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Naringin protects against diabetic cataracts in rats by modulating oxidative stress and inflammation

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Abstract: This study investigated the efficacy of naringin, a bioflavonoid recognized for its antioxidative and anti-inflammatory properties, in preventing the development of diabetic cataracts. Streptozotocin (STZ)-induced diabetic rats and age-matched controls were treated either with naringin or a vehicle for 12 weeks. Cataract formation scores, oxidative stress markers, proinflammatory cytokines, and the transcription factor Nrf2 and NF-κB activities in the lens were assessed. Diabetic rats treated with naringin had a significantly reduced incidence and severity of cataracts compared to vehicle-treated counterparts. Vehicle-treated diabetic rats had elevated oxidative stress and inflammation in the lens, characterized by decreased Nrf2 activity and increased NF-κB activity. Naringin treatment effectively mitigated these detrimental effects by enhancing Nrf2-mediated antioxidant defenses and suppressing NF-κB-driven inflammatory responses. Importantly, naringin did not significantly alter blood glucose levels in diabetic rats, indicating its cataract-preventive effects were independent of glycemic control. Naringin has a protective role against the onset and progression of diabetic cataracts by modulating key oxidative and inflammatory pathways in the lens. These findings suggest it is a promising therapeutic agent for preventing diabetic cataracts.

Keywords: diabetic cataract, naringin, oxidative stress, inflammation, Nrf2, NF-κB

INTRODUCTION

Diabetic cataracts are a major consequence of diabetes mellitus and one of the leading causes of vision impairment worldwide [1]. Individuals with diabetes have a substantially higher risk of developing cataracts, often at an earlier age compared to non-diabetic populations [2]. The pathogenesis of diabetic cataracts is multifactorial, involving chronic hyperglycemia-induced metabolic and physiological alterations in the lens [3]. Hyperglycemia results in the buildup of advanced glycation end products (AGEs), osmotic stress from sorbitol accumulation, and the activation of multiple metabolic pathways, which contribute to the development of lens opacity [4]. Oxidative stress and inflammation are critical in diabetic cataract development [5, 6]. High glucose concentrations enhance the generation of reactive oxygen species (ROS), overwhelming the lens's antioxidant defense systems, and causing oxidative damage to proteins, lipids, and DNA [7]. Oxidative damage results in structural and functional alterations of lens proteins, contributing to lens opacity [8]. Concurrently, hyperglycemia induces a chronic low-grade inflammatory state characterized by proinflammatory signaling pathway activation and increased levels of inflammatory cytokines [9]. Inflammation exacerbates oxidative stress and promotes apoptosis of lens epithelial cells, further advancing cataract formation [10]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor governing the expression of antioxidant enzymes and cytoprotective proteins [11]. Activation of Nrf2 enhances the cellular antioxidant capacity, protecting against oxidative damage and delaying the progression of diabetic complications [12]. Nuclear factor kappa B (NF-κB) is a critical transcription factor regulating inflammatory responses [13]. Hyperactivation of NF-κB increases proinflammatory gene expression, leading to inflammation-associated damage commonly observed

in diabetic tissues [14]. Modulating these pathways represents a strategic therapeutic approach to mitigate oxidative stress and inflammation in diabetic cataracts.

Naringin, a bioflavonoid predominantly found in citrus fruits like grapefruits and oranges, exhibits potent antioxidative and anti-inflammatory properties [15], including free radical scavenging, lipid peroxidation suppression, and enhanced expression of antioxidant enzymes, like superoxide dismutase and glutathione peroxidase [16]. Naringin additionally regulates inflammatory responses by inhibiting the NF-κB signaling pathway and reducing the production of proinflammatory cytokines such as TNF-α and IL-6 [17,18]. These properties suggest that naringin could effectively counteract the oxidative stress and inflammation implicated in diabetic cataract formation. Despite the known beneficial effects of naringin on oxidative stress and inflammation, its potential protective role against diabetic cataracts has not been extensively explored. The specific mechanisms by which naringin could influence Nrf2 and NF-κB pathways in diabetic cataracts remain unclear. Insight into these mechanisms is vital for developing targeted therapies to prevent or delay cataract formation in diabetic patients. This study evaluates the effectiveness of naringin in preventing diabetic cataract development and progression in STZ-induced diabetic rats. We hypothesize that naringin treatment enhances Nrf2-mediated antioxidant defenses and suppresses NF-κB-mediated inflammatory responses in the lens, thereby ameliorating cataract formation without significantly altering blood glucose levels. This study elucidates the therapeutic potential of naringin and its underlying mechanisms, contributing to the development of effective interventions for diabetic cataracts.

