Molecular Vision 2025; 31:396-409 <http://www.molvis.org/molvis/v31/396>
Received 13 September 2024 | Accepted 3 October 2025 | Published 5 October 2025

Ginger extract and selenium supplementation: A promising approach to improve diabetic retinopathy

Xiaohan Huang, Jing Li, Shaohua Tang, Jing Xu, Jiong Liu

Department of Ophthalmology, Beijing Jishuitan Hospital, Capital Medical University, Beijing, China

Purpose: Diabetic retinopathy results from damage to small blood vessels and retinal neurons due to the overproduction of reactive oxygen species and overexpression of TRPM2 and TRPV1. Hence, inhibition of these events by ginger and selenium may reduce diabetes-induced ocular damage. Therefore, the aim of this study was to investigate the therapeutic effects of ginger, selenium, and their combinations on apoptosis, inflammation, insulin resistance, oxidative damage, and the expression of TRPM2 and TRPV1.

Methods: Seventy-two adult male Wistar rats were divided into nine groups as follows: control, diabetes, diabetes-ginger (100 mg/kg), diabetes-selenium (50, 100, and 150 µg/kg), and diabetes-ginger (100 mg/kg)–selenium (50, 100, and 150 µg/kg).

Results: Diabetes increased the expression of protein and genes of TRPM2 and TRPV1, and it induced oxidative damage by increasing malondialdehyde levels and decreasing superoxide dismutase, glutathione peroxidase, and catalase enzyme activities. Diabetes induced apoptosis by increasing BAX and caspase-3 gene expression and decreasing Bcl2 in eye tissue when compared to the control group. However, treatment with ginger (100 mg/kg), selenium (50, 100, and 150 µg/kg), and their combinations improved these situations in the diabetic groups compared to the diabetic group.

Conclusions: Diabetes induced retinopathy by inducing oxidative damage, inflammation, apoptosis, and upregulation of TRPM2 and TRPV1. However, treatments with selenium, ginger, and their combinations improved diabetic retinopathy by inhibiting oxidative damage, inflammation, and apoptosis and downregulating protein and gene expression of TRPM2 and TRPV1. The results of this study suggest that ginger and selenium can be a good treatment to inhibit the progression of diabetic retinopathy.

Diabetic retinopathy is a common complication of diabetes that affects the eyes, leading to vision impairment or even blindness if left untreated. Diabetic retinopathy develops due to prolonged exposure to high blood glucose levels, causing damage to the blood vessels supplying the retina [1,2]. This damage triggers a cascade of events leading to oxidative stress, inflammation, and apoptosis in retinal cells. These processes contribute to the progression of diabetic retinopathy [2]. Transient receptor potential melastatin 2 (TRPM2) and transient receptor potential vanilloid 1 (TRPV1) are two ion channels that have been implicated in the pathogenesis of diabetic retinopathy [3,4]. TRPM2 is a calcium-permeable ion channel that plays a role in oxidative stress–induced cell death [4]. In diabetic retinopathy, increased oxidative stress is observed due to high glucose levels and impaired antioxidant defense mechanisms. Studies have shown that TRPM2 expression and activity are upregulated in the retina of diabetic animals and patients [5,6]. Overactivation of TRPM2 contributes to retinal cell death and inflammation through calcium influx and activation of downstream signaling pathways [2,6]. TRPV1 is a nonselective cation channel primarily known for its role in pain sensation. However, recent studies have suggested its involvement in diabetic retinopathy as well [7,8]. Activation of TRPV1 has been shown to promote inflammation, oxidative stress, and vascular dysfunction, all of which are key features of diabetic retinopathy [8,9]. In animal models of diabetes, increased TRPV1 expression and activity have been observed in the retina, leading to neurovascular damage [10]. Overall, TRPM2 and TRPV1 overactivation due to diabetes contributes to oxidative stress, inflammation, and cell death in the retina [11]. Targeting these ion channels may hold therapeutic potential for managing this sight-threatening complication of diabetes. However, further research is needed to fully understand their precise roles and develop effective treatments targeting these channels.

Recent studies suggest that herbal and nutrient supplementations, such as ginger and selenium supplementation, may hold potential in mitigating these detrimental effects by targeting specific molecular pathways [12,13]. Ginger (*Zingiber officinale*) has been widely recognized for its anti-inflammatory, antioxidant, and antiapoptotic properties [13,14]. Its active components, such as gingerols and shogaols, have demonstrated protective effects against various diseases

Correspondence to: Jiong Liu, Department of Ophthalmology, Beijing Jishuitan Hospital, Capital Medical University, No. 68 Huinanbei Road, Changping District, Beijing, 102208, China; email: doctor2023t@sina.com

[15]. Recent studies have shown that ginger reduced inflammation markers and inhibited oxidative stress by scavenging free radicals [16-18]. Selenium is an essential trace element known for its antioxidant properties. Some studies suggest that selenium supplementation may have a protective effect against diabetic retinopathy by inhibiting oxidative stress and inflammation [18-20]. Oxidative stress is known to contribute to the development and progression of diabetic retinopathy. Selenium acts as a cofactor for some antioxidant enzymes, such as glutathione peroxidase, which helps neutralize harmful free radicals [9,20]. By enhancing the antioxidant defense system, selenium may potentially inhibit the oxidative stress associated with diabetic retinopathy. Additionally, selenium has been shown to have anti-inflammatory properties. Chronic inflammation plays a crucial role in the pathogenesis of diabetic retinopathy [21]. By modulating inflammatory mediators, selenium potentially inhibits inflammation in the retina and the progression of diabetic retinopathy. However, it is important to note that more research is needed to establish a definitive relationship between selenium and diabetic retinopathy [22]. The optimal dose of selenium supplementation for the prevention or management of diabetic retinopathy is unclear. It is always recommended to consult with a health care professional before starting any supplementation regimen. Recent research suggests that combining ginger with some trace elements, such as zinc and selenium, enhances their individual therapeutic effects on diabetic retinopathy. These supplementations may target TRPM2 and TRPV1 ion channels, which play crucial roles in oxidative stress, autophagy, and apoptosis in retinal cells in patients with diabetes. Therefore, the aim of this study was to investigate the anti-inflammatory, antioxidative, and antiapoptotic effects of different doses of selenium in combination with ginger extract in the eye tissue of diabetic rats.

METHODS

Ginger extract preparation and its high-performance liquid chromatography analysis: Ginger, or the rhizome of *Z. officinale* Roscoe, was purchased fresh from an herbal store. After washing and cutting into small pieces, it was dried in a laboratory environment, and 500 g of its powder was used to prepare the extract. Preparation of ginger (*Z. officinale* Roscoe) extraction was done according to the method described by Akbari et al. [17]. The prepared extract was stored in the refrigerator (4 °C) for a maximum of 2 days. High-performance liquid chromatography (HPLC) analysis was performed to determine the levels of ginger bioactive compounds [6], -gingerol [8], -gingerol, and [10] -gingerol and [6] -shogaol. The analysis protocol was based on the study by Ma and Li [15]. HPLC was performed by a device equipped with a UV detector (2500 KNAUER, Wissenschaftliche Geräte GmbH, Berlin, Germany) set at 245 nm and consisted of a Smartline pump.

Animals and study plan: Seventy-two adult male Wistar rats (10-12 weeks, 230-260 g) were divided into nine groups as follows: control (C), diabetes (D), diabetes-selenium (50, 100, and 150 µg/kg; DSE50, DSE100, and DSE150), diabetes-ginger extract (100 mg/kg; DG100), and diabetes-ginger extract (100 mg/kg)–selenium (50, 75, and 100 µg/kg; DGSE50, DGSE100, and DGSE150). Rats were kept in a controlled laboratory environment and had free access to chow and water. Diabetes type 2 was induced by feeding a high-fat diet (HFD, 48% fat, 25% protein, 27% carbohydrate) for 3 weeks and injecting a single dose of streptozocin (STZ, 50 mg/kg) solution intraperitoneally [23,24]. One week after STZ injection, fasting blood glucose was measured using a glucometer to confirm diabetes. Blood glucose above 200 mg/dl was considered diabetic. After that, the grouping was fixed and treatment was provided by ginger extract and selenium supplementation. Ginger extract and selenium supplementation were dissolved in normal saline and then administered by gavage for 28 days. In the DGSE50, 100, and 150 groups, the ginger (100 mg/kg) was first dissolved in saline (1 ml), and then doses of 50 mg/kg, 100 mg/kg, and 150 mg/kg selenium were added to it. The changes in body weight and food intake were also measured weekly. This research was approved by the animal ethical committee of Jiangxi Medical College, Shangrao, China (Approval No. 203142.14).

Sampling preparation: After the last treatment session, animals were anesthetized by injecting the ketamine (65 mg/kg) and xylazine (5 mg/kg) combination. The eye tissue was removed after collecting a blood sample from the heart. The left eye was used for histopathological evaluation, and the right eye was used for biochemical and molecular evaluation. Sera were prepared by centrifuging for 15 min at 360 ×g.

