



ORIGINAL ARTICLE

Geraniin ameliorates streptozotocin-induced diabetic retinopathy in rats via modulating retinal inflammation and oxidative stress



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KEYWORDS

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Abstract

Background: Diabetic retinopathy (DR) is the major complication of diabetes, which causes acquired vision loss in the working-age group population.

Objective: Here, we planned to address the therapeutic roles of geraniin against the streptozotocin (STZ)-challenged DR in rats

Methodology: The DR was induced in the animals by 60 mg/kg of STZ, and then treated with 25 mg/kg of geraniin for 60 days. Later, bodyweight, food consumption, and blood glucose levels were investigated. The levels of antioxidants, MMP-9, MCP-1, and VEGF, and inflammatory cytokine status were measured using marker-specific kits. The morphometric study was conducted to assess the retinal thickness. The pancreatic tissues were analyzed microscopically.

Results: Geraniin reduced the blood glucose (270.36 ± 81 mg/dL), hemoglobin, and enhanced bodyweight (261.93 ± 72 g) in the DR rats. The antioxidant levels in the STZ-challenged DR rats were substantially improved by geraniin. Geraniin also decreased inflammatory cytokines, MCP-I,

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MMP-9, and VEGF levels and enhanced the retinal thickness. A histological study demonstrated that geraniin reduced the pancreatic islet cell damage in STZ-induced DR rats.

Conclusion: Our outcomes witnessed that geraniin reduced retinal inflammation and oxidative stress in the STZ-induced DR rats.

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1. Introduction

Diabetic retinopathy (DR) is a common problem among diabetic patients and a major cause of acquired vision loss in the working-age population (Yamagishi and Matsui, 2011). The occurrence of DR among diabetic patients was estimated at 35 %, and almost 10 % of patients among them had the risk of vision loss (Sayres et al., 2019; Hu et al., 2012). DR results in the detachment and edema of the retina, which further directs to vision loss. Diabetic patients are more likely to suffer from chronic vascular damage caused by high blood sugar levels, and they are at a higher risk of developing diabetes (Barot et al., 2013). The exact etiology of DR is not yet known, but the causes may comprise hyperglycemia, inflammation, oxidative stress, and depletion of antioxidants that could initiate the DR progression (Abougambou and Abougambou, 2015; Zheng et al., 2018). The uncontrolled hyperglycemia due to diabetes leads to oxidative stress via facilitating increased glucose oxidation, protein kinase-C and polyol fluctuations, and elevated end products of glycation (Rains and Jain, 2011; Singh et al., 2011). The eye cells are more vulnerable to oxidative stress than other body tissues because of the high levels of membrane-bound polyunsaturated fatty acids and higher glucose oxidation and oxygen utilization. The depletion of antioxidant defensive mechanisms and improved oxidative stress are the crucial contributors to hyperglycemia-mediated injury (Cao et al., 2014). The increased energy needs during diabetes and retinal exposure to light could elevate the DR risks. Hence, adjusting the oxidative and antioxidant proportions could be a hopeful approach to prevent DR-provoked injuries (Vakifahmetoglu-Norberg et al., 2017).

Chronic inflammation at the retinal blood vessels is caused by arteriosclerotic plate depositions caused by high blood glucose levels, making them vulnerable to microrupture, which results in fluid leakage into the retina. If this condition is left undiagnosed, there could be new vessel growth that alters the retinal microvasculature and this consequently triggers the detachment of retina (Aylward, 2005; Kern, 2007). NF- κ B activates many genes involved in disease progression during DR progression, enhancing inflammatory regulators such as cytokines and intercellular adhesion molecules-1 (ICAM-1) (Leal et al., 2010). These regulators simultaneously control the BRB permeability combined with leukostasis (Zhong and Kowluru, 2011). Angiogenesis is one of the imperative pathological features of DR progression. The elevated expression of angiogenic proteins like VEGF is tightly connected with diabetes-provoked neurodegeneration and oxidative stress. Calcium dobesilate is a well-known angioprotective and vasoactive drug, which is extensively employed to treat DR at the local ocular and systematic level with hopeful outcomes (Cai et al., 2017). For many years, laser photocoagulation has been considered as the gold standard therapeutic option to treat the DR. However, much recent evidence has shown that the intravitreal administration of anti-VEGF agents has the potential to alleviate the DR and is hence currently being explored broadly (Wells et al., 2016). Nonetheless, not all the DR patients responded well to the anti-VEGF drugs and also experienced several adverse effects. Hence, the discovery of novel therapeutic agents with minimal adverse effects from natural sources to treat the DR were highly demanded.