MATERIALS AND METHODS

Animals and the experimental design

Eight-week-old male Wistar rats, weighing between 200 and 250 g, were acquired from the animal facility at Xi'an Aier Ancient City Eye Hospital, Shaanxi Province, China. Before commencing the experiments, the animals underwent a one-week acclimatization period under standard laboratory conditions in a controlled environment with a 12-h light/dark cycle, temperature maintained at 22±2°C, and relative humidity set at 55±5%. The rats had unrestricted access to standard laboratory chow and water. All experimental protocols were reviewed and approved by the Xi'an Aier Ancient City Eye Hospital, Shaanxi Province, China, and were performed under ARRIVE guidelines. The rats were randomly divided into four groups: Control + Vehicle (C+V): non-diabetic rats receiving vehicle treatment (n=20 lenses), Control + Naringin (C+N): non-diabetic rats receiving naringin treatment (n=20 lenses), Diabetic + Vehicle (D+V): diabetic rats receiving vehicle treatment (n=19 lenses), and Diabetic + Naringin (D+N): diabetic rats receiving naringin treatment (n=20 lenses).

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 60 mg STZ/kg body weight. The STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5) and administered following an overnight fasting period. Control rats received an equal volume of the citrate buffer. Fasting blood sugar (FBS) levels were measured 72 h after the injection using a glucometer (Accu-Chek, Roche Diagnostics). Rats with fasting blood glucose concentrations >250 mg/dL were identified as diabetic and included in the study.

Naringin administration

One week after the STZ injection, rats assigned to the naringin-treated groups (C+N and D+N) were given naringin at a dose of 50 mg/kg body weight daily orally via gavage for 12 weeks, dissolved in a 0.5% carboxymethylcellulose (CMC) solution. Rats in the vehicle-treated groups (C+V and D+V) received an equal volume of 0.5% CMC solution.

Assessment of cataract formation

Cataract progression was monitored bi-weekly by slit-lamp-microscopy (SL-15, Kowa Company Ltd.) after pupil dilation with 1% tropicamide ophthalmic solution. Cataract severity was graded on a scale from Stage 0 to Stage 5 based on lens opacity (Supplementary Fig. S1), as follows: stage I: clear lens with no opacity, stage II: peripheral vesicles or vacuoles, stage III: initial cortical opacity, stage IV: diffuse central opacity, and

stage V: mature cataract with complete opacity. After the 12-week treatment period, the rats were anesthetized using a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg), blood samples were collected and centrifuged to obtain serum for FBS measurement, followed by euthanasia by cervical dislocation. Both eyes were enucleated, and the lenses were extracted for biochemical and molecular analyses.

Biochemical assays

FBS levels were measured using the Crystal Chem Inc. Rat Glucose Assay Kit (Crystal Chem. Inc. Netherlands). Lens tissues were homogenized in ice-cold phosphate-buffered saline (PBS) pH 7.4 and centrifuged at 10000×g for 15 min at 4°C to obtain clear supernatants for analysis. The supernatants were used to assess oxidative stress markers and antioxidant enzyme activities. Malondialdehyde (MDA) levels, indicative of lipid peroxidation, were measured using a commercially available ELISA kit (Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's instructions. The activities of antioxidant enzymes, glutathione peroxidase (GPx), and catalase (CAT), and reduced glutathione (GSH) levels, were determined using specific ELISA kits (Bioassay Technology Laboratory) following the provided protocols. Absorbance readings were taken using a microplate reader (FLUOstar Omega Multifunctional). Protein concentrations in the samples were measured using the Bradford assay with bovine serum albumin as the standard. Naringin effects on the serum levels of proinflammatory cytokines were assessed using specific ELISA kits (Bioassay Technology Laboratory).

RNA extraction and Real-Time quantitative PCR

Total RNA was isolated from lens tissues using TRIzol™ reagent (Invitrogen, USA). The quality and purity of the RNA were assessed with a NanoDrop™ 2000c spectrophotometer (Thermo Scientific, USA), with A260/A280 ratios within the acceptable range of 1.8 to 2.0. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) in a 20 µL reaction mixture, following the manufacturer's protocol. RT quantitative PCR was performed using SYBR™ Green Master Mix (Applied Biosystems, USA) on a QuantStudio™ 3 RT-PCR System (Applied Biosystems, USA). The gene expression levels of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α, and transcription factors Nrf2 and NF-κB, were quantified. β-actin served as the housekeeping gene for normalization of gene expression data. Primer sequences used for amplification are listed in Supplementary Table S1.

The PCR protocol included an initial denaturation step at 95°C for 10 min to activate the DNA polymerase, followed by 40 cycles with the following temperature profile: denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Melting curve analysis was performed after the amplification cycles to verify the specificity of the PCR products and confirm the absence of nonspecific amplifications or primer-dimer artifacts. Gene expression was analyzed using the comparative Ct method ($2^{-\Delta\Delta Ct}$), with beta-actin serving as the reference gene to standardize the expression of the target genes.

Statistical analysis

Data are expressed as the mean±standard deviation (SD). Statistical analysis was performed using GraphPad Prism (ver. 8.3.0, GraphPad Software, San Diego, CA). Differences among multiple groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was defined as $P<0.05$.