Biochemical measurement: Glucose and insulin levels as metabolic parameters in serum, as well as tumor necrosis factor-α (TNF-α) and interleukin (IL) 1β in eye tissue, were measured by enzyme-linked immunosorbent assay kits (Shanghai Crystal Day Biotech Co., Shanghai, China) according to the manufacturer's instructions. The levels of oxidative biomarkers, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), were measured by the methods as previously described in eye tissue [2].

Table 1. Sequences of primers used to quantify mRNA of target genes.

Gene	Forward	Reverse
TRPV1	CAAGGCACTTGCTCCATTTG	TCTGTGGCCCAATTTCGA
TRPM2	GACAGCAACCACTCCCACTT	CTCCAACACCACGCAGACA
Bcl2	GCTACGAGTGGGATACTGGAGATGA	ACAGCGGGCGTTCGGTTG
Bax	AGGGTGGCTGGGAAGGC	TGAGCGAGGCGGTGAGG
Caspase-3	GCAGCAGCCTCAAATTGTTGAC	TGCTCCGGCTCAAACCATC
GAPDH	ATGACTCTACCCACGGCAAG	TACTCAGCACCAGCATCACC

Molecular measurement:

Real-time PCR—The expression of Bax, Bcl2, TRPV1, TRPM2, and caspase 3 genes as target genes was measured in eye tissue. The sequence of primers (Table 1) for target and control genes was extracted from previous studies [25-27]. TRIzol LS Reagent (ThermoFisher Scientific, Waltham, MA) was used to extract RNA from eye tissue. Complementary DNA synthesis was performed with a Prime ScriptTM-RT reagent kit (Takara Bio Inc., Shiga, Japan). Real-time PCR reaction was run by a SYBR Green/qPCR Master Mix (Fermentas, ThemoFisher Scientific) on a Rotor Gene 6000 (Qiagen, Netherlands). The expression of target genes was also calculated using the $2^{-\Delta\Delta Ct}$ method [28].

Western blot—Western blot analysis was performed based on the standard method in eye tissue. The samples were homogenized in RIPA buffer, and the total protein concentration was measured by the Bradford method. Primary antibody (TRPM2: NB110-81601SS; TRPV1: NB100-1617) exposure was performed at 4 °C for 1 night. The secondary antibody (rabbit IgG) was also used. Quantification was done using the relative immunoreactivity levels of the target proteins against β-actin using the Box Gel Imagination System (Syngene, UK).

Histological examination: The left eye was fixed in a 10% formalin solution after being removed from the skull for 3 days. Eyes were then embedded in paraffin. Sections with a thickness of 5 µm were prepared from paraffin blocks and stained with hematoxylin and eosin. Tissue sections were examined by a histologist using a light microscope (×40) without knowledge of their details. Histological examination was performed according to a previous study [29].

Statistical analysis: Statistical analysis was performed by one-way analysis of variance followed by a post hoc Tukey test with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). The least significant p level was <0.05. The results were reported as mean ± standard deviation (SD).

RESULTS

HPLC results: HPLC analysis of ginger extract showed that it contains high amounts of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol. The levels of 6-gingerol and 6-shogaol were the highest in ginger powder, followed by 8-gingerol and 10-gingerol (Table 2 and Figure 1).

Body weight and metabolic parameter results: Induction of diabetes by feeding HFD and injecting STZ increased body weight and fasting blood glucose, as well as decreased insulin in the D group compared to the C group ($p < 0.05$, Figures 2 and Figure 3). Treating with ginger (100 mg/kg) and selenium (50, 100, and 150 µg/kg) daily significantly attenuated the HFD-STZ-induced harm on body weight, insulin, and fasting glucose compared to the untreated diabetic (D) group ($p < 0.05$, Figure 2). Moreover, there was no significant difference between the DG100 group and DSE50, DSE100, and DSE150 groups with regard to these parameters ($p > 0.05$, Figure 2 and Figure 3). Additionally, the combination of ginger (100 mg/kg) with different doses of selenium (50, 100, and 150 µg/kg) significantly improved body weight, insulin levels, and glucose levels in the DGSE50, DGSE100, and DGSE150 groups compared to the D, DG100, DSE50, DSE100, and DSE150 groups ($p < 0.05$, Figure 2 and Figure 3). Although we looked for a dose-dependent response, a comparison of the glucose and insulin results in the DGSE50, DGSE100, and DGSE150 groups or DSE50, DSE100, and DSE150 groups showed that increasing the dose of selenium did not cause a significant change ($p > 0.05$, Figure 3).

Oxidative biomarker results: Diabetes increased the MDA level and decreased GPx, CAT, and SOD levels in eye tissue

Table 2. HPLC analysis results of ginger extract.

Retention time (min)	Compound	Concentration (µg/ml)
5.23475	6-gingerol	34.712
8.61324	8-gingerol	8.124
10.84594	10- gingerol	6.413
19.23647	6-shaogol	46.478

in the D group compared to the C group ($p < 0.05$, Figure 4). In contrast, treating with ginger (100 mg/kg) and selenium (50, 100, and 150 µg/kg) daily significantly attenuated oxidative stress by decreasing the MDA level and increasing SOD, GPx, and CAT levels in the DG100, DSE50, DSE100, and DSE150 groups compared to the D group ($p < 0.05$, Figure 4). SOD and CAT levels had a significant increase in the DG100 group compared to the DSE50 and DSE100 groups. Additionally, there were no significant changes in MDA level as a by-product of lipid peroxidation in the DG100 and DSE150 groups, as well as the DG100 and DSE150 groups, regarding CAT and SOD levels. However, GPx activity increased in the DSE50, DSE100, and DSE150 groups in a dose-dependent manner, and there was no significant difference between the DG100 and DSE150 groups. Additionally, the combination of ginger (100 mg/kg) with different doses of selenium (50, 100, and 150 µg/kg) significantly improved SOD, CAT, and GPx levels and attenuated the MDA level in eye tissue in the DGSE50, DGSE100, and DGSE150 groups compared to the D, DSE50, DSE100, and DSE150 groups ($p < 0.05$, Figure 4). However, there was no significant difference among the DG100, DSE100, DSE150, and DGSE50 groups regarding SOD, CAT, GPx, and MDA values in eye tissue ($p > 0.05$).

Inflammatory marker results: HFD-STZ–induced diabetes increased significantly TNF-α and IL-1β levels in eye tissue in the D group compared to the C group ($p < 0.05$, Figure 5). In contrast, treating with ginger (100 mg/kg) and different doses of selenium (50, 100, and 150 µg/kg) daily attenuated significantly inflammation intensity by decreasing TNF-α and IL-1β values in the DG100, DSE50, DSE100, and DSE150 groups compared to the D group ($p < 0.05$, Figure 5). IL-1β and TNF-α values had a significant decrease in the DG100 group compared to the DSE50 and DSE100 groups but had no significant difference with the DSE150 group. IL-1β and TNF-α values attenuated in the DSE50, DSE100, and DSE150 groups in a dose-dependent manner, and the lowest values were observed in the DSE150 group, among others. However, there was no significant difference between the DG100 group and the DSE100 and DSE150 groups ($p > 0.05$, Figure 5). Additionally, the combination of ginger (100 mg/