Geraniin is a crystalline tannin found in several medicinal plant species from the Sapindaceae, Geranium, and Euphobiaceae families, e.g., *Nephelium lappaceum*. Geraniin is reported to exhibit numerous biological actions like antioxidants (Lin et al., 2008), antihypertensive

and antihyperglycaemic properties (Palanisamy et al., 2011). It was also highlighted that the geraniin revealed potent hepatoprotective (Aayadi et al., 2017), anti-inflammatory (Wang et al., 2016; Okabe et al., 2001), anticancer (Wang et al., 2017), antitumor (Guo et al., 2018), insulin sensitizing (Perera et al., 2020), and anti-atherosclerosis (Lin et al., 2022 Jul). In contrast, the therapeutic role of geraniin in the DR has yet to be investigated. As a result, we conducted this study to address the therapeutic role of geraniin against the streptozotocin (STZ)-triggered DR in rats via ameliorating oxidative stress and inflammation.

2. Materials and methods

2.1. Chemicals

Geraniin, STZ, citrate buffer (CB), and other chemicals were purchased from Sigma Aldrich, USA. The marker-specific assay kits were procured from Thermofisher and R&D Systems, USA.

3. Experimental animals

The 6–8 week old Wistar rats (male breed) weighing approximately 230 ± 30 g were chosen for this study and purchased from the institutional animal house. The experimental protocol has been approved by the institutional ethics committee at Shandong First Medical University, Shandong, China. Rats were caged with the utmost care in organized laboratory conditions, which were conserved with a 22–26 °C temperature, 40–70 % air moisture, and a 12-h dark-light sequence. All rats were administered pure water and standard rat chow during the study period. Before the experiments started, all of the rats were in the lab for a week to get used to it.

3.1. Experimental design

The rats were distributed into four groups ($n = 6$). Group I was control rats received only 0.1 M of CB instead of STZ. Group II rats were administered with 60 mg/kg of STZ (dissolved in 0.1 M of CB) to provoke the DR and were considered as a DR control. After 48 h of STZ administration, the blood glucose of rats was examined, and the animals with 200 mg/dl or above were deliberated as diabetic. Group III were diabetic rats provided orally with 25 mg/kg of geraniin for 60 days. Group IV were diabetic rats provided with 350 mg/kg of metformin for 60 days (standard drug). Finally, the blood was gathered and utilized for the biochemical assessments.

3.2. Measurement of changes in the blood glucose, glycosylated hemoglobin (HbA1c), food intake, and bodyweight

The average food intake levels of all the experimental animals were measured and tabulated carefully. The body weight of animals was detected using a sensitive weighing balance and

values were tabulated. The blood glucose in the treated animals was examined with the help of a glucometer. The HbA1c levels in the blood samples of rats were examined using an assay kit according to the protocols described by the manufacturer (ThermoFisher, USA).

3.3. Measurement of enzymatic and non-enzymatic antioxidant levels

The activities of superoxide dismutase (SOD) and SOD/catalase (CAT) in the retina of experimental animals were investigated using marker-specific kits using manufacturer's guidelines (ThermoFisher, USA). The levels of glutathione (GSH) and GSH/oxidized glutathione (GSSG) in the retina were investigated using assay kits by the manufacturer's guidelines (ThermoFisher, USA).

3.4. Determination of VEGF, MCP-1, and MMP-9 levels

The angiogenic protein MCP-1, VEGF and MMP-9 status in the experimental rats were investigated using assay kits as per the described protocols by the manufacturer (R&D Systems, USA). Each assay was done in triplicate and the outcomes were depicted as ng/l.

3.5. Assay of pro-inflammatory cytokine levels

The inflammatory cytokines, i.e., TNF- α , IL-6, IL-1 β , IFN- γ , IL-8, IL-12, IL-2, IL-3, and IL-10 contents in the serum of

experimental animals were assayed using marker specific kits in accordance with the recommended protocols of the manufacturer (ThermoFisher and MyBioSource, USA, respectively). Each assay was done in triplicate and the outcomes were depicted as ng/l.

3.6. Morphometric analysis

The morphometric assessment was conducted on the retinal tissues of experimental rats with the aid of a computer-assisted image analysis technique. The thickness of the retina and the cells in the ganglion cell layer (GCL) was detected using a morphometric study using the Image J software.

3.7. Histopathological study

The pancreatic tissues were removed from the experimental animals and processed with 10 % formalin for 24 h and then dehydrated by the addition of isopropyl alcohol/xylene for 12 h. Afterward, pancreatic tissues were paraffin fixed and cut into 5 mm size. The sections were then stained with H&E and the changes in pancreatic islet cells were assayed using an optical microscope at 40 \times magnification.

