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Research Article

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Protective Effects Against Diabetic Retinopathy and Cataractogenesis Through Attenuation of Hyperglycemia and Inflammation in Streptozotocin-Induced Rats: A Siddha Drug Flueggea virosa (Vellaipula)

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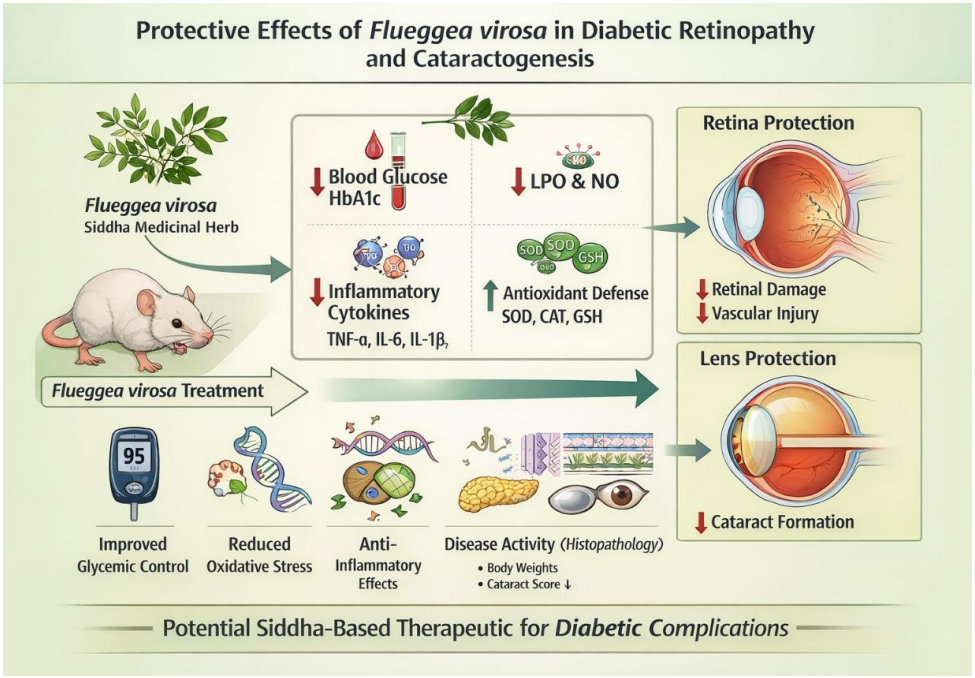
Abstract

Diabetic retinopathy and cataractogenesis are two major microvascular sequelae of diabetes mellitus that are primarily caused by chronic hyperglycemia and inflammation. This study evaluated Flueggea virosa capacity to shield Wistar albino rats from streptozotocin (STZ)-induced diabetic retinopathy and cataractogenesis. After STZ was used to induce diabetes, animals were administered different doses of Flueggea virosa extract for 19 weeks. The disease progression was assessed using body weight, cataract score, and retinal damage score. Biochemical markers that were evaluated included blood glucose, glycated haemoglobin (HbA1c) and histological analyses were performed on the pancreas, retina, and lens. STZ-induced diabetic rats showed significant weight loss, elevated glucose and HbA1c levels and severe retinal and lens damage. Flueggea virosa therapy significantly enhanced glycaemic control, reduced pro inflammatory markers and maintained tissue architecture in a dose-dependent manner. The findings demonstrate the potent anti-inflammatory, antihyperglycemic, and antioxidant qualities of Flueggea virosa.

In conclusion, Flueggea virosa (A Siddha drug (Vellaipula) has promising therapeutic potential as a Siddha-based treatment for diabetic retinopathy and cataractogenesis.