RESULTS

Assessment of blood glucose concentration

To evaluate whether the effects of naringin were independent of glycemic control or not, fasting blood glucose levels were monitored throughout the study period (Fig. 1). Diabetic rats (D+V and D+N) exhibited significantly elevated blood glucose levels compared to control rats (C+V and C+N) ($P<0.0001$). There was no significant difference in blood glucose between the D+V and D+N groups ($P=0.45$), indicating that naringin did not significantly alter hyperglycemia in diabetic rats. This suggests that the protective effects of naringin on cataract formation were primarily due to its antioxidative and anti-inflammatory properties rather than changes in blood glucose levels.

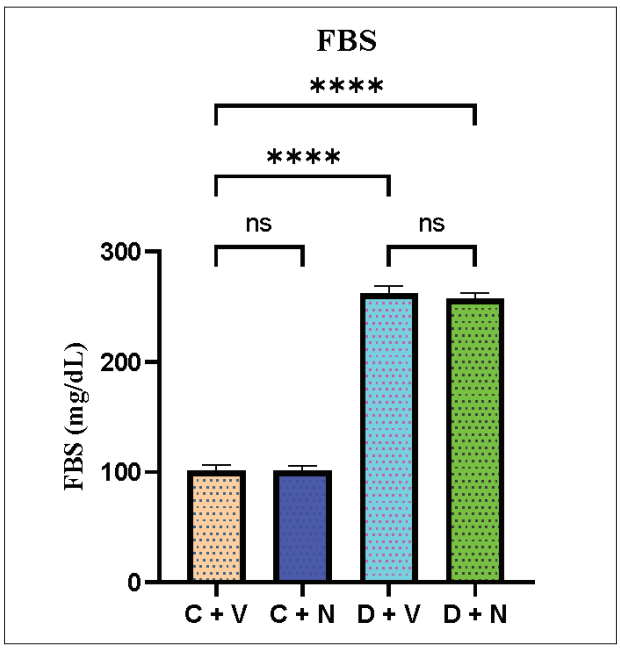

Fig.1. FBS levels at the end of treatment. Diabetic rats (D+V and D+N) exhibited significantly elevated blood glucose concentrations compared to control rats (C+V and C+N) (P<0.0001). There was no significant difference in blood glucose levels between the D+V and D+N groups (P=0.45). Statistical significance was determined using Tukey's multiple comparisons test. Significance levels are indicated as *P<0.05, **P<0.01P, ***P<0.001, and ****P<0.0001.

Naringin reduces cataract formation in diabetic rats

At the end of the 12-week treatment period, cataract formation was observed only in diabetic rats. Notable differences in cataract scores were detected among the experimental groups (Table 1, Supplementary Fig. S1). In the control groups (C+V and C+N), all lenses remained clear (score 0), indicating no cataract development. Diabetic rats treated with vehicle (D+V) exhibited pronounced cataract formation, with only 2 out of 20 clear lenses. Most of the lenses in the D+V group progressed to higher cataract stages. Diabetic rats treated with naringin (D+N) exhibited lower incidence and severity of cataract formation. In the D+N group, 6 out of 20 lenses remained clear, and fewer lenses progressed to advanced cataract stages compared to the D+V group. These results suggest that naringin delayed the onset and slowed the progression of cataracts in diabetic rats.

Table 1. Effects of naringin treatment on diabetes mellitus-induced cataract formation score

Cataract score	D+V (n=20)	D+N (n=20)	P
<3	7	15	0.025*
≥3	13	5	
Total	20	20	

The Kruskal-Wallis test was used to compare the groups. *P<0.05 was considered as a statistically significant difference. V – vehicle; N – naringin; D – diabetes; n – number of lenses.

Naringin modulates oxidative stress markers in diabetic lenses

To evaluate the effect of naringin on lens oxidative stress, we measured MDA levels, the activities of antioxidant enzymes GPx and CAT, and GSH levels (Fig. 2).

MDA levels

In diabetic rats treated with the vehicle (D+V), MDA levels were significantly elevated compared to control rats receiving the vehicle (C+V) (P<0.0001), indicating enhanced lipid peroxidation associated with oxidative stress. Administration of naringin to diabetic rats (D+N) resulted in a significant reduction of MDA levels compared to the D+V group (P=0.0004), bringing the levels close to those of the control group (C+V *vs* D+N, P=0.84). A modest but significant decrease in MDA was observed in control rats treated with naringin (C+N) compared to the C+V group (P=0.006).

GPx activity

The activity of GPx was markedly decreased in the D+V group compared to the C+V group (P<0.0001), reflecting compromised antioxidant defenses in diabetic conditions. Naringin treatment significantly enhanced GPx activity in diabetic rats (D+N) compared to those receiving the vehicle (P<0.0001). Despite this improvement, GPx activity in the D+N group remained significantly lower than in the control group (P<0.0001), suggesting that the restoration was partial. There was no significant difference in GPx activities between the C+V and C+N groups (P=0.12).