3.8. Statistical analysis

The outcomes were interpreted statistically using GraphPad Prism software (ver.7.0). Outcomes were illustrated as

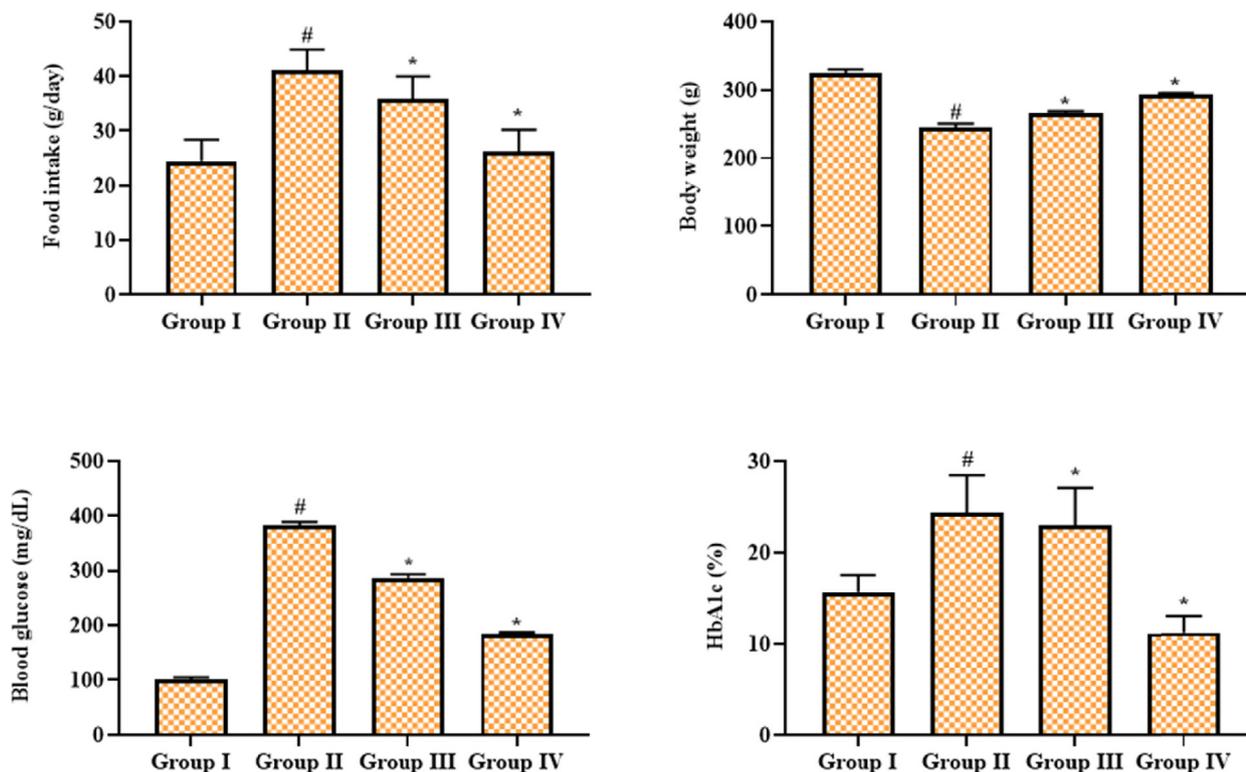


Fig. 1 Effect of geraniin on the bodyweight, blood glucose, hemoglobin, and food intake in the STZ-challenged DR rats. Group I: Control; Group II: STZ-induced DR rats; Group III: STZ-induced DR + 25 mg/kg of geraniin treated rats; Group IV: STZ-induced DR + 350 mg/kg of metformin treated rats. Values are represented as the mean \pm SD of triplicates assessed statistically by one-way ANOVA and Tukey's multiple comparison assay. '#' reveals significance at $p < 0.05$ from control and '*' reveals significance at $p < 0.01$ from STZ group.

mean \pm SD of triplicates, which were investigated using one-way ANOVA and Tukey's post hoc assay. Significance was fixed at 'p' < 0.05.

4. Results

4.1. Geraniin treatment modulates the changes in the STZ-provoked DR rats

Fig. 1 demonstrates the impacts of geraniin on bodyweight, food consumption, HbA1c, and blood glucose in the STZ-provoked DR rats. The improved bodyweight, HbA1c, and blood glucose were evidenced in the STZ-triggered DR rats, which is in contrast to the control. STZ-induced rats also had a lower bodyweight than control. Conversely, the 25 mg/kg of geraniin treatment appreciably suppressed the food consumption, HbA1c, and blood glucose in the STZ-challenged DR rats. The bodyweight of the STZ-challenged rats was also improved by the geraniin treatment. These results were found to be consistent with the results of standard drug metformin treatment.