Keywords: Cataract Formation, Flueggea Virosa, Ocular Damage, Pro Inflammatory Cytokines And Streptozutasin Induction



1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The global prevalence of diabetes has risen dramatically in recent decades, posing a major public health challenge worldwide. Long-term hyperglycemia leads to the development of various microvascular complications, among which diabetic retinopathy and cataractogenesis are the leading causes of visual impairment and blindness [1,2]. These ocular complications significantly reduce the quality of life and impose a substantial socioeconomic burden, particularly in developing countries. Microaneurysms, haemorrhages, vascular leakage, and eventually neovascularisation are the hallmarks of diabetic retinopathy (DR), a progressive neurovascular disease of the retina [3]. Numerous metabolic mechanisms, such as the polyol pathway's activation, the production of advanced glycation end products (AGEs), the activation of protein kinase C, and elevated oxidative stress, are responsible for the retinal damage caused by chronic hyperglycemia [4,5]. Because it produces too many reactive oxygen species (ROS), which harm retinal cells, interfere with mitochondrial function, and cause apoptosis, oxidative stress is a key factor in the pathophysiology of DR [6]. Furthermore, by encouraging leukostasis and vascular permeability, inflammatory mediators including cytokines and chemokines worsen retinal injury [7,8].

Another significant consequence of diabetes is cataractogenesis, which is characterised by lens opacification that impairs vision [9]. Osmotic stress, oxidative damage, and protein aggregation inside the lens are all strongly associated with the development of diabetes cataracts [10]. When the polyol pathway is activated, sorbitol builds up in the lens, causing osmotic imbalance and swelling of the lens fibres [11]. Additionally, oxidative stress plays a role in the oxidation, cross-linking, and aggregation of lens proteins, which eventually results in lens opacity [12]. Oxidative stress and inflammation together are thought to be important factors in the development of cataractogenesis and diabetic retinopathy [13]. Glycaemic management, laser therapy, intravitreal injections, and surgical procedures are the main current treatment approaches for diabetic ocular problems. However, these methods frequently have drawbacks such high expense, invasiveness, and other adverse consequences [14,15]. Furthermore, they do not entirely stop the progression of the disease, underscoring the necessity of supplementary and alternative therapeutic strategies. Plant-based treatments have gained popularity recently because of their anti-inflammatory, anti-oxidant, and multi-targeted pharmacological characteristics [16].

Medicinal herbs have long been used in traditional medical systems like Siddha to treat chronic illnesses like diabetes and its consequences [17]. Bioactive substances with strong anti-inflammatory and antioxidant properties, such as flavonoids, alkaloids, and phenolic compounds, are abundant in these herbal preparations [18]. These characteristics make them attractive options for treating and preventing complications from diabetes. A popular medicinal plant in traditional medicine, Flueggea virosa has drawn notice for its wide range of pharmacological effects. It is recognised to have cytoprotective, anti-inflammatory, antidiabetic, and antioxidant qualities [19]. Numerous bioactive components, including as lignans, flavonoids, and terpenoids, have been identified by phytochemical analyses as contributing to its medicinal potential. Its effectiveness in lowering oxidative stress and enhancing metabolic parameters in experimental models has been shown in earlier research [20]. Its ability to reduce diabetic retinopathy and cataractogenesis, however, has not been well investigated.

By selectively destroying pancreatic β-cells, streptozotocin (STZ)-induced diabetes is a commonly used experimental model that closely resembles clinical diabetic complications, resulting in hyperglycemia and related tissue damage [21]. This model is very helpful for assessing the therapeutic potential of new medicines and researching the pathophysiological mechanisms behind diabetic complications. In this regard, the goal of the current study was to examine how Flueggea virosa protects diabetic retinopathy and cataractogenesis in rats with STZ-induced diabetes. The study's main objectives are to determine how oxidative stress and inflammation contribute to the development of disease and whether the plant extract can lessen these pathological processes. This work attempts to give experimental proof for the therapeutic potential of Flueggea virosa as a Siddha-based remedy for diabetic ocular problems by fusing traditional knowledge with contemporary scientific methods.