CAT activity

CAT activity was significantly reduced in the D+V group compared to the C+V group (P<0.0001). Diabetic rats treated with naringin (D+N) showed a

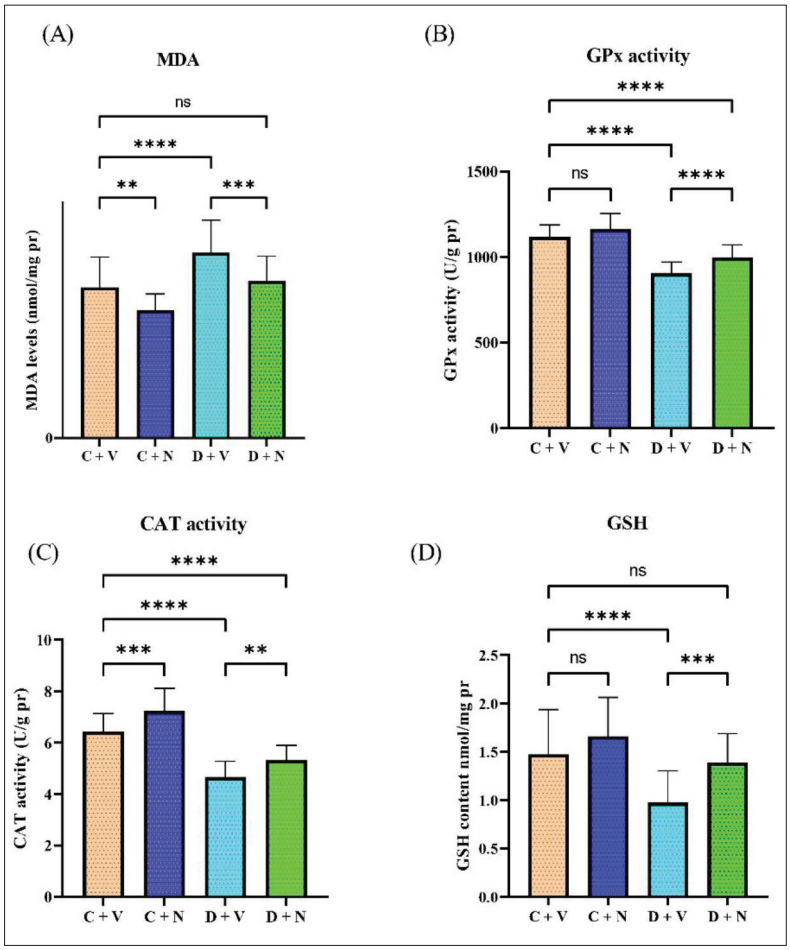

Fig.2. Naringin attenuates oxidative stress markers in rat lens tissues. *A* – malondialdehyde (MDA) levels; *B* – glutathione peroxidase (GPx) activity; *C* – catalase (CAT) activity; *D* – reduced glutathione (GSH) levels of control rats administered vehicle (C+V), control rats administered naringin (C+N), diabetic rats administered vehicle (D+V), and diabetic rats administered naringin (D+N). Data are expressed as the mean±standard deviation (SD). Statistical significance was determined using Tukey's multiple comparisons test. Significance levels are indicated as *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

significant increase in CAT activity compared to the D+V group (P=0.0027). However, CAT activity in the D+N group was significantly lower than in the control group (P<0.0001). Control rats administered naringin (C+N) exhibited a significant elevation in CAT activity compared to the C+V group (P=0.0001).

GSH levels

GSH levels were markedly lower in the D+V group compared to the C+V group (P<0.0001), highlighting the depletion of this crucial antioxidant in diabetic lenses. However, treatment of diabetic rats (D+N) with naringin restored GSH levels to values similar to the control group, with no significant difference observed between the D+N and C+V groups (P=0.81). Additionally, the D+N group demonstrated significantly increased GSH levels compared to the D+V group (P=0.0003). No significant difference in GSH levels was detected between the C+V and C+N groups (P=0.23).

Naringin modulates inflammatory cytokine in diabetic lenses

To assess the inflammatory response associated with diabetic cataract formation, we evaluated the mRNA expression levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α in the lenses of all experimental groups, and cytokine levels in the rat sera (Figs. 3 and 4). In diabetic rats treated with vehicle (D+V), IL-1β expression was significantly elevated compared to control rats receiving the vehicle (C+V) (P<0.0001), indicating a pronounced inflammatory response. Administration of naringin to diabetic rats (D+N) resulted in a substantial reduction in IL-1β levels compared to the D+V group (P<0.0001). However, IL-1β expression in the D+N group remained higher than in the control group (P<0.0001). There was no significant difference in IL-1β expression between the control groups treated with vehicle and naringin (C+V *vs* C+N, P=0.8).