4.2. Geraniin treatment improved the antioxidant levels in the retinal tissues of STZ-challenged DR rats

Fig. 2 shows the effects of geraniin on the antioxidants in the retina of STZ-challenged DR animals. When compared to control, STZ-administered DR rats had lower SOD and SOD/CAT activity as well as lower GSH and GSH/GSSG contents.

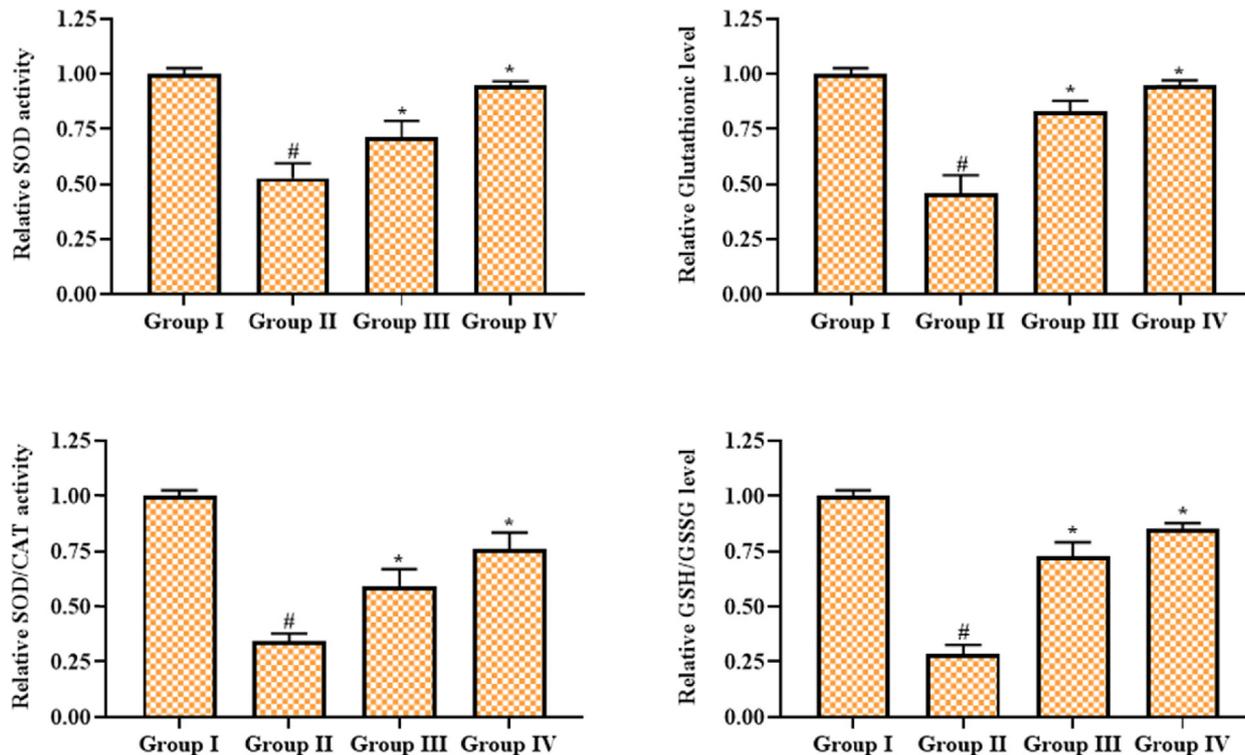


Fig. 2 Effect of geraniin on the antioxidant levels in the retinal tissues of STZ-provoked DR rats. Group I: Control; Group II: STZ-induced DR rats; Group III: STZ-induced DR + 25 mg/kg of geraniin treated rats; Group IV: STZ-induced DR + 350 mg/kg of metformin treated rats. Values are represented as the mean \pm SD of triplicates assessed statistically by one-way ANOVA and Tukey's multiple comparison assay. '#' reveals significance at p < 0.05 from control and '*' reveals significance at p < 0.01 from STZ group.

The 25 mg/kg of geraniin administration remarkably enhanced the SOD and SOD/CAT activities and GSH and GSH/GSSG contents in the STZ-provoked DR rats, which proves the antioxidant action of geraniin. The outcomes of geraniin treatment were slightly similar to those of metformin treatment.

4.3. Geraniin treatment decreased the angiogenic and inflammatory markers in the serum of STZ-challenged DR rats

Fig. 3 exhibits the levels of the angiogenic protein VEGF and the inflammatory proteins MMP-9 and MCP-1 in the experimental rats. The STZ-provoked rats displayed improved levels of VEGF, MCP-I, and MMP-9 in the serum. Nonetheless, these elevations were remarkably decreased by the geraniin treatment. The 25 mg/kg of geraniin appreciably reduced the levels of VEGF, MCP-I, and MMP-9. Metformin also decreased the amount of VEGF, MCP-I, and MMP-9 in the serum of DR rats that had been given STZ.