2. Materials and Methods

2.1. Chemicals and kits

Streptozutasin (purity ≥ 96.0%) from Bio Corporals Chennai Tamil Naidu India, Metformin (purity 98.0–101.5%) from Apollo pharmacy Chennai and other chemicals, assay kits and reagents from Sigma - Aldrich, USA. All of the other compounds were of analytical grade. The following rat ELISA assay kits, such as TNF-α, IL-6, IL-1β, were bought from Synergy scientific services Pvt Ltd, Chennai, India.

2.2. Procurement and preparation of Flueggea virosa leaves extract

Leaves of Flueggea virosa (A Siddha drug (Vellaipula) were collected from Aralvaymozhi, Kanyakumari district (August 2021), and authenticated (Voucher No. F16092101V). The leaves were washed, shade-dried, and powdered. About 100 g of the powder was subjected to Soxhlet extraction using 500 mL methanol at ~65 °C for 24 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying to obtain a semisolid mass (yield: 73 g). The extract was stored safely and suspended in normal saline at required concentrations for pharmacological studies.

2.3. Experimental Animals

All animal experiments were performed using healthy male Wistar albino rats weighing 150–200 g. The animals were procured from TANUVAS, Madhavaram, Chennai, India (Registration No: 190/GO/Re/SL/2000/CPCSEA). Prior to the initiation of the study, the animals were acclimatized to laboratory conditions for a period of 7 days. The study was conducted in the animal house facility of the Siddha Central Research Institute, Arumbakkam, Chennai, India (Registration No: 512/GO/R/S/01/CPCSEA). The animals were housed under standard laboratory conditions with a 12 h light/dark cycle, controlled temperature (22 ± 2 °C), and relative humidity of 40–70%. Rats were provided with standard pellet diet and water ad libitum throughout the experimental period. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC No: 198/PHARMA/SCRI,2018), and all procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Development of Streptozotocin-Induced Diabetic Retinopathy and Cataractogenesis Model and Treatment Regimen in Rats

Streptozotocin (STZ)-induced diabetic retinopathy and cataractogenesis model was developed in Wistar albino rats. Healthy rats (150–200 g) were fasted overnight (18 h) with free access to water prior to induction of diabetes. Diabetes was induced by a single intraperitoneal injection of STZ (45 mg/kg body weight), freshly dissolved in 0.05 M citrate buffer (pH 4.5). To prevent initial drug-induced hypoglycemia, rats were provided with 10% glucose solution overnight after STZ administration [22]. After 72 h of STZ injection, fasting blood glucose (FBG) levels were measured using a glucometer. Rats with FBG levels ≥250 mg/dL were considered diabetic and included in the study (Day 0). The total duration of the experimental study was 19 weeks. Flueggea virosa was administered orally suspended in 0.5% CMC at three different dosages as 200, 400 and 600 mg/kg BW per animal, whereas standard drug Metformin was administered at a dose of 500 mg/kg BW. All dosages were given to the animals from week 16 to week 19. Six groups of fourteen rats each (n=14) were randomly selected from the grouping of rats.

- ***Group I:*** Normal control (received normal diet and distilled water)
- ***Group II:*** Diabetic control (received vehicle only, STZ alone without *Flueggea virosa*)
- ***Group III:*** STZ+ 0.5% CMC standard drug (Metformin 500 mg/kg, oral)
- ***Group IV:*** STZ + Low dose of 0.5% CMC *Flueggea virosa* extract (200 mg/kg, oral)
- ***Group V:*** STZ + Mid dose of 0.5% CMC *Flueggea virosa* extract (400 mg/kg, oral)
- ***Group VI:*** STZ +High dose of 0.5% CMC *Flueggea virosa* extract (600 mg/kg, oral)

All the groups except normal control were challenged with STZ from Day 1 to week 19

On the 19th week, which was the termination day, blood was drawn aseptically and stored in a freezer at -80^{0}C for further biochemical and ELISA analysis. Diabetic and non-diabetic animals were subjected to death by co2 asphyxiation, retina and pancreas organs weights were measured. A distal portion or segments of retina and pancreas tissues were preserved at room temperature in 10% buffered formalin for histopathological and morphometric study for lens assessment. For experimental design see Figure.1a.