IL-6 expression was markedly increased in the D+V group relative to the C+V group (P<0.0001). Naringin treatment of diabetic rats (D+N) significantly decreased IL-6 levels compared to the D+V group (P=0.0003). Despite this reduction, IL-6 expression in the D+N group remained slightly elevated compared to controls (P=0.012). No significant difference was observed between the C+V and C+N groups for IL-6 expression (P=0.27).

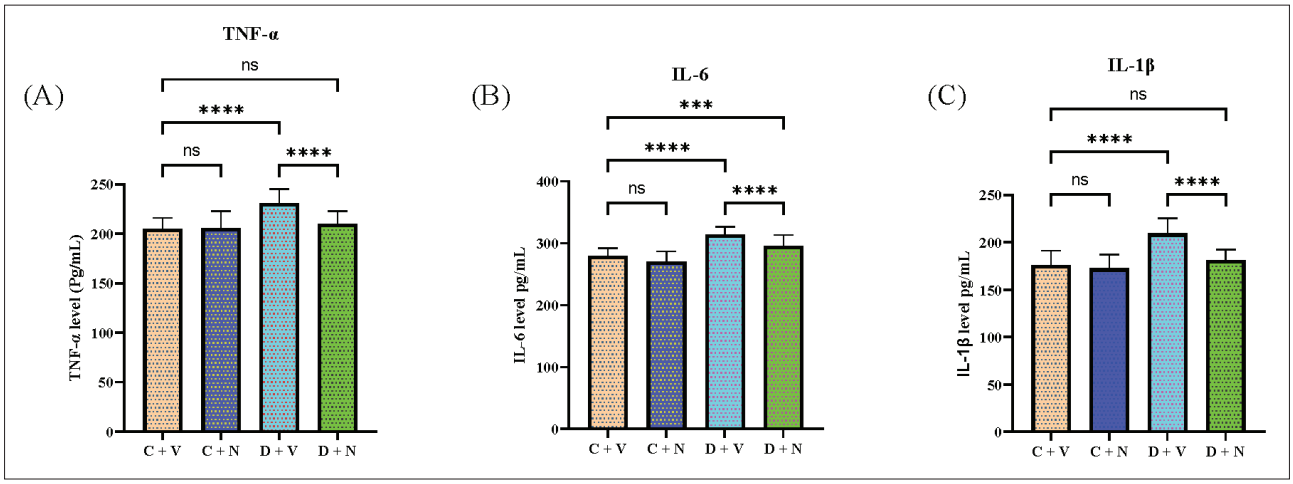

Fig.3. Naringin affects the expression of pro-inflammatory cytokines and transcription factors in rat lens tissues. mRNA expression levels of **A** – interleukin-1β (IL-1β); **B** – interleukin-6 (IL-6); **C** – tumor necrosis factor-alpha (TNF-α); **D** – nuclear factor erythroid 2-related factor 2 (Nrf2); **E** – nuclear factor kappa B (NF-κB) are presented for control rats treated with vehicle (C+V), control rats treated with naringin (C+N), diabetic rats treated with vehicle (D+V), and diabetic rats treated with naringin (D+N). Gene expression levels were normalized to β-actin and are expressed as fold change relative to the control group. Data are shown as the mean±SD. Statistical significance was determined using Tukey's multiple comparisons test. Significance levels are indicated as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