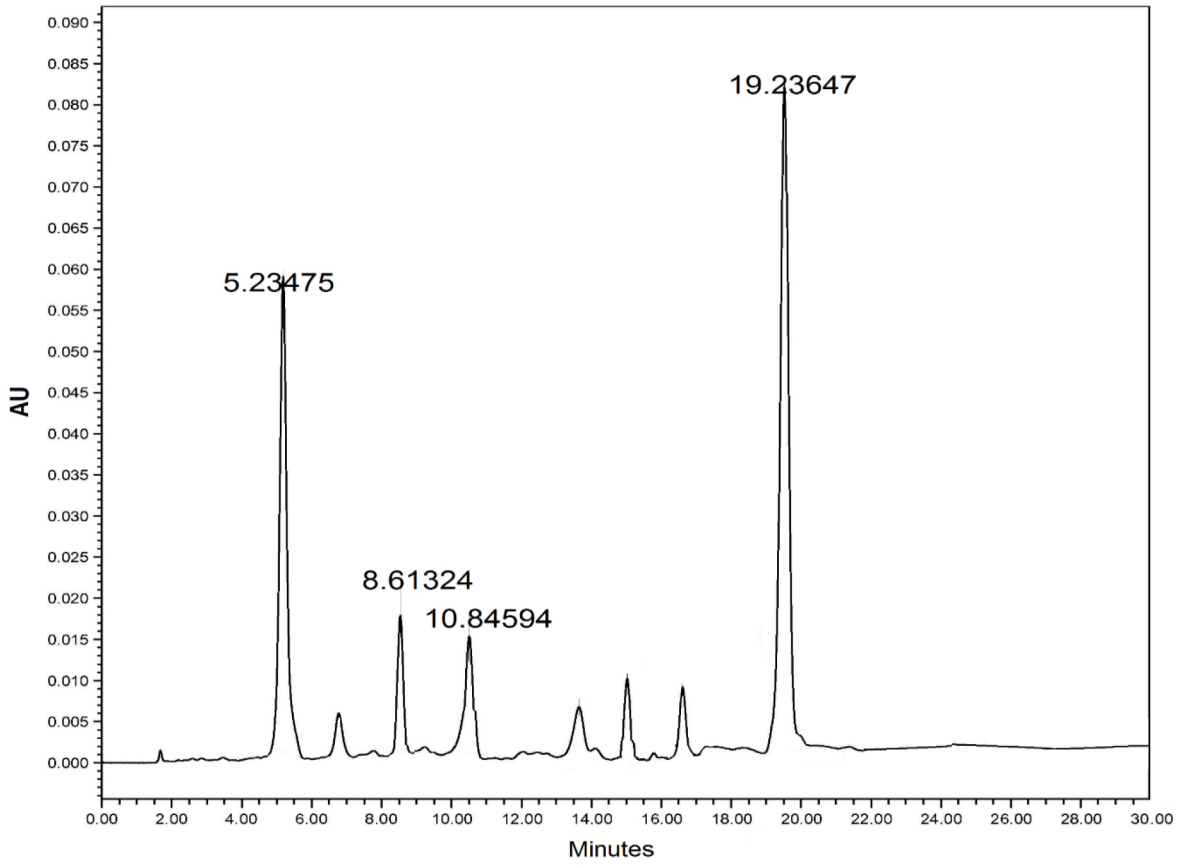

Figure 1. HPLC analysis of ginger extract. Representative HPLC chromatogram shows the identification and quantification of major bioactive compounds in the ginger extract.

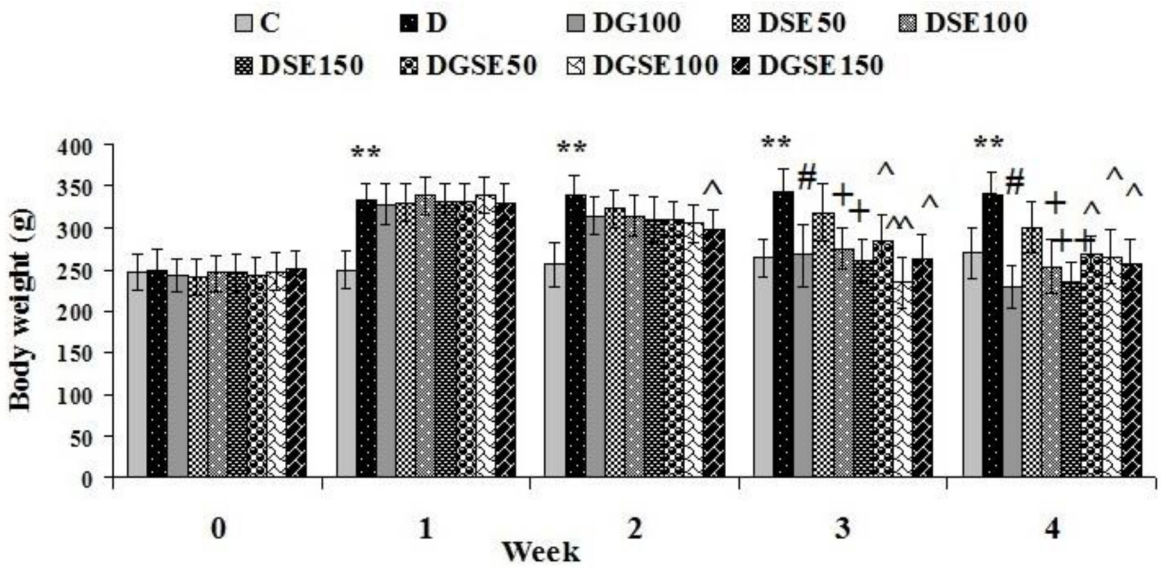

Figure 2. Body weight changes were monitored throughout the study period in all experimental groups. Data are presented as mean ± SD (n = 6). Week zero represents the baseline measurement prior to the induction of diabetes. Statistical significance is denoted as follows: □□p < 0.01 indicates a comparison with the control (C) group; #p < 0.05 and +p < 0.05 indicate a comparison with the diabetic (D) group; ++p < 0.01 and ^^p < 0.01 indicate a comparison with the diabetic (D) group; ^p < 0.05 indicates a comparison with the diabetic (D) group.

kg) with different doses of selenium (50, 100, and 150 µg/kg) significantly attenuated IL-1β and TNF-α values in eye tissue in the DGSE50, DGSE100, and DGSE150 groups in a dose-dependent manner compared to the D, DSE50, DSE100, and DSE150 groups ($p < 0.05$, Figure 5). However, there was no significant difference among the DG100, DSE100, DSE150, and DGSE50 groups regarding IL-1β and TNF-α values in eye tissue ($p > 0.05$, Figure 5).

Apoptosis results: HFD-STZ decreased Bcl2 expression gene and increased Bax and Cas-3 expression genes in eye tissue in the D group compared to the C group ($p < 0.05$, Figure 6). In contrast, treating with ginger (100 mg/kg) and selenium (50, 100, and 150 µg/kg) daily for 28 days significantly increased Bcl2 expression gene and decreased Bax and Cas-3 expression genes in the DG100, DSE50, DSE100, and DSE150 groups compared to the D group ($p < 0.05$, Figure 6). The expression of Bax and Cas-3 genes significantly decreased and the expression of the Bcl2 gene significantly increased in the DG100 group compared to the DSE50 and DSE100 groups. Additionally, Bcl2 expression in eye tissue had no significant difference in the DG100 group compared with the DSE150 group. In contrast, the expression of Bax and Cas-3 had a significant decrease in the DG100 group compared to the DSE150 group. The expression of Bax and Cas-3 decreased in the DSE50, DSE100, and DSE150 groups in a dose-dependent manner, and the lowest expression was observed in the DSE150 group, among others. However, the Bcl2 expression in eye tissue significantly increased in the DSE50, DSE100, and DSE150 groups, and the highest expression was observed in the DSE150 group, among others. Although there was a dose-dependent response to Bcl2, Bax, and Cas-3 expression in the DSE50, DSE100, and DSE150 groups, the DSE100 and DSE150 groups had no significant difference together. Additionally, treating with the combination of ginger (100 mg/kg) with different doses of selenium (50, 100, and 150 µg/kg) daily for 28 days significantly attenuated HFD-STZ–induced apoptosis in eye tissue in the DGSE50, DGSE100, and DGSE150 groups compared to the D, DSE50, DSE100, and DSE150 ($p < 0.05$, Figure 6). The expression of Bcl2 had a significant increase and the expression of Bax and Cas-3 had a significant decrease in the DGSE50, DGSE100, and DGSE150 groups compared to the D, DG100, DSE50, DSE100, and DSE150 groups. However, the expression of these genes in eye tissue had no significant changes in the DG100 and DSE150 groups compared to the DGSE50 group ($p > 0.05$, Figure 6).

Molecular results of TRPM2 and TRPV1: HFD-STZ administration increased the protein and gene expression of TRPV1 and TRPM2 in eye tissue in the D group compared to the C group ($p < 0.05$, Figure 7 and Figure 8). However, treatment of diabetic rats with ginger (100 mg/kg), selenium (50, 100, and 150 µg/kg), and their combinations daily for 28 days significantly decreased their gene and protein expression in eye tissue in the DG100, DSE50, DSE100, and DSE150 groups

compared to the D group ($p < 0.05$, Figure 3 and Figure 4). The expression of TRPV1 and TRPM2 genes and proteins significantly decreased in the DG100 group compared to the DSE50 and DSE100 groups and had no significant difference with the DSE150 group. Although there was a dose-dependent decrease in protein and gene TRPV1 and TRPM2 expression in the DSE50, DSE100, and DSE150 groups, the DSE100 and DSE150 groups had no significant difference together. Additionally, treatment of diabetic rats with the combination of ginger (100 mg/kg) with different doses of selenium (50, 100, and 150 µg/kg) daily for 28 days significantly attenuated protein and gene TRPV1 and TRPM2 expression in eye tissue in the DGSE50, DGSE100, and DGSE150 groups compared to the D, DG100, DSE50, DSE100, and DSE150 ($p < 0.05$, Figure 7 and Figure 8). However, protein and gene TRPV1 and TRPM2 expression in eye tissue had no significant changes in the DG100 and DSE150 groups compared to the DGSE50 group ($p > 0.05$, Figure 7 and Figure 8).

Histological evaluations: Histological evaluation of retinal tissue in diabetic rats was done by an optical microscope at ×40. Diabetic retinopathy symptoms, including edema (yellow line), detachment of the retinal layers (black line and green arrow), and inflammatory cell infiltration (blue arrow), can be seen in the prepared tissue sections (Figure 9, the diabetic group). The use of ginger extract (100 mg/kg), selenium (50, 100, and 150 µg/kg), and their combination greatly reduced the severity of these injuries and symptoms (Figure 9, the treated diabetic group).