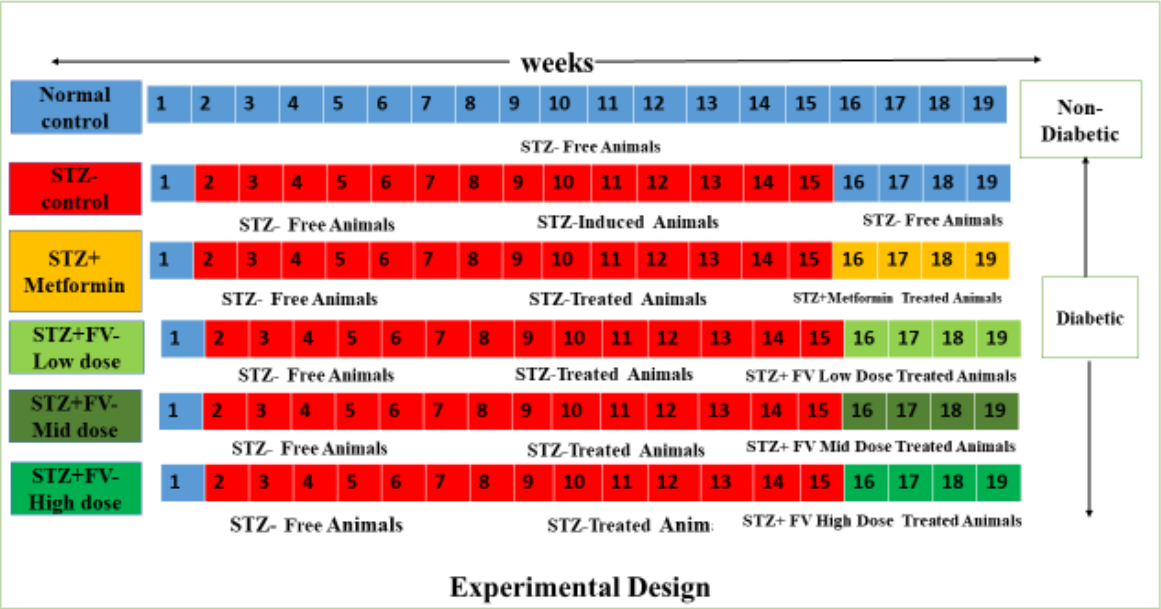


Figure 1a: Experimental Design

2.5. Determination of body and organ weights

The weights of animals were recorded until the study's termination. At the end of the experimental period, all animals were fasted overnight and sacrificed under appropriate anaesthesia. Following sacrifice, vital organs including the pancreas and eyes were carefully excised, blotted dry to remove excess blood, and weighed immediately using a calibrated digital weighing balance. The absolute organ weight of each organ was recorded individually and expressed in grams (g).

2.6. Assessment of disease activity index (DAI)

Animals were monitored regularly for changes in Disease Activity Index (DAI), which comprised variations in body weight, blood glucose levels, retinal alterations, and cataract progression from day 1 to the end of the experimental period (see Table 1). The DAI was calculated at the end of the intervention by summing the scores of individual parameters as follows: Body weight loss was assessed by recording the body weights using a digital weighing balance. The percentage reduction in body weight was scored as: 0 = no change or increase; 1 = 1–5% loss; 2 = 5.1–10% loss; 3 = 10.1–20% loss; and 4 = >20% loss. Blood glucose levels were measured using a glucometer at regular intervals. The scores were assigned as: 0 = normal levels; 1 = mild hyperglycemia; 2 = moderate hyperglycemia; 3 = severe hyperglycemia; and 4 = very severe hyperglycemia. Retinal changes were evaluated by ophthalmoscopic examination following pupil dilation. The scoring was based on the severity of retinal abnormalities: 0 = normal retina; 1 = mild vascular dilation; 2 = moderate changes with early edema; 3 = severe changes with hemorrhages; and 4 = extensive damage with neovascularization. Cataract progression was assessed using flashlight. The degree of lens opacity was graded as: 0 = clear lens; 1 = peripheral opacity; 2 = nuclear opacity; 3 = diffuse opacity; and 4 = mature cataract. At the end of the experimental period, animals were sacrificed, and eyeballs were enucleated for further histopathological examination. Retinal and lenticular tissues were processed and examined for structural alterations, which further supported the DAI findings. The overall DAI score was calculated by summing the individual parameter scores, providing a comprehensive measure of disease severity and progression [23]. The eye photographs were taken for examined the gross macroscopic appearance on STZ- induced rats by alternative week's Figure.1b.

