loss.²⁰ Loss of GSH decreases the redox buffering capacity and increase the oxidative stress sensitivity of the crystalline lens. Another proposed mechanism is that the loss of intracellular calcium (Ca^{2+}) homeostasis could lead to the development of cataracts by increasing cytosolic Ca^{2+} levels and thus activating calpain. Previous studies have shown that calpain-induced β -crystalline proteolysis could play an important role in lens maturation and cataractogenesis.¹²

Several studies demonstrated that selenite-induced cataractogenesis could be inhibited by antioxidant substances, for example, caffeic acid phenethyl ester, 2-ketoglutarate, and *Ocimum sanctum* extract.²¹⁻²³ These studies proposed that the

anti-cataractogenic effect was provided by protecting normal antioxidant levels. A previous study also investigated the anti-cataractogenic effect of green tea (*Camellia sinensis*) and revealed that green tea had a significant anti-cataract potency because of its ability to support the antioxidant defense system.²⁴ Doganay et al. revealed that resveratrol had a protective effect against selenite induced cataract in rats. The authors proposed that an increase in GSH levels and a decrease in malondialdehyde (MDA) levels in rats that received resveratrol indicated the protective effect.²⁵ Yagci et al showed that melatonin, an endogenous antioxidant, could prevent the development of cataracts induced by selenite in rats. They demonstrated that MDA and other oxidative stress markers such as xanthine oxidase and protein carbonyls decreased in the melatonin-treated group.²⁶ Other antioxidant agents such as saffron, C-phycoyanin, α -lipoic acid and fisetin, ebselen, cysteamine, pirenixine, acetyl L-carnitine, vitamin E, disulfonic stilbene and hydrogen-rich saline, N-acetylcysteine, caffeine, broccoli, hesperetin and coenzyme Q have previously been reported to inhibit selenite-induced cataract development.^{27–41}

Sildenafil, a phosphodiesterase-5 (PDE-5) enzyme inhibitor, has been used increasingly in clinical practice. Sildenafil is commonly used to treat erectile dysfunction and pulmonary hypertension (PH), because it induces vasodilatation. Previous studies have shown that sildenafil also inhibits platelet aggregation, and has antioxidant and anti-inflammatory properties.^{42,43} Recent human and animal studies have revealed that sildenafil could improve the clinical course of congestive heart failure and ovarian ischemia/reperfusion injury because of its antioxidant properties.^{44–49} However, the effect of sildenafil on cataract development has not yet been investigated.

Sildenafil shows its primary effect through increasing cellular cGMP by inhibiting PDE-5. Previous studies considered that cGMP had antioxidant effect in two main ways. The first mechanism has been explained that increased cGMP activates protein kinase G (PKG), which inhibits nitric oxide (NO) synthase through a negative feedback mechanism.^{50,51} Ito et al.⁵² demonstrated that short term inhibition of NO synthase could prevent selenite-induced cataracts by reducing oxidative stress. The second mechanism has been explained as the activation of PKG leading to opening of mitochondrial potassium-ATP channels and blocking Ca^{++} oscillation from the endoplasmic reticulum.⁵³ Moreover, the opening of mitochondrial potassium-ATP channels results in inhibition of mitochondrial calcium import.^{53,54} Consequently, cytosolic free calcium is reduced and thus calpains, accused of causing cataract formation by increasing oxidative stress, are inactivated.⁵³ Furthermore, as the third possible mechanism, some researchers revealed that cGMP could contribute to antioxidant activity by increasing antioxidant gene expression.^{55,56}

In 1996, a new oxidative stress biomarker was identified in the plasma of patients with chronic uremia known as AOPP. The levels of which correlate with highly oxidized protein concentrations, especially albumin.¹⁹ Recent studies evaluating the anti-cataractogenic effects of varying substances on the selenite-induced cataract model commonly used MDA as an oxidative stress marker.^{57–59} However, two different studies evaluating the accuracy of oxidative markers emphasized that AOPP was more accurate in reflecting oxidative stress than MDA.^{19,60} Considering that cataractogenesis mainly consists

of lens protein oxidation and precipitation, we thought that AOPP could better reflect the precipitation of oxidized lens proteins causing cataracts than MDA, and, therefore, we preferred AOPP to demonstrate the level of protein oxidation in the current study.

Sulfhydryl (SH) groups are composed of a sulfur atom and a hydrogen atom linked to a carbon atom. TSH is the sum of both intracellular and extracellular SH groups, which are in a free form in glutathione or bound to plasma proteins, particularly albumin. TSH is the major part of the antioxidant defense system.⁶¹ SH are found in glutathione (GSH) in plasma, and in cysteine (CysSH), cysteinylglycine (CysGlySH) and homocysteine (HcySH), which are involved in the antioxidant system.⁶² Although several studies^{27,34,63} assessing the anti-cataractogenic effects of varying substances on selenite-induced cataract model commonly used glutathione levels to reflect antioxidant status, we considered TSH levels as being a more sensitive marker than glutathione because of the aforementioned reasons; therefore, we preferred TSH levels to reflect antioxidant activity.

NOx is a well-known indicator of oxidative stress.⁶⁴ Previous studies revealed that higher levels of NO and NOx were obtained in the event of oxidative stress and they also demonstrated that NO and NOx might be associated with cataract development.^{52,65} Furthermore, considering that sildenafil increases cGMP by inhibiting PDE-5, and thus cGMP activates PKG, we thought that activated PKG could inhibit NO synthase through a negative feedback mechanism, similar to the views of other researchers.^{50,51} Therefore, we preferred NOx levels to better reflect the effect of sildenafil on oxidative stress induced by selenite.

In the current study, the lower levels of oxidative markers, including AOPP and NOx, and the higher TSH levels indicated the higher antioxidant activity in rats treated with low dose sildenafil. All these laboratory findings were able to explain the lesser cataract development in rats treated with low-dose sildenafil. Otherwise, high-dose sildenafil showed no antioxidant activity, which was supported by the higher incidence of cataract development and higher levels of protein oxidation markers in rats that received high-dose sildenafil. The low (0.7 mg/kg) and high (1.4 mg/kg) doses of sildenafil in this study were determined by referencing previously used doses to prevent organ ischemia-reperfusion injury.^{14,49} Some researchers reported that low-dose (0.7 mg/kg) and high-dose (1.4 mg/kg) sildenafil showed similar antioxidant activity,¹⁴ but others reported that low-dose sildenafil had greater antioxidant activity than sildenafil at high doses, similar to the present study.⁴⁹

The present study demonstrates that low-dose sildenafil is more favorable to prevent cataract development than high-dose sildenafil, and underlines the possible mechanisms of the anti-cataract effect of sildenafil. However, experimental selenite cataracts differ from actual human cataracts as mentioned earlier. Therefore, the outcomes obtained in our experimental study cannot be directly extrapolated to the development of human cataracts. Furthermore, in our study, sildenafil was administered orally, but further studies are needed to clarify the optimal application route and dosage of sildenafil.

In conclusion, our preliminary study is encouraging, but further investigations that aim to determine whether a similar anticataractogenic potential can be revealed in humans and clarify the exact molecular mechanisms of the protective effects of sildenafil on cataract development should be designed.

Declaration of Interest

The authors report no conflicts of interest.

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