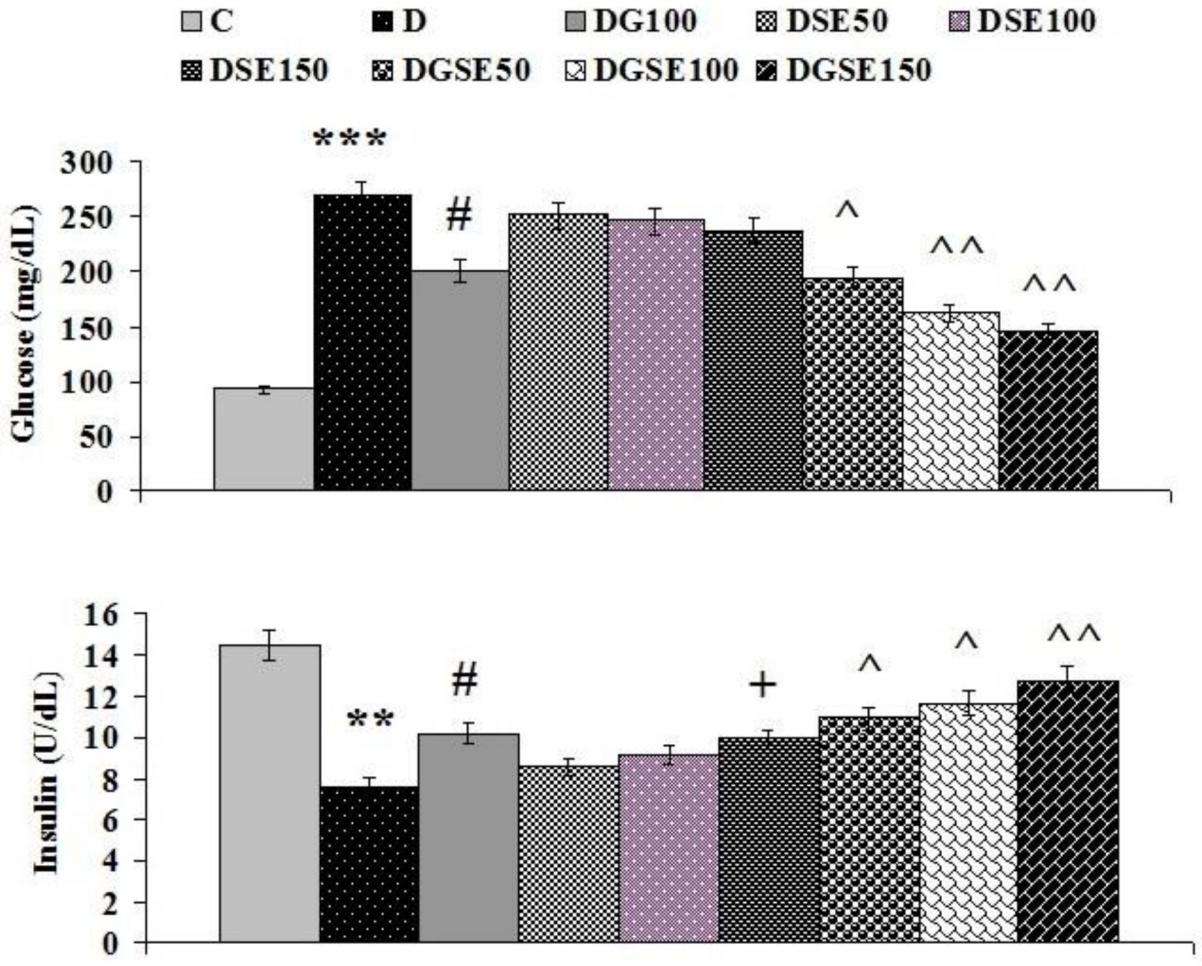

Figure 3. Serum levels of insulin and glucose were measured in all experimental groups at the conclusion of the study. Values are presented as mean ± SD (n = 6 per group). **Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Significant differences among multiple groups are indicated by different lowercase letters ($p < 0.05$). Additionally, asterisks denote comparisons with the control (C) group: □□$p < 0.01$ and *$p < 0.001$. Symbols indicate comparisons with the diabetic (D) group: #$p < 0.05$, +$p < 0.05$, ^^$p < 0.01$, and ^$p < 0.05$.

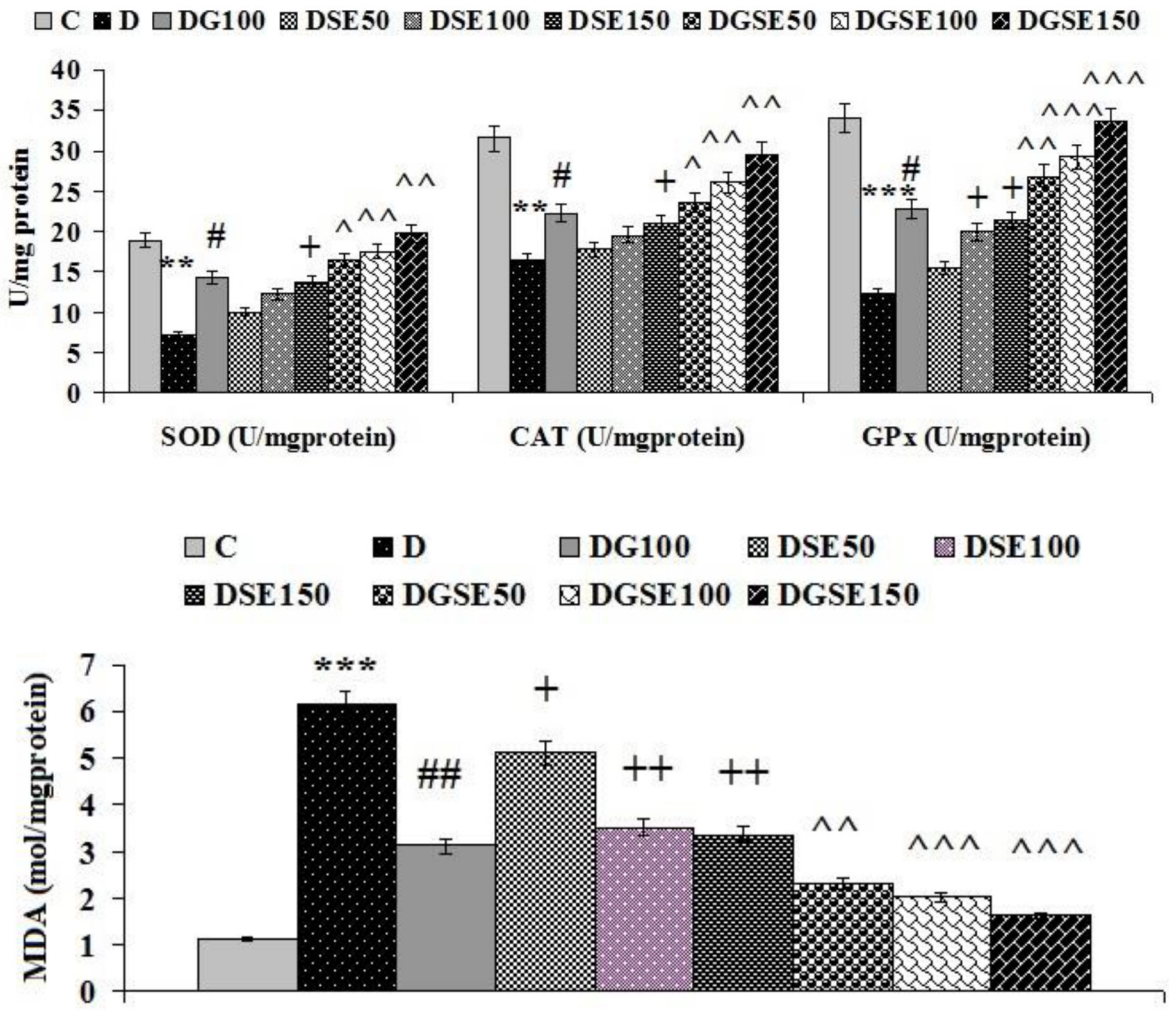

Figure 4. The activities of antioxidant enzymes (SOD, CAT, GPx) and the level of lipid peroxidation (MDA) were measured in the ocular tissue of control and treated groups. All data are expressed as the mean ± SD (n = 6).**Asterisks denote statistically significant differences compared to the control (C) group: □□p < 0.01 and *p < 0.001.Symbols indicate statistically significant differences compared to the untreated diabetic (D) group: #p < 0.05, ##p < 0.01, +p < 0.05, ++p < 0.01, ^p < 0.05, ^^p < 0.01, and ^^^p < 0.001.

DISCUSSION

HFD-STZ–induced diabetes increased protein and gene expression of TRPM2 and TRPV1, and it induced inflammation and oxidative damage by increasing the amount of TNF-α, IL-1β, and MDA and decreasing the amount of SOD, GPx, and CAT enzymes. It also induced apoptosis by increasing the expression of BAX and caspase 3 genes and decreasing Bcl2 in eye tissue. However, treatment with ginger extract, different doses of selenium, and their combinations improved these situations.