Score	Body Weight (% Reduction)	Blood Glucose Level	Retinal Changes (Ophthalmoscopy)	Cataract Score (Lens Opacity)
0	No change / increase	Normal (<120 mg/dL)	Normal retina, no abnormalities	Clear lens
1	1–5% loss	Mild increase (120–200 mg/dL)	Mild vessel dilation, no edema	Peripheral opacity
2	5.1–10% loss	Moderate (200–300 mg/dL)	Moderate dilation + mild edema	Nuclear opacity
3	10.1–20% loss	High (300–400 mg/dL)	Severe dilation + hemorrhages	Diffuse opacity
4	>20% loss	Very high (>400 mg/dL)	Neovascularization + severe edema	Mature cataract

Table 1: Disease Activity Index (DAI) Scoring Criteria (0–4 Scale)

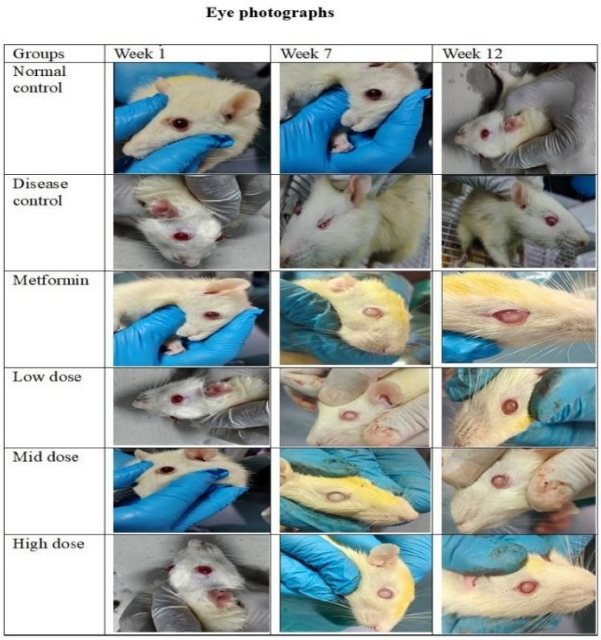


Figure 1b: Representative Eye photographs from different groups

2.7. Assessment of Histopathological and Morphological Findings in Retina, Lens and Pancreas

Retinal and lenticular alterations such as inflammatory cell infiltration, retinal layer disorganization, vascular abnormalities, edema, and lens fiber degeneration were considered as key histopathological features for the evaluation of Histopathological Activity Index (HAI) (Table 2) [24,25]. At the end of the experimental period, eyeballs and pancreas from all experimental groups were carefully enucleated and fixed in 10% phosphate-buffered formalin. The tissues were then processed and embedded in paraffin. Sections of approximately 4 µm thickness were obtained using a microtome (e.g., Leica Microsystems), followed by staining with hematoxylin and eosin (H&E) for histopathological examination. The stained sections were examined under a light microscope, and photomicrographs were captured using 10X and 20X objectives. The retinal sections were evaluated for changes in retinal thickness, integrity of retinal layers (ganglion cell layer, inner and outer nuclear layers), vascular alterations, and inflammatory infiltration. Lens sections were examined for fiber disorganization, protein aggregation, vacuole formation, and degree of opacity. For morphometric analysis, retinal thickness and cellular architecture were quantitatively assessed in multiple microscopic fields (at least 40–50 fields per group) at 10X magnification. Measurements were performed using image analysis software to ensure accuracy and reproducibility. Histopathological evaluation was carried out by an investigator blinded to the experimental groups to eliminate bias.