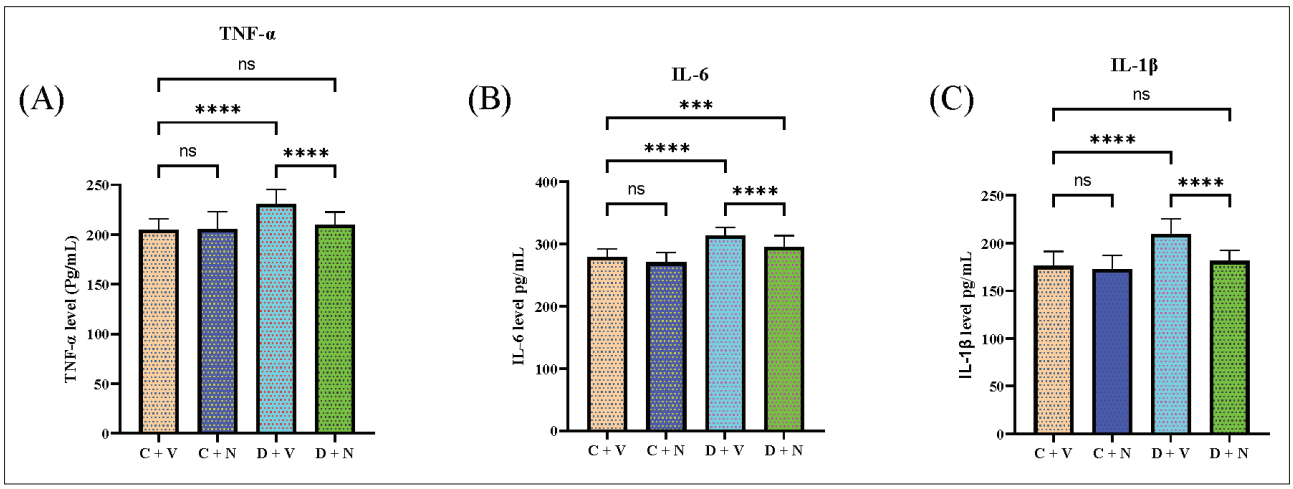

Fig.4. Naringin effects on the serum levels of pro-inflammatory cytokines of control rats treated with vehicle (C+V), control rats treated with naringin (C+N), diabetic rats treated with vehicle (D+V), and diabetic rats treated with naringin (D+N). Data are shown as the mean±SD. Statistical significance was determined using Tukey's multiple comparisons test. Significance levels are indicated as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

TNF-α levels were also significantly higher in the D+V group compared to the C+V group (P<0.0001). Naringin administration to diabetic rats (D+N) led to a significant decrease in TNF-α expression compared to the D+V group (P<0.0001). TNF-α levels in the D+N group were slightly above the control group (P=0.024). There was no significant difference in TNF-α expression between the C+V and C+N groups (P=0.97).

A similar pattern was observed when the inflammatory cytokines were measured in the rat sera (Fig. 4). Thus, naringin significantly reduced the overexpression of proinflammatory cytokines in the lens caused by diabetes, although it did not fully restore their levels to those of non-diabetic controls. The lack of significant differences between the control groups suggests that naringin does not adversely affect cytokine expression under normal physiological conditions.

Naringin influences Nrf2 and NF-κB expression in diabetic lenses

To further investigate the molecular mechanisms underlying naringin's antioxidative and anti-inflammatory effects, we assessed the expression levels of Nrf2 and NF-κB in lens tissues of all experimental groups (Fig. 5). In diabetic rats treated with the vehicle (D+V), a slight reduction in Nrf2 expression was observed compared to control rats receiving the vehicle (C+V), approaching statistical significance (P=0.05). Administration of naringin to diabetic rats (D+N) led to a significant upregulation of Nrf2 expression relative to both the D+V group (P<0.0001) and the control group (P<0.0001), indicating that naringin enhances Nrf2 expression in diabetic conditions. No significant difference in Nrf2 expression was detected between the control groups treated with vehicle and naringin (C+V vs. C+N, P=0.86), suggesting that naringin does not affect Nrf2 levels under normal physiological conditions.

Regarding NF-κB expression, the D+V group exhibited significantly elevated levels compared to the C+V group (P<0.0001), indicating activation of proinflammatory signaling pathways in the diabetic lens. Naringin treatment of diabetic rats (D+N) effectively suppressed NF-κB expression compared to the D+V group (P<0.0001), reducing it to levels not significantly different from controls (C+V *vs* D + N, P=0.16). This suggests that naringin can reverse the diabetes-induced upregulation of NF-κB. There was no significant difference in NF-κB expression between the control groups (C+V vs. C+N, P=0.4), indicating that naringin does not alter NF-κB levels in non-diabetic rats.

These findings show that naringin modulates key transcription factors involved in oxidative stress and inflammation. By upregulating Nrf2, naringin enhances the antioxidant defense mechanisms in the diabetic lens. Concurrently, the downregulation of NF-κB by naringin alleviates proinflammatory signaling. Together, these molecular effects contribute to the protective role of naringin against diabetic cataract formation.

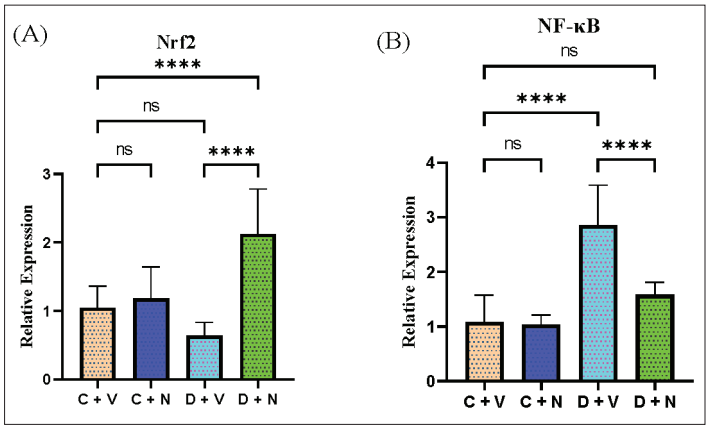

Fig.5. Naringin effects on the expression of transcription factors **A** – Nrf2, and **B** – NF-κB in rat lens tissues of control rats treated with vehicle (C+V), control rats treated with naringin (C+N), diabetic rats treated with vehicle (D+V), and diabetic rats treated with naringin (D+N). Gene expression levels were normalized to β-actin and are expressed as fold change relative to the control group. Data are shown as the mean±SD. Statistical significance was determined using Tukey's multiple comparisons test. Significance levels are indicated as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

DISCUSSION

This study demonstrates that naringin prevents the development and advancement of diabetic cataracts in STZ-induced diabetic rats by modulating oxidative stress and the inflammatory response in the lens. Naringin treatment significantly reduced cataract formation and severity, as evidenced by lower cataract scores than in vehicle-treated diabetic rats. The protective effects of naringin are associated with enhanced antioxidant defense mechanisms, increased expression of Nrf2, and inhibition of proinflammatory cytokines and NF-κB expression.