TRPM2 and TRPV1 channels are upregulated in diabetic retinopathy and contribute to the progression of retinopathy by inducing oxidative damage, apoptosis, and inflammation in retinal cells [30-32]. It has been reported that TRPV1, TRPV4, and TRPM2 channels are activated by oxidative stress [33,34]. DNA damage caused by free radicals and PARP-1 activation also causes the activation of the TRPM2 channel [35]. Our results in the diabetic group showed that protein and gene expression of TRPM2 and TRPV1 are parallel with changes in factors involved in causing oxidative damage, inflammation, and apoptosis. Previous studies have shown that TRP channels are sensitive to reduced intracellular thiol levels and can be inhibited by antioxidant treatment. Increasing intracellular thiol enhances cell survival by improving mitochondrial function and reducing related apoptotic cascades. In fact, these findings clearly indicate that improving the function of TRPM2 and TRPV1 channels

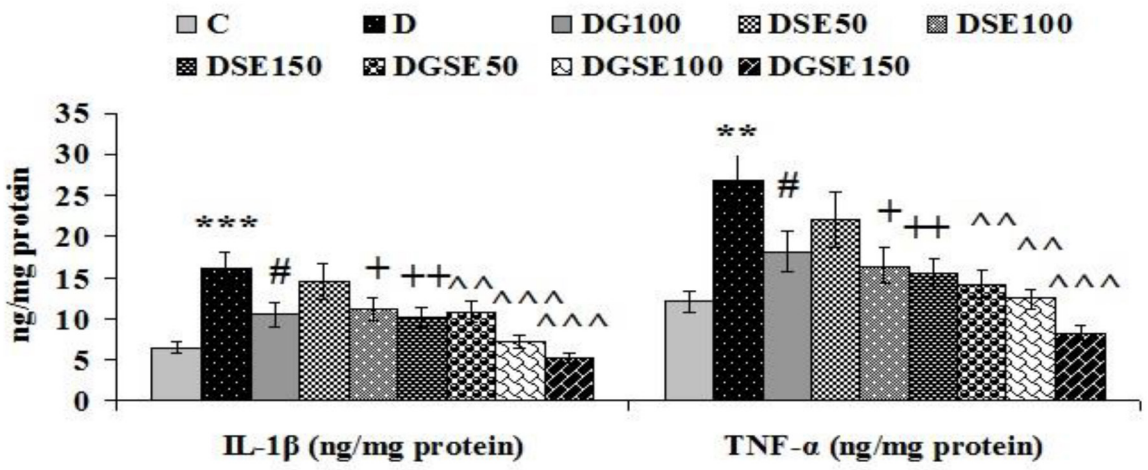

Figure 5. The concentrations of the pro-inflammatory cytokines IL-1β and TNF-α were quantified in the ocular tissue of all experimental groups. Data are presented as the mean ± SD (n = 6). Statistical significance is indicated as follows: double asterisks ($p < 0.01$) and triple asterisks ($p < 0.001$) denote a significant difference compared to the control (C) group; the symbols #, +, and ^ denote significant differences compared to the untreated diabetic (D) group at $\#p < 0.05$, $\#\#p < 0.01$, $+p < 0.05$, $++p < 0.01$, $\hat{}p < 0.05$, $\hat{}\hat{}p < 0.01$, and $\hat{}\hat{}\hat{}p < 0.001$.

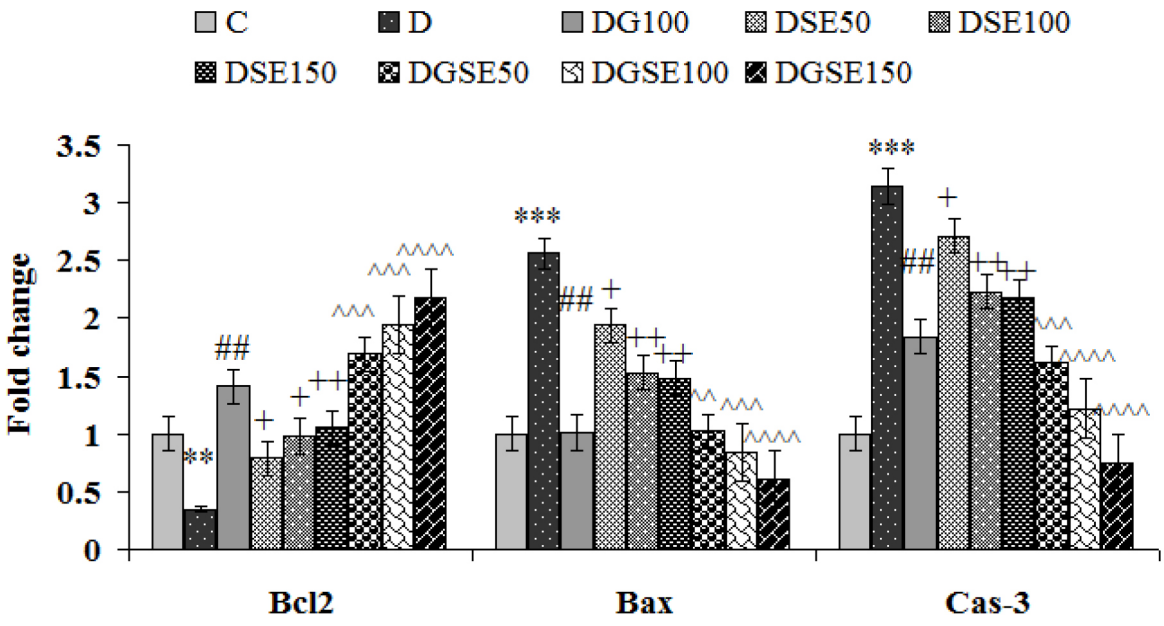

Figure 6. The effects of ginger extract, selenium, and their combinations on the ocular mRNA expression of apoptosis-related genes (Bcl2, Bax, and Caspase-3) were evaluated in diabetic rats.Gene expression levels were measured by quantitative real-time PCR, and data are presented as the mean ± SD (n=6).**Asterisks indicate statistically significant differences compared to the control (C) group: $**p < 0.01$ and $*p < 0.001$.Symbols denote statistically significant differences compared to the untreated diabetic (D) group: $\#\#p < 0.01$, $+p < 0.05$, $++p < 0.01$, $\hat{}\hat{}p < 0.01$, $\hat{}\hat{}\hat{}p < 0.001$, and $\hat{}\hat{}\hat{}\hat{}p < 0.0001$.

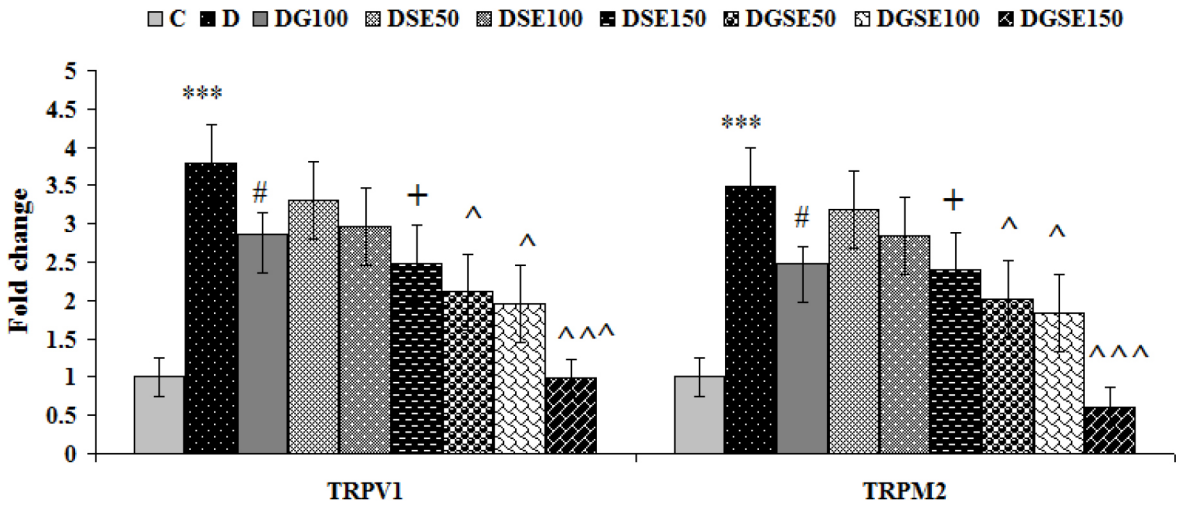

Figure 7. Treatments with ginger extract, selenium, and their combinations modulated the ocular mRNA expression of TRPV1 (**A**) and TRPM2 (**B**) in diabetic rats.Gene expression was analyzed by quantitative real-time PCR, and results are shown as the mean ± SD (n=8). Triple asterisks (*p < 0.001) indicate a statistically significant difference compared to the control (**C**) group.**The symbols #, +, and ^ denote significant differences compared to the untreated diabetic (**D**) group at #p < 0.05, +p < 0.05, ^p < 0.05, and ^^^p < 0.001.