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Inflammatory Cell Infiltration (Retina)	Absence	Mild infiltration in inner retinal layers	Moderate infiltration reaching outer layers	Marked infiltration with retinal edema	Severe and extensive infiltration with structural damage
Retinal Thickness / Edema	Normal retina	Mild thickening, slight edema	Moderate thickening with visible edema	Severe thickening with hemorrhages	Extensive edema with neovascularization
Vascular Changes (Retina)	Normal vessels	Mild dilation	Moderate dilation + शुरुआती microaneurysms	Severe dilation + hemorrhages	Neovascularization and vascular disorganization
Lens (Cataract) Changes	Clear lens	Peripheral opacity	Nuclear opacity	Diffuse opacity	Mature cataract (complete opacity)
Photoreceptor / Retinal Cell Damage	Normal architecture	Mild cell disorganization	Moderate degeneration	Severe degeneration	Extensive cell loss and layer disruption
Epithelial / Retinal Layer Integrity	Normal layers	Focal disruption	Zonal damage	Diffuse damage	Complete disorganization

Table 2: Histopathological & Ocular Disease Activity Index (DAI)

2.8. Estimation of Glycated Hemoglobin (HbA1c) Levels

Using blood samples taken from experimental rats at the end of the trial, blood glucose levels were determined using a standard glucose estimation kit based on the glucose oxidase–peroxidase (GOD-POD) method. Following the manufacturer's instructions, glycated haemoglobin (HbA1c) levels were measured using a commercially available diagnostic kit based on the ion-exchange resin or immunoturbidimetric method. A spectrophotometer was used to measure the absorbance, and the results were reported as percentage (%) for HbA1c and mg/dL for glucose. Both short-term and long-term glycaemic management were evaluated using these metrics [26].

2.9. Estimation of Inflammatory Cytokines in Serum

Inflammatory factors TNF-α, IL-1β, and IL-6 were measured in the serum using a sandwich ELISA kit. Serum samples were incubated with a biotin conjugate solution, followed by streptavidin-HRP. After incubation, the absorbance was read spectrophotometrically at a wavelength of 450 nm using a microplate ELISA reader. All cytokines' concentrations were expressed as ng/ml in serum [27,28].

2.10. Statistical Analysis

Using GraphPad Prism, version 11.00 (85) software, one-way ANOVA was used for all statistical analyses. Using the STZ control group as a reference, post hoc Dunnett's multiple comparison procedures were used to compare the groups. The mean ± standard error of the mean (SEM) was used to express the results. When the p-value was less than 0.05, statistical significance was taken into consideration.

3. Results

3.1. Effect of *Flueggea virosa* on symptoms and organ weights of STZ- induced Diabetic Retinopathy rats

The effects of *Flueggea virosa* extract on body and organ weights were evaluated in rats with streptozotocin-induced diabetic retinopathy. The diabetes control group's significant reduction in body weight (Figure. 2a), pancreas, and eye weights ($p < 0.001$ against normal control) (Figure. 2b) demonstrated severe metabolic and tissue damage. Metformin treatment significantly improved body and organ weights ($p < 0.01$ vs. diabetes control). When *Flueggea virosa* extract was compared to the diabetes control group, the low dose showed a small improvement ($p < 0.05$), the mid dose showed a considerable restoration ($p < 0.01$), and the high dose showed a significant recovery of body and organ weights ($p < 0.001$). These findings suggest that *Flueggea virosa* considerably lessens organ damage and diabetes-related weight loss, particularly at higher dosages.