Diabetic cataract formation is a multifactorial process, with oxidative stress and inflammation playing crucial roles in lens opacification [19,20]. Hyperglycemia-induced oxidative stress leads to the accumulation of ROS, which damage lens proteins, lipids, and DNA [21]. Excessive generation of ROS overwhelms the antioxidant defense systems, resulting in lipid peroxidation, protein aggregation, and apoptosis of lens epithelial cells [22,23]. In this study, diabetic rats exhibited elevated levels of MDA, a marker of lipid peroxidation, and decreased activities of antioxidant enzymes GPx, CAT, and reduced GSH levels. These findings align with previous reports indicating that diabetes impairs the lens's antioxidant defense system, making it susceptible to oxidative damage [7,24]. Naringin treatment mitigated oxidative stress in diabetic lenses by reducing MDA levels and enhancing the GPx, CAT, and GSH activities. The restoration of antioxidant enzyme activities suggests that naringin enhances the

lens's capacity to neutralize ROS, thereby preventing oxidative damage. These results are consistent with earlier studies demonstrating the antioxidative properties of naringin in various diabetic complications [15,16]. For instance, naringin has been reported to alleviate oxidative stress in diabetic nephropathy [16], diabetic cardiomyopathy [25], and diabetic neuropathy [26]. Its ability to scavenge free radicals and upregulate antioxidant enzymes may underlie its protective effects against oxidative stress-induced lens damage.

Inflammation is another important factor contributing to diabetic cataractogenesis. Proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, are upregulated under diabetic conditions, intensifying oxidative stress and triggering apoptosis in lens epithelial cells [27]. In this study, the lenses of diabetic rats exhibited significantly elevated IL-1β, IL-6, and TNF-α expression levels. Treatment with naringin substantially decreased the expression of these cytokines, demonstrating its antiinflammatory effects. This aligns with previous findings where naringin suppressed inflammatory responses in diabetic models [28,29].

Naringin's antiinflammatory properties have been attributed to its ability to suppress the activation of NF-κB, a crucial transcription factor regulating inflammatory gene expression [30,31]. Nrf2 and NF-κB pathway modulation by naringin provides insight into the molecular mechanisms underlying its antioxidative and antiinflammatory actions. Nrf2 is a transcription factor that regulates the expression of antioxidant and cytoprotective genes. Its activation boosts cellular antioxidant capacity, protecting against oxidative stress [32]. Naringin upregulated Nrf2 expression in diabetic lenses, suggesting it activates the Nrf2 pathway to bolster antioxidant defenses. This is supported by earlier studies where naringin activated Nrf2 signaling in diabetic complications [33,34]. Naringin enhances Nrf2-mediated antioxidant responses in diabetic nephropathy [26] and attenuates oxidative stress in diabetic cardiomyopathy through Nrf2 activation [25]. Conversely, NF-κB is a key regulator of inflammatory responses, and its activation leads to the transcription of proinflammatory genes [13]. The elevated expression of NF-κB observed in diabetic lenses indicates the activation of inflammatory pathways contributing to cataract formation. Naringin treatment significantly decreased NF-κB expression, indicating inflammatory signaling inhibition. Previous studies have reported similar findings where naringin inhibited NF-κB activation, thereby reducing inflammation [35]. Naringin's inhibitory effect on NF-κB may be due to its ability to prevent the degradation of IκBα, the inhibitor of NF-κB, thereby blocking NF-κB nuclear translocation [31].

Interestingly, naringin did not significantly alter blood glucose levels in diabetic rats, suggesting that its protective effects are independent of glycemic control. This is an important finding, as maintaining tight glycemic control is often challenging in diabetic patients, and therapies that protect without affecting glucose levels are valuable. Naringin's direct action on oxidative stress and inflammation pathways offers a targeted approach to preventing diabetic cataracts. These results agree with previous research demonstrating the beneficial effects of natural antioxidants in preventing diabetic cataracts. Curcumin and quercetin have been shown to delay cataract formation by modulating oxidative stress and inflammatory responses [36,37]. Similarly, resveratrol has been reported to protect against diabetic cataracts by activating Nrf2 and inhibiting NF-κB pathways [38]. These compounds, like naringin, highlight the potential of phytochemicals in managing diabetic complications through antioxidative and antiinflammatory mechanisms. Moreover, the role of Nrf2 and NF-κB crosstalk in diabetic complications has been increasingly recognized [39]. Nrf2 activation enhances antioxidant defenses and inhibits NF-κB-mediated inflammatory responses. Conversely, NF-κB activation can suppress Nrf2 activity, exacerbating oxidative stress. Naringin's ability to modulate Nrf2 and NF-κB pathways suggests that it may restore the balance between oxidative stress and inflammation in diabetic lenses.