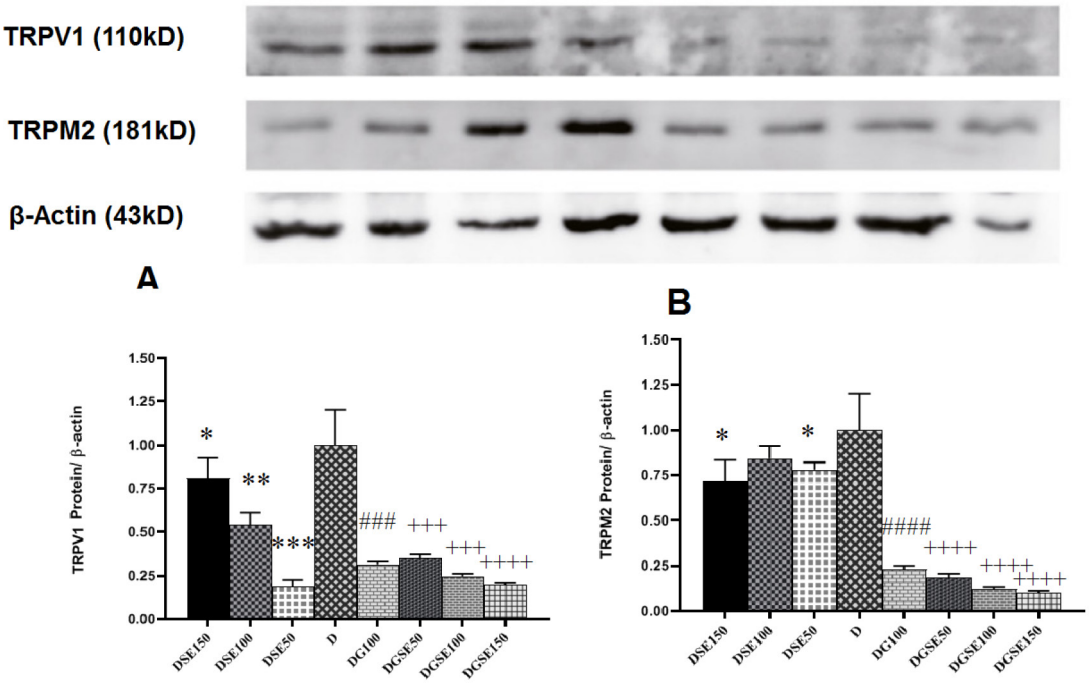

Figure 8. Western blot analysis demonstrates the effects of ginger extract, selenium, and their combinations on the protein levels of TRPV1 (**A**) and TRPM2 (**B**) in the ocular tissue of diabetic rats. Data from densitometric analysis are presented as the mean ± SD (n=3 independent experiments).**Statistical significance for comparisons with the untreated diabetic (D) group is indicated by asterisks and symbols: *p < 0.05, **p < 0.01, *p < 0.001, ###p < 0.001, ####p < 0.0001, +++p < 0.001, and ++++p < 0.0001.

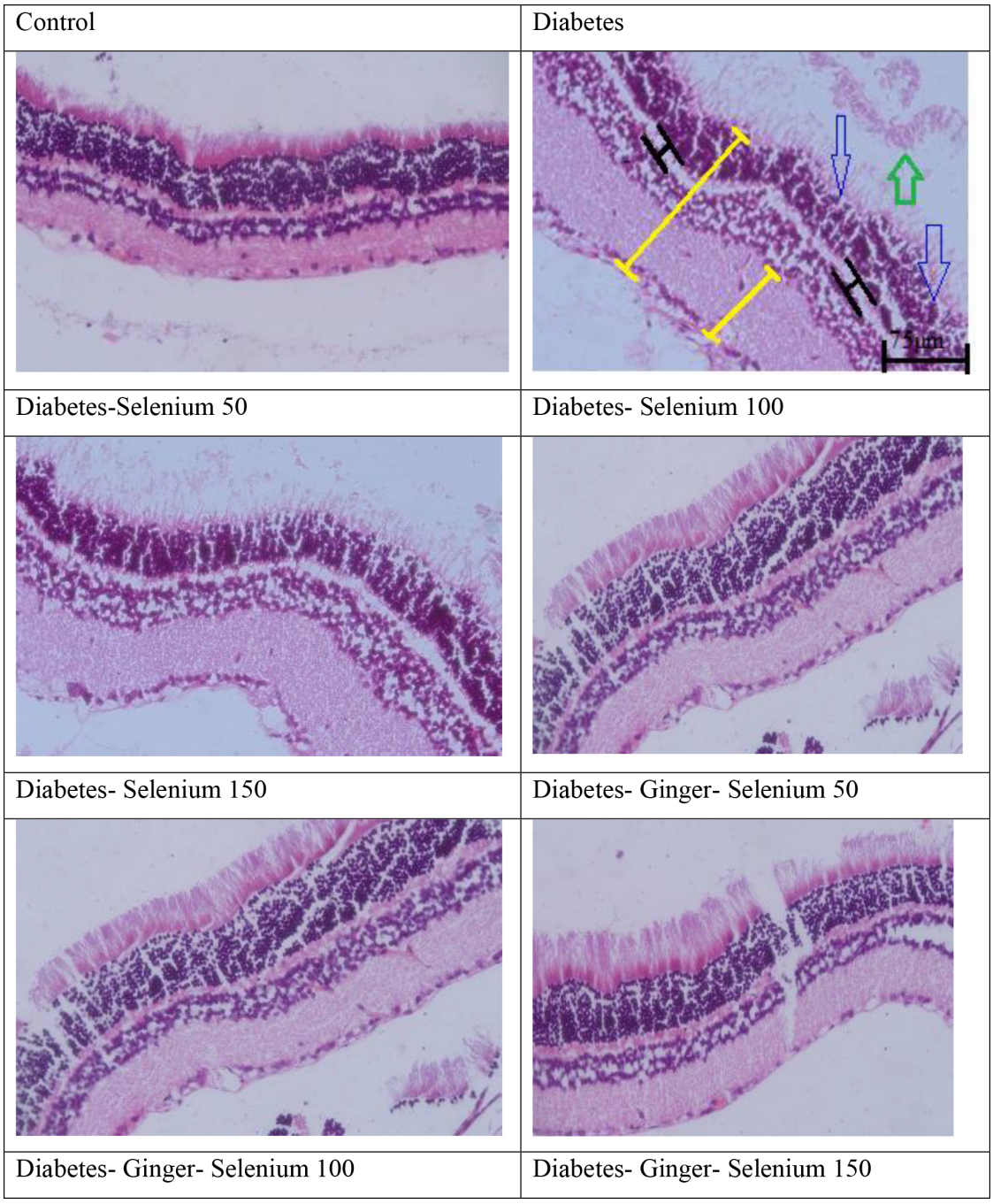

Figure 9. Representative photomicrographs of retinal tissue sections illustrate the histopathological changes across the experimental groups. Induction of diabetes with HFD-STZ caused significant damage to the retinal structure and induced retinopathy. Treatment with ginger extract, various doses of selenium, and their combinations ameliorated these diabetes-induced pathological changes. Images are representative of findings from 5 rats per group. Scale bar is provided.

and maintaining the appropriate concentrations of antioxidant precursors, such as thiol, can prevent the occurrence of events after their dysfunction (i.e., oxidative damage, inflammation, and apoptosis). In support of this evidence, the results of western blot analysis showed that the activity of TRPM2 and TRPV1 channels improved in a dose-dependent manner by treating with different doses of selenium. However, treatment with selenium in doses of 50 and 100 µg/kg did not have much effect on the expression of the genes of these channels, and the 150-µg/kg dose was able to significantly change their

expression. It seems that this effect is due to indirect gene changes resulting from selenium consumption. Probably, in this case, selenium changed the expression of TRPM2 and TRPV1 genes through its intracellular effects. Selenium inhibits activation of TRP channels and intracellular calcium signaling. In addition, the reduction of intracellular calcium decreases mitochondrial depolarization and prevents excessive production of free radicals. Supplementation with selenium, in line with our results, prevents apoptotic cell death by reducing caspase 3 and 9 activities. Selenium is also a cofactor for the antioxidant GPx in reducing excessive reactive oxygen species production in cytosolic compartments. Therefore, the treatments with selenium at different doses improved diabetic retinopathy by inhibiting oxidative damage, inflammation, and apoptosis via the downregulation of TRPM2 and TRPV1 activity.

Under these conditions, ginger appears to have inhibited the protein and gene expression of TRPM2 and TRPV1 channels, as well as factors contributing to oxidative damage and inflammation [14,17,36]. Ginger's bioactive compounds, such as gingerols and shaogols, are directly and indirectly responsible for inhibiting inflammation and oxidative damage [12,15]. These compounds also exert genomic and nongenomic control over the activity of enzymes involved in sugar and lipid metabolism, insulin action, and the modulation of pathways like the pentose phosphate pathway [12,15,37]. Reducing glucose levels and improving serum insulin levels are likely key factors in mitigating diabetes-related complications, particularly retinopathy. Consistent with its effects on glucose metabolism and insulin function, ginger's free radical scavenging ability likely contributed to reduced reactive oxygen species levels. This, in turn, appears to have further inhibited inflammation and oxidative damage, in addition to reducing TRPM2 and TRPV1 protein and gene expression.