Lens opacity score was used to evaluate the advancement of cataracts (see Figure.2c). In all groups, there was no discernible cataract development at week 0. Rats treated with STZ, however, had severe cataractogenesis, as evidenced by a significant rise in cataract score from 1.83 ± 0.17 at week 4 to 3.83 ± 0.17 at week 19 ($p < 0.0001$). Conversely, the FV-treated groups showed a dose-dependent reduction in cataract severity. At week 19, the cataract score was 2.67 ± 0.21 for the FV low-dose group, 1.83 ± 0.31 for the mid-dose group, and a substantial drop to 1.17 ± 0.17 ($p < 0.0001$) for the high-dose group. The standard drug-treated group (metformin) had a reduced cataract score of 1.33 ± 0.21, just like the FV high-dose group.

Rats treated with STZ showed a gradual increase in retinal damage scores (Figure. 2d). Severe retinal degeneration was evident in the diabetic control group, which increased from 1.67 ± 0.21 at week 4 to 3.67 ± 0.21 at week 19 ($p < 0.0001$). In a dose-dependent manner, FV treatment dramatically decreased retinal damage. The FV low-dose group scored 2.50 ± 0.22 at week 19, compared to 1.67 ± 0.21 for the mid-dose group and a significant decrease to 1.17 ± 0.17 ($p < 0.0001$) for the high-dose group. With a retinal score of 1.33 ± 0.21, the metformin-treated group likewise showed substantial protection. Significant weight loss, an increase in cataract development, and progressive retinal impairment were the outcomes of STZ therapy. *Flueggea virosa* therapy, however, considerably and dose-dependently reduced these alterations. With results similar to those of the conventional drug-treated group, the high-dose FV group demonstrated greatest protection, suggesting its ability to prevent cataractogenesis and diabetic retinopathy.

In streptozotocin (STZ)-induced diabetic rats, the effects of *Flueggea virosa* on physiological, ocular, and organ-specific parameters were evaluated throughout the trial. The introduction of diabetes resulted in a significant reduction in body weight, a drop in the weights of the eyes and pancreas, and a significant increase in cataractogenesis and retinal damage when compared to the normal control group. Treatment with *Flueggea virosa* at all dose levels (low, mid, and high) demonstrated a dose-dependent improvement in all assessed parameters when paired with the widely used drug metformin. (a) Body weight changes: Diabetic control rats steadily lost weight during the study, which is indicative of tissue atrophy and metabolic imbalance. In contrast, rats administered *Flueggea virosa* demonstrated a significant recovery in body weight; the high-dose group displayed values that were nearly normal and comparable to those of the standard-treated group. (b) Eye and pancreatic organ weights: STZ-induced diabetic rats had lower eye and pancreatic weights, suggesting both structural and functional damage. Treatment with *Flueggea virosa* significantly boosted these organ weights, suggesting improved cellular integrity and protection against tissue degradation, particularly in ocular tissues and pancreatic β-cells. (c) Cataract score: The diabetic control animals' cataract scores gradually increased, indicating the development of cataracts and severe lens opacity. *Flueggea virosa* administration significantly slowed the development of cataracts, and the high-dose group showed notable protection against lens opacification. (d) Retinal damage score: The diabetic control group's retinal damage score significantly increased, indicating vascular damage and retinal degeneration. *Flueggea virosa* treatment dramatically reduced retinal damage scores, suggesting that the structure and function of the retina were preserved. Overall, *Flueggea virosa* treatment showed a dose-dependent protective effect, with the high dose demonstrating superior efficacy across all parameters when compared to low and

mid doses. The results are expressed as mean ± SEM (n = 14). ###p < 0.001; ##p < 0.01; #p < 0.05 compared to normal control group; *p < 0.05, **p < 0.01, ***p < 0.001 compared to diabetic control group; ns, non-significant.