While the findings are promising, certain limitations should be acknowledged. The study utilized an STZ-induced rat model for type 1 diabetes. The efficacy of naringin in type 2 diabetes, which involves insulin resistance and other metabolic disturbances, warrants investigation. Additionally, the study focused on the expression levels of Nrf2 and NF-κB at the mRNA level. Assessing the protein levels and the activity of downstream target genes could offer a more comprehensive insight into the underlying molecular mechanisms. Western blotting and immunohistochemistry could provide insights into transcription factor localization and activation. Furthermore, the bioavailability and

pharmacokinetics of naringin in ocular tissues remain to be elucidated. Naringin is known to have limited bioavailability, primarily due to its poor absorption and rapid metabolism [40]. Strategies to improve its bioavailability, such as nanoformulations or co-administration with bio-enhancers, could potentiate its therapeutic effects [40]. Future studies should also explore the potential synergistic effects of naringin with other antioxidants or antiinflammatory agents.

Translating these findings into clinical practice holds considerable potential. Diabetic cataracts remain a major cause of vision impairment, and current treatments are limited to surgical interventions. Pharmacological agents that can prevent or delay cataract formation are highly desirable. Naringin, a natural compound with a favorable safety profile, could be an attractive candidate for therapeutic development. Clinical trials evaluating the efficacy of naringin in diabetic patients are necessary to validate its potential benefits.

CONCLUSION

Naringin effectively prevents the development and advancement of diabetic cataracts in rats by boosting antioxidant defenses and reducing inflammatory responses in the lens. The modulation of Nrf2 and NF-κB pathways is a key mechanism underlying its protective effects. Naringin's ability to act independently of blood glucose levels makes it a promising therapeutic candidate for preventing diabetic cataracts. These results expand our understanding of the role of natural compounds in managing diabetic complications and lay the groundwork for future research and potential clinical applications.

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Author contributions: Conceptualization, GM, and YX; methodology, LM; software, LM; validation, YX; formal analysis, GM; investigation, LM; visualization, LM; supervision, GM; project administration, YX. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: Authors declare no potential conflicts of interest.

Data availability: Data underlying the reported findings have been provided as a raw dataset available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Meng%20et%20al_Dataset.pdf

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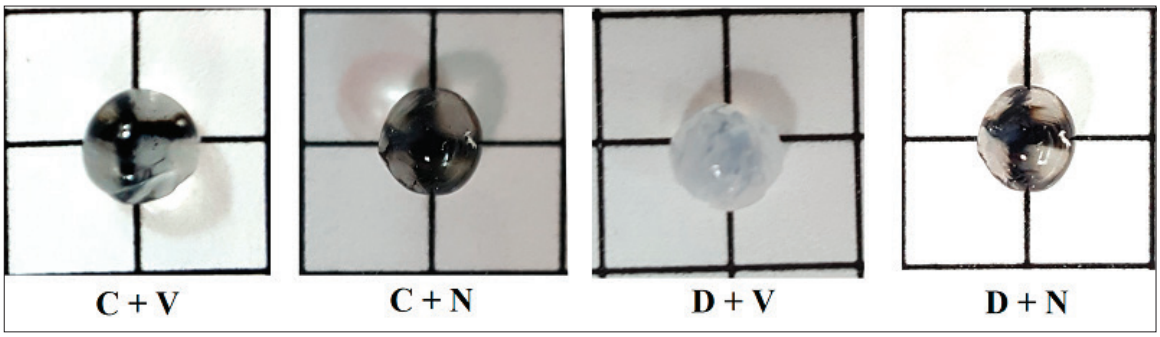
SUPPLEMENTARY MATERIAL

Supplementary Table S1. Sequences of primers used for target gene amplification and the internal control (β-actin) in RT PCR analysis

Gene	Forward sequence (5'→3')	Reverse sequence (5'→3')
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
IL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Nrf2	GAGACGGCCATGACTGAT	TGCTTGCTGAATGTGAGG
NF-κB	GAGGCCCAGTGTGGTGTG	GAGGAGCAGGAGGAGGAGG
β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA



Supplementary Fig. S1. Effects of naringin treatment on lens opacity. Notable differences in cataract scores were detected between the experimental groups. C+V: Control + Vehicle; C+N: Control + Naringin; D+V: Diabetic + Vehicle; D+N: Diabetic + Naringin.