Oxidative stress, apoptosis, and inflammation are believed to play a significant role in the progression of diabetic retinopathy [12,38-40]. Oxidative stress is a major contributor to retinal cell damage in diabetic retinopathy [38]. Our results showed that treatment with selenium at different doses inhibited MDA levels and improved SOD, CAT, and GPx levels in eye tissue in diabetic rats. Selenium acts as a cofactor in the thiol structure as a main precursor of various antioxidants such as GSH (glutathione) and Se-GPx (selenium-dependent glutathione peroxidase). A decrease in thiol level causes a disturbance in the activity of glutathione peroxidase and is one of the reasons for the disturbance in the antioxidant defense mechanisms. The findings of the diabetic groups treated with ginger extract, different doses of selenium, and their combination showed that ginger with selenium works synergistically to scavenge free radicals, reduce lipid peroxidation, and restore antioxidant enzyme activity. By inhibiting oxidative damage, these supplements may help preserve retinal cell function. Our results, in line with previous studies [14,36,41-43], showed that 100 mg/kg of ginger extract indicates a potential antioxidative activity in diabetic animal models and patients with diabetes. Moreover, ginger extract exhibited anti-inflammatory and antiapoptotic activities, as previously reported, due to the presence of bioactive compounds such as gingerols and shaogls [14,15,36].

Treatment with the combination of ginger (100 mg/kg) and different doses of selenium (50, 100, 150 µg/kg) downregulated protein and gene expression of TRPM2 and TRPV1, as well as inhibited oxidative damage, inflammation, and apoptosis in eye tissue of diabetic rats. There is limited research specifically investigating the synergistic effects of ginger and selenium on inhibiting apoptosis, oxidative damage, and inflammation or even TRPM2 and TRPV1 activity. However, both ginger and selenium have been individually studied for their potential antiapoptotic and inflammatory properties and inhibition of oxidative damage [30,32,37]. As discussed, ginger and selenium have both been studied for their potential anti-inflammatory and antioxidant effects [15,17,36,44]. When used together, they may exhibit synergistic effects in inhibiting inflammation and oxidative damage. Ginger contains various bioactive compounds such as gingerols, shogaols, and paradols, which have been shown to possess anti-inflammatory properties by inhibiting the production of proinflammatory molecules such as cytokines and prostaglandins [14,37]. These compounds have been shown to inhibit apoptosis by modulating various signaling pathways involved in cell death regulation [12,15,37]. For example, ginger extract has been found to suppress apoptosis by reducing the expression of proapoptotic proteins (e.g., Bax) and increasing the expression of antiapoptotic proteins (e.g., Bcl-2) [12]. Ginger extract can also improve the levels of some trace elements and total homocysteine, as well as prevent oxidative damage induced by ethanol in rat eye and testes [17,36]. Ginger also exerts its antiapoptotic effects by reducing oxidative stress and inflammation, which are known triggers for apoptosis [45]. Moreover, ginger contains compounds called piperine [37,44], which may enhance the absorption and bioavailability of certain nutrients, including selenium. By consuming ginger along with selenium-rich foods or supplements, the body can better absorb and utilize selenium for its anti-inflammatory and antioxidant effects [46]. Additionally, individual responses for ginger may vary based on factors such as dosage, form of supplementation (e.g., fresh ginger, ginger extract, selenium supplements), and overall health status [47].

While there is no specific research on the combined effects of ginger and selenium on inhibiting apoptosis, therefore, it is plausible that their individual antiapoptotic properties may synergistically contribute to enhanced protection against cell death. However, further studies are needed to explore this potential synergy and elucidate the underlying mechanisms involved. Overall, the combination of ginger and selenium works synergistically to inhibit inflammation by reducing proinflammatory molecules, enhancing antioxidant activity, modulating immune responses, and improving nutrient absorption. This combination may provide greater protection against inflammatory conditions and oxidative damage compared to using ginger or selenium alone [36]. Selenium has been shown to inhibit apoptosis by regulating multiple signaling pathways involved in cell death regulation [9,30]. For instance, selenium supplementation has been found to increase the expression of antiapoptotic proteins (e.g., Bcl-2) and decrease the expression of proapoptotic proteins (e.g., Bax) [9,30]. Additionally, selenium can enhance the activity of antioxidant enzymes, thereby reducing oxidative stress–induced apoptosis. However, it is important to note that more research is needed to establish a clear relationship between selenium supplementation and its effects on diabetic retinopathy in humans. Additionally, individual responses to selenium supplementation may vary, and it should be used under medical supervision [48]. Overall, there is some evidence suggesting the potential benefits of selenium for individuals with diabetic retinopathy. Therefore, the combination of ginger together with selenium in different doses has the potential to inhibit oxidative damage, inflammation, and apoptosis, thereby downregulating TRPM2 and TRPV1 channels. An important point regarding high doses of selenium is the toxic effects. Although we conducted a dose-response study to determine the healing effects of selenium, we did not observe toxic effects in high doses of selenium (100 and 150 µg/kg) in this study. There were also no significant differences between them in some parameters. This lack of significant difference in doses of 100 and 150 µg/kg may indicate the occurrence of cell tolerance or the preparation of cell defense mechanisms to initiate damaging events. In other words, taking these doses for more than the duration of this study or a higher dose may be toxic.

Conclusion: It can be concluded that diabetes due to HFD-STZ plays a significant role in developing retinopathy. However, treatment with ginger, selenium, and the combination of ginger with different doses of selenium could improve diabetic retinopathy by downregulating TRPM2 and TRPV1 expression and inhibiting oxidative damage, inflammation, and apoptosis. These data clearly demonstrate that the use of herbal and nutritional supplements, including essential elements such as a ginger-selenium combination, plays a very important role in inhibiting the progression and delaying the onset of diabetic retinopathy.

REFERENCES

1. Nentwich MM, Ulbig MW. Diabetic retinopathy - ocular complications of diabetes mellitus. World J Diabetes 2015; 6:489-99. [PMID: 25897358].
2. Rasoulinejad SA, Akbari A, Nasiri K. Interaction of miR-146a-5p with oxidative stress and inflammation in complications of type 2 diabetes mellitus in male rats: Anti-oxidant and anti-inflammatory protection strategies in type 2 diabetic retinopathy. Iran J Basic Med Sci 2021; 24:1078-86. [PMID: 34804425].
3. Kaneko Y, Szallasi A. Transient receptor potential (TRP) channels: a clinical perspective. Br J Pharmacol 2014; 171:2474-507. [PMID: 24102319].
4. Adhya P, Sharma SS. Redox TRPs in diabetes and diabetic complications: Mechanisms and pharmacological modulation. Pharmacol Res 2019; 146:104271[PMID: 31096011].
5. Hu W, Ding Y, Li Q, Shi R, He Y. Transient receptor potential vanilloid 4 channels as therapeutic targets in diabetes and diabetes-related complications. J Diabetes Investig 2020; 11:757-69. [PMID: 32129549].
6. Calderon GD, Juarez OH, Hernandez GE, Punzo SM, De la Cruz ZD. Oxidative stress and diabetic retinopathy: development and treatment. Eye (Lond) 2017; 31:1122-30. [PMID: 28452994].
7. Zhang BY, Zhang YL, Sun Q, Zhang PA, Wang XX, Xu GY, Hu J, Zhang HH. Alpha-lipoic acid downregulates TRPV1 receptor via NF-κB and attenuates neuropathic pain in rats with diabetes. CNS Neurosci Ther 2020; 26:762-72. [PMID: 32175676].
8. Chen L, Wang H, Xing J, Shi X, Huang H, Huang J, Xu C. Silencing P2X7R Alleviates Diabetic Neuropathic Pain Involving TRPV1 via PKCε/P38MAPK/NF-κB Signaling Pathway in Rats. Int J Mol Sci 2022; 23:14141
9. Kahya MC, Nazıroğlu M, Övey İS. Modulation of Diabetes-Induced Oxidative Stress, Apoptosis, and Ca^{2+} Entry Through TRPM2 and TRPV1 Channels in Dorsal Root Ganglion and Hippocampus of Diabetic Rats by Melatonin and Selenium. Mol Neurobiol 2017; 54:2345-60. [PMID: 26957303].
10. Moraes RDA, Webb RC, Silva DF. Vascular Dysfunction in Diabetes and Obesity: Focus on TRP Channels. Front Physiol 2021; 12:645109[PMID: 33716794].
11. Lakk M, Young D, Baumann JM, Jo AO, Hu H, Križaj D. Polymodal TRPV1 and TRPV4 Sensors Colocalize but Do Not Functionally Interact in a Subpopulation of Mouse Retinal Ganglion Cells. Front Cell Neurosci 2018; 12:353-[PMID: 30386208].
12. Luo L, Zhu S, Akbari A, Tan B. Ginger could improve gestational diabetes by targeting genes involved in nutrient metabolism, oxidative stress, inflammation, and the

WNT/β-Catenin/GSK3β signaling pathway. Nat Prod Commun 2022; •••:17-.

13. Ahmed Mustafa Z, Hamed Ali R, Rostum Ali D, Abdulkarimi R, Abdulkareem NK, Akbari A. The combination of ginger powder and zinc supplement improves the fructose-induced metabolic syndrome in rats by modulating the hepatic expression of NF-κB, mTORC1, PPAR-α SREBP-1c, and Nrf2. J Food Biochem 2021; 45:e13546[PMID: 33145794].