4.4. Geraniin treatment reduced the inflammatory cytokines level in the serum of STZ-challenged DR rats

Fig. 4 displays the inhibitory actions of geraniin on the inflammatory cytokine content in the serum of STZ-challenged DR animals. The levels of inflammatory cytokines, i.e., TNF- α , IL-6, IL-1 β , IFN- γ , IL-8, IL-12, IL-2, IL-3, and IL-10 were found elevated in the serum of DR rats, which is in contrast to the control. Interestingly, the drastic reductions in the contents of TNF- α , IL-6, IL-1 β , IFN- γ , IL-8, IL-12, IL-2, IL-3,

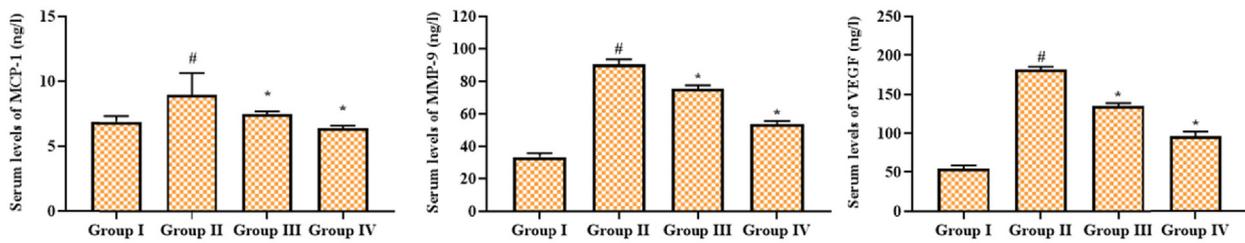


Fig. 3 Effect of geraniin on the angiogenic marker levels in the serum of STZ-challenged DR rats. Group I: Control; Group II: STZ-induced DR rats; Group III: STZ-induced DR + 25 mg/kg of geraniin treated rats; Group IV: STZ-induced DR + 350 mg/kg of metformin treated rats. Values are represented as the mean \pm SD of triplicates assessed statistically by one-way ANOVA and Tukey's multiple comparison assay. '#' reveals significance at $p < 0.05$ from control and '*' reveals significance at $p < 0.01$ from STZ group.

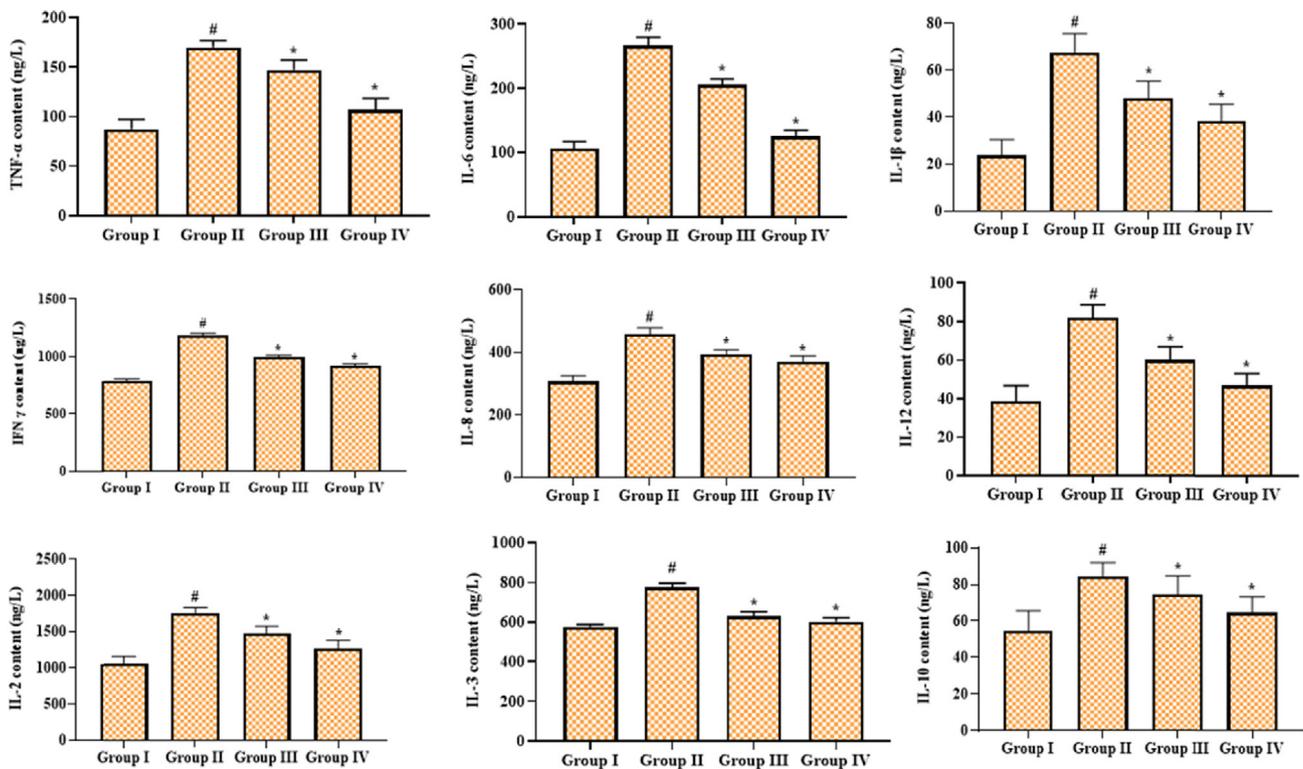


Fig. 4 Effect of geraniin on the inflammatory cytokines level in the serum of STZ-challenged DR rats. Group I: Control; Group II: STZ-induced DR rats; Group III: STZ-induced DR + 25 mg/kg of geraniin treated rats; Group IV: STZ-induced DR + 350 mg/kg of metformin treated rats. Values are represented as the mean \pm SD of triplicates assessed statistically by one-way ANOVA and Tukey's multiple comparison assay. '#' reveals significance at $p < 0.05$ from control and '*' reveals significance at $p < 0.01$ from STZ group.

and IL-10 were witnessed in the 25 mg/kg of geraniin administered to DR animals. Hence, it was clear that the geraniin could decrease the inflammatory cytokine level, which is found to be similar to the outcomes of metformin.

4.5. Geraniin enhanced the retinal thickness and cell number in CGL of STZ-challenged DR rats

Fig. 5 demonstrates the findings of morphometric analysis of the retinas of experimental rats. The findings demonstrated that retinal thickness and the number of cells in the CGL of the retina were diminished in the DR rats. Fascinatingly, ger-

aniin effectively modulated these changes in the STZ-challenged animals. The increased retinal thickness and cell numbers in the CGL were witnessed in the retinal tissues of 25 mg/kg of geraniin administered DR rats. The metformin also moderated these alterations in the STZ-challenged DR rats.

4.6. Geraniin treatment improved the pancreas histology of the STZ-challenged DR rats

Fig. 6 displays the histological changes of pancreas tissues from control and treated rats. The control rats demonstrated

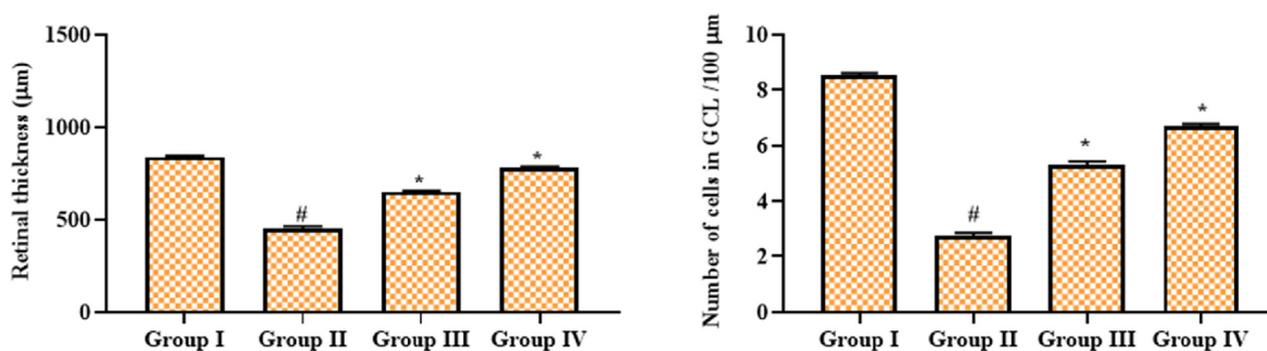


Fig. 5 Effect of geraniin on the retinal thickness and cell numbers in GCL of STZ-challenged DR rats. Group I: Control; Group II: STZ-induced DR rats; Group III: STZ-induced DR + 25 mg/kg of geraniin treated rats; Group IV: STZ-induced DR + 350 mg/kg of metformin treated rats. Values are represented as the mean \pm SD of triplicates assessed statistically by one-way ANOVA and Tukey's multiple comparison assay. '#' reveals significance at $p < 0.05$ from control and '*' reveals significance at $p < 0.01$ from STZ group.