14. Fathi R, Akbari A, Nasiri K, Chardahcherik M. Ginger (*Zingiber officinale* roscoe) extract could upregulate the renal expression of *NRF2* and *TNFα* and prevents ethanol-induced toxicity in rat kidney. Avicenna J Phytomed 2021; 11:134-45. [PMID: 33907672].

15. Ma H, Li J. The ginger extract could improve diabetic retinopathy by inhibiting the expression of e/iNOS and G6PDH, apoptosis, inflammation, and angiogenesis. J Food Biochem 2022; 46:e14084[PMID: 35060143].

16. Xue W, Tang Q, Yang L. The combination of ginger and zinc supplement could improve lead-induced reproductive dysfunction by inhibiting apoptosis mediated by oxidative damage and inflammation. Andrologia 2022; 54:e14577[PMID: 36574600].

17. Akbari A, Nasiri K, Heydari M, Mosavat SH, Iraji A. The protective effect of hydroalcoholic extract of Zingiber officinale Roscoe (Ginger) on ethanol-induced reproductive toxicity in male rats. J Evid Based Complementary Altern Med 2017; 22:609-17. [PMID: 29228791].

18. Akbari A, Nasiri K, Heydari M, Nimrouzi M, Afsar T. Ameliorating potential of ginger (Zingiber officinale Roscoe) extract on liver function and oxidative stress induced by ethanol in male rats. Zahedan J Res Med Sci 2019; 21:.

19. González de Vega R, García M, Fernández-Sánchez ML, González-Iglesias H, Sanz-Medel A. Protective effect of selenium supplementation following oxidative stress mediated by glucose on retinal pigment epithelium. Metallomics 2018; 10:83-92. [PMID: 29119175].

20. She C, Shang F, Cui M, Yang X, Liu N. Association between dietary antioxidants and risk for diabetic retinopathy in a Chinese population. Eye (Lond) 2021; 35:1977-84. [PMID: 33009517].

21. Mattmiller SA, Carlson BA, Sordillo LM. Regulation of inflammation by selenium and selenoproteins: impact on eicosanoid biosynthesis. J Nutr Sci 2013; 2:e28[PMID: 25191577].

22. Huang Z, Rose AH, Hoffmann PR. The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 2012; 16:705-43. [PMID: 21955027].

23. Mousavi SM, Gorji FZ, Fallahmohammadi Z, Nasiri K, Akbari A. Endurance training promotes the browning of WAT by enhancing the NPFF pathway in the hypothalamus in rats with type 2 diabetes. Iran J Basic Med Sci 2025; 28:575-83. [PMID: 40666184].

24. Zhang M, Lv X-Y, Li J, Xu Z-G, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008;2008.

25. Chu C, Zavala K, Fahimi A, Lee J, Xue Q, Eilers H, Schumacher MA. Transcription factors Sp1 and Sp4 regulate TRPV1 gene expression in rat sensory neurons. Mol Pain 2011; 7:44-[PMID: 21645329].

26. Xiao Q, Deng B, Akbari A, Liu Q, Zhu B. The ketogenic diet could improve the efficacy of curcumin and Oldenlandia diffusa extract in the treatment of gastric cancer by increasing miR340 expression and apoptosis mediated by autophagy, oxidative stress, and angiogenesis. J Food Biochem 2022; 46:e14407[PMID: 36219718].

27. Evtushenko AA, Voronova IP, Kozyreva TV. Effect of Long-Term Adaptation to Cold and Short-Term Cooling on the Expression of the TRPM2 Ion Channel Gene in the Hypothalamus of Rats. Curr Issues Mol Biol 2023; 45:1002-11. [PMID: 36826010].

28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Δ Δ C(T)) Method. Methods 2001; 25:402-8. [PMID: 11846609].

29. Dongare S, Gupta SK, Mathur R, Saxena R, Mathur S, Agarwal R, Nag TC, Srivastava S, Kumar P. Zingiber officinale attenuates retinal microvascular changes in diabetic rats via anti-inflammatory and antiangiogenic mechanisms. Mol Vis 2016; 22:599-609. [PMID: 27293376].

30. Yüksel E, Nazıroğlu M, Şahin M, Çiğ B. Involvement of TRPM2 and TRPV1 channels on hyperalgesia, apoptosis and oxidative stress in rat fibromyalgia model: Protective role of selenium. Sci Rep 2017; 7:17543-[PMID: 29235496].

31. Demirdaş A, Nazıroğlu M, Övey İS. Duloxetine Reduces Oxidative Stress, Apoptosis, and Ca^{2+} Entry Through Modulation of TRPM2 and TRPV1 Channels in the Hippocampus and Dorsal Root Ganglion of Rats. Mol Neurobiol 2017; 54:4683-95. [PMID: 27443158].

32. Balaban H, Nazıroğlu M, Demirci K, Övey İS. The Protective Role of Selenium on Scopolamine-Induced Memory Impairment, Oxidative Stress, and Apoptosis in Aged Rats: The Involvement of TRPM2 and TRPV1 Channels. Mol Neurobiol 2017; 54:2852-68. [PMID: 27021021].

33. Nazıroğlu M. A novel antagonist of TRPM2 and TRPV4 channels: Carvacrol. Metab Brain Dis 2022; 37:711-28. [PMID: 34989943].

34. Bai JZ, Lipski J. Differential expression of TRPM2 and TRPV4 channels and their potential role in oxidative stress-induced cell death in organotypic hippocampal culture. Neurotoxicology 2010; 31:204-14. [PMID: 20064552].

35. Yamamoto S, Shimizu S. Targeting TRPM2 in ROS-coupled diseases. Pharmaceuticals (Basel) 2016; 9:57-[PMID: 27618067].

36. Akbari A, Nasiri K, Heydari M. Ginger (*Zingiber officinale* Roscoe) extract can improve the levels of some trace elements and total homocysteine and prevent oxidative

damage induced by ethanol in rat eye. Avicenna J Phytomed 2020; 10:365-71. [PMID: 32850293].

37. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). Foods (Basel, Switzerland). 2019;8(6).

38. Kang Q, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. Redox Biol 2020; 37:101799[PMID: 33248932].

39. Li B, Li J, Akbari A, Baziyar P, Hu S. Evaluation of Expression of Cytochrome P450 Aromatase and Inflammatory, Oxidative, and Apoptotic Markers in Testicular Tissue of Obese Rats (Pre)Treated with Garlic Powder. Evid Based Complement Alternat Med 2023; 2023:4858274[PMID: 36644444].

40. Gao J, Akbari A, Wang T. Green tea could improve elderly hypertension by modulating arterial stiffness, the activity of the renin/angiotensin/aldosterone axis, and the sodium-potassium pumps in old male rats. J Food Biochem 2022; 46:e14398[PMID: 36181277].

41. Samad MB, Mohsin MNAB, Razu BA, Hossain MT, Mahzabeen S, Unnoor N, Muna IA, Akhter F, Kabir AU, Hannan JMA. 6-Gingerol, from Zingiber officinale, potentiates GLP-1 mediated glucose-stimulated insulin secretion pathway in pancreatic β-cells and increases RAB8/RAB10-regulated membrane presentation of GLUT4 transporters in skeletal muscle to improve hyperglycemia in Lepr$^{db/db}$ type 2 diabetic mice. BMC Complement Altern Med 2017; 17:395-[PMID: 28793909].

42. Mandal P, Das A, Majumdar S, Bhattacharyya T, Mitra T, Kundu R. The efficacy of ginger added to ondansetron for preventing postoperative nausea and vomiting in ambulatory surgery. Pharmacognosy Res 2014; 6:52-7. [PMID: 24497743].

43. Li Y, Tran VH, Duke CC, Roufogalis BD. Gingerols of Zingiber officinale enhance glucose uptake by increasing cell surface GLUT4 in cultured L6 myotubes. Planta Med 2012; 78:1549-55. [PMID: 22828920].

44. Atal N, Bedi KL. Bioenhancers: Revolutionary concept to market. J Ayurveda Integr Med 2010; 1:96-9. [PMID: 21836795].

45. Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and protective properties of Zingiber officinale (ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: a brief review. Evid Based Complement Alternat Med 2012; 2012:516870[PMID: 23243452].

46. Bapte PS, Pansambal SS, Borgave S. Biosynthesized selenium nanoparticles: an excellent bait for antioxidant therapy. Journal of Nanostructures. 2022; 12:178-93. .

47. Anh NH, Kim SJ, Long NP, Min JE, Yoon YC, Lee EG, Kim M, Kim TJ, Yang YY, Son EY, Yoon SJ, Diem NC, Kim HM, Kwon SW. Ginger on Human Health: A Comprehensive Systematic Review of 109 Randomized Controlled Trials. Nutrients 2020; 12:157-[PMID: 31935866].

48. Minich WB. Selenium Metabolism and Biosynthesis of Selenoproteins in the Human Body. Biochemistry (Mosc) 2022; 87:Suppl 1S168-102. [PMID: 35501994].