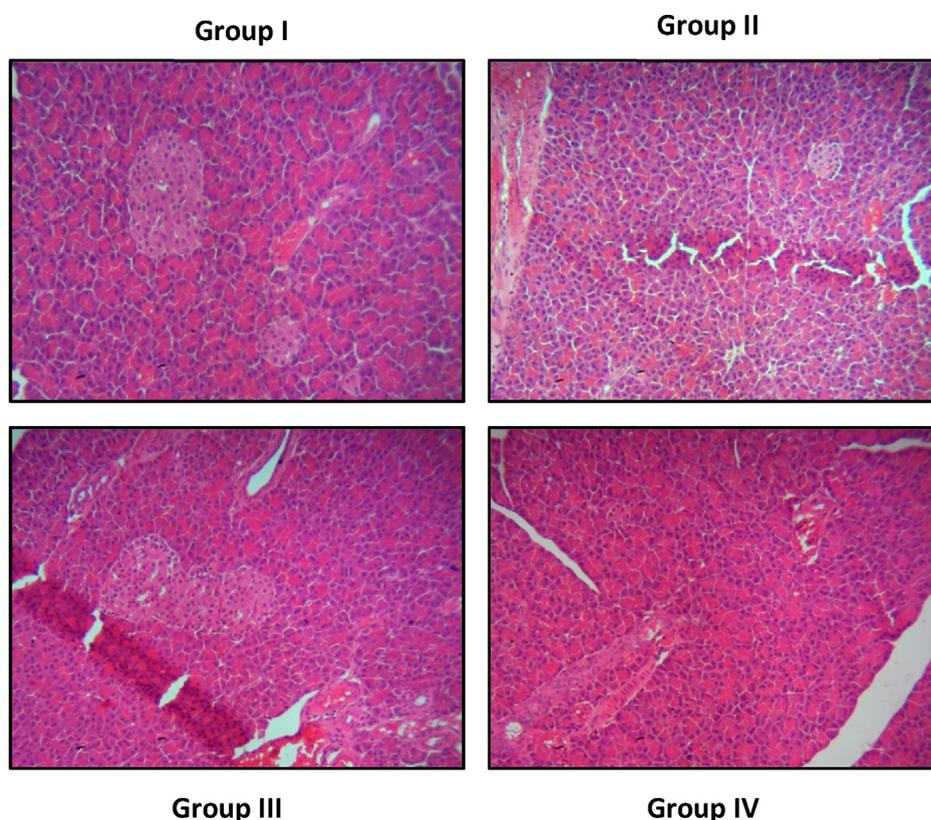


Fig. 6 Effect of geraniin on the pancreas histology of the STZ-provoked DR rats. Group I: control animals demonstrated the normal histological appearance. Group II: STZ-challenged DR animals demonstrated the inflammatory signs and pancreatic islets contractions in the pancreas. Group III & IV: The reduced pancreatic lesions and improved islets appearances were noted in the geraniin and metformin treated animals, respectively.

the normal histological appearance without inflammatory signs. Conversely, the inflammatory symptoms and inflammatory cell infiltrations, as well as pancreatic islet contractions were observed in the pancreas of STZ-provoked DR rats. However, these histological alterations were remarkably ameliorated by the geraniin treatment. The 25 mg/kg of geraniin demonstrated reduced pancreatic lesions and improved islet

appearances in the STZ-challenged DR rats, which is consistent with the results of metformin treatment.

5. Discussion

DR is a major difficulty faced by diabetic patients, which is responsible for their blindness. DR has a complex pathogene-

sis, but it is widely thought that chronic hyperglycemia could be the initiator of DR (Rui et al., 2017). The changes in the retinal microvascular system are the distinctive feature of DR. The diabetic neurodegeneration of the retina comprises retinal cell apoptosis that further leads to the retinal layer thinning and loss of neuronal activity (Sohn et al., 2016). The pathological features of DR are elevated vascular permeability, neovascularization, and edema. In particular, the main pathological feature is the elevated vascular permeability. This occurrence is intimately related to the expression of inflammatory mediators (Funatsu et al., 2009). The retinal neurovascular system was primarily affected during DR that further provoked inflammation, plasma leakage, neurodegeneration, and BRB integrity loss. DR also develops retinal edema, neovascularization, vision loss, and ultimately blindness (Abcouwer, 2013).

There is strong evidence that oxidative stress and local inflammation are actively involved in the progression of DR and play a critical role in the disorder's development. In vascular inflammation, the participation of several pro-inflammatory factors like TNF- α and IL-1 β were identified (Williams et al., 2013). It has already been reported that inflammation is a crucial regulator of DR progression, and DR is primarily recognized as the inflammatory ailment (Semeraro et al., 2015). Increased production of pro-inflammatory cytokines is a major cause of DR development, and inflammation actively contributes to DR development. Several retinal pathological and biochemical complications are connected with diabetes, which is consistent with inflammation (Jousseaume et al., 2004). A number of studies have demonstrated that the elevated leukocyte adhesion to the retinal vessels in experimental diabetic models and the status of several pro-inflammatory regulators were elevated in the retina to activate the pro-inflammatory response. Such elevated leukostasis and inflammatory mediators may play a role in BRB breakdown, which is an early event in the development of DR (Jousseaume et al., 2001).

In inflammatory conditions, several regulators like chemokines and cytokines are actively involved. Inflammation is fundamentally involved in the progression of DR. General pro-inflammatory mediators like TNF- α , IL-6, and IL-1 β were primarily augmented in the retinal tissues and serum of diabetic patients as well as animal models. In diabetic animal models of DR, inflammatory cytokines such as IL-2, IL-3, IL-8, and IFN- γ were found to be elevated (Liu et al., 2012; Li et al., 2012). It has already been stated that IL-1 increases retinal capillary cell apoptosis by activating the NF- κ B cascade. Additionally, IL-1 β also linked with angiogenesis and improved vascular permeability, and promotes retinal cell apoptosis, and the mechanism is aggravated in hyperglycemic situations (Santiago et al., 2020). During DR development, IL-1 β is activated and further improves the vascular permeability due to leukocyte adhesion and retinal capillary cell apoptosis. TNF- α is an important inflammatory regulator that causes cell differentiation, apoptosis, and inflammation (Wu et al., 2017). TNF- α is required for the breakdown of BRB in DR, and it may improve BRB breakdown and leukostasis (Huang et al., 2011). TNF- α inhibition has been shown in preliminary studies to suppress inflammatory responses. Similarly, the level of IL-1 β was also augmented during DR (Mukhopadhyay et al., 2006). Here, our findings showed that geraniin remarkably

suppressed the inflammatory cytokines in the serum of DR rats.

Oxidative stress due to the disproportion of free radical development and antioxidant mechanisms is tightly connected with the damage of several biological macromolecules like proteins, carbohydrates, nucleic acids, and lipids and participates in the stimulation of numerous ailments like diabetes, eye diseases, neurodegenerative ailments, glaucoma, and DR (Sies et al., 2017; Masuda et al., 2017). Our outcomes confirmed that the geraniin remarkably enhanced the antioxidants in the DR rats.

Uncontrolled oxidative stress enhances the VEGF expression that eventually translocate to the nucleus to enhance the pro-inflammatory mediators' expression like ICAM-1 and MCP-1 (Behl and Kotwani, 2015). During diabetic conditions, ICAM-1 improves the leukocyte chemoattraction in the vascular membranes of the retina. TNF- α regulates ICAM-1 expression and improves leukostasis and BRB injury in DR (Zhang et al., 2011). ICAM-1 is a vital player that mediates the leukocytes' adhesion to the endothelial cells, and augmented ICAM-1 was mentioned to be connected with DR initiation and development. The well-known pro-inflammatory mediators IL-1 β and TNF- α have been linked to the development of DR (Ugur et al., 2013; Capita and Soares, 2016). The adhesion of leukocytes is the major cause of retinal endothelial cell damage and apoptosis. It directs to capillary occlusion, local ischemia, and vessel non-perfusion. Consequently, it elevates the VEGF and ICAM-1 and aggravates vascular leakage, finally resulting in the DR. ICAM-1 actively participates in the migration of inflammatory cells and controls the expression of inflammatory mediators. Hence, the alteration in the ICAM-1 level is closely connected with inflammation (Miyamoto et al., 1999).

VEGF is one of the crucial proteins that improves angiogenesis. It has already been discovered that an increased level of VEGF in the retina is closely linked to the development of DR (Rezzola et al., 2019). In recent decades, several clinical reports have identified that the expressions of inflammatory factors are found augmented in the retina of DR rats. Overexpression of inflammatory markers such as VEGF, TNF- α and IL-1 β may disrupt BRB homeostasis and lead to hypoxia and ischemia (Wang et al., 2018). It was identified that the vitreous VEGF expressions were augmented in the DR patients (Abu El-Asrar et al., 2012). Our results have demonstrated that the geraniin treatment efficiently decreased the VEGF, MCP-1, and MMP-2 status in the serum of STZ-challenged DR rats.

6. Conclusion

Our results showed that geraniin inhibited retinal inflammation and oxidative stress in the STZ-triggered DR rats. The findings also proved that geraniin could alleviate the retinal inflammation, lower retinal MCP-1, ICAM-1, and VEGF levels, and ameliorate retinal oxidative stress, in that way, lessening the DR development. As a result, geraniin could be considered as a therapeutic agent to treat DR patients, followed by additional research in the future to elucidate the systematic role of geraniin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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