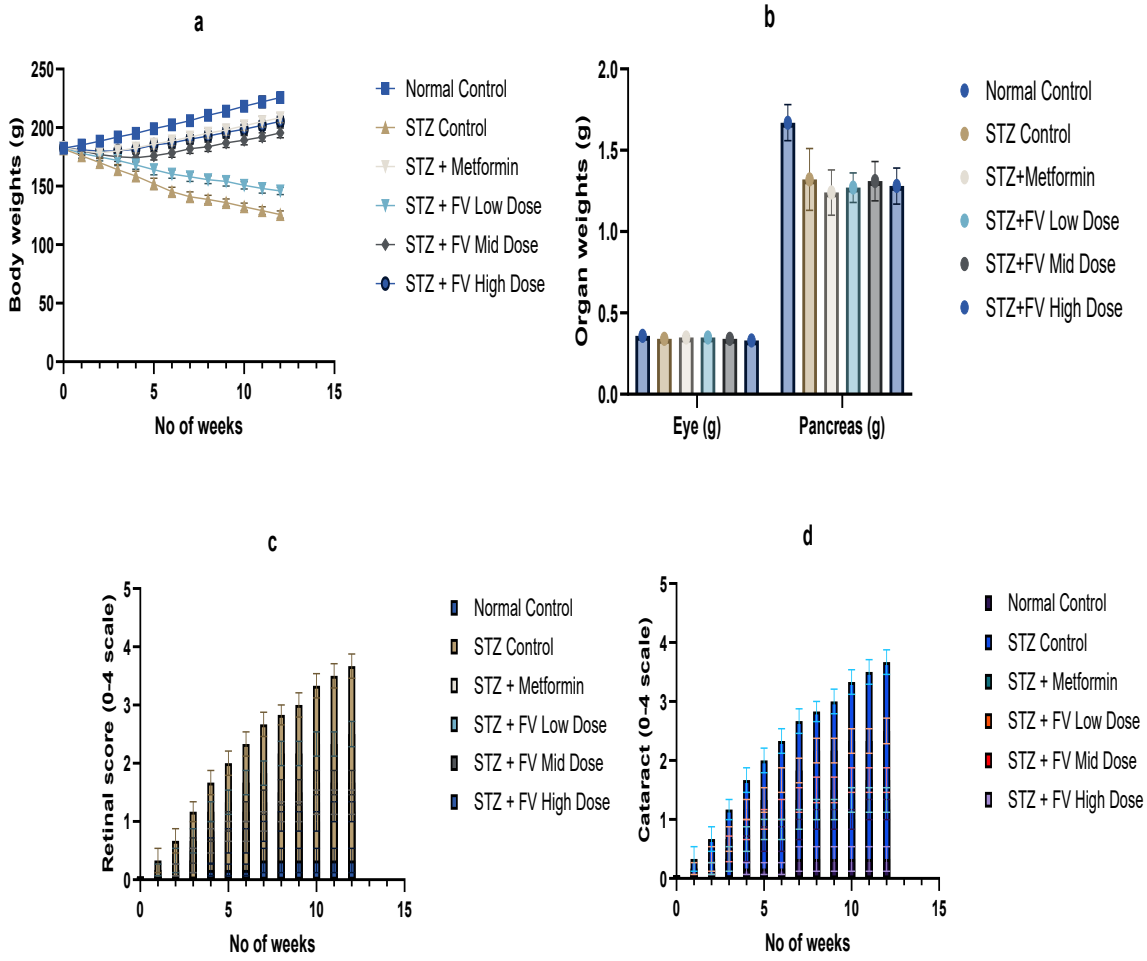


Figure 3: Effect of *Flueggea virosa* on body weight, organ weights, cataract score, and retinal score in STZ-induced diabetic rats

3.2. Effect of *Flueggea virosa* on retina, lens and pancreas histology of STZ induced rats

Our histological study employed haematoxylin and eosin (H&E) staining to assess the degree of tissue damage in the retina, lens, and pancreas. There were no discernible pathological changes in the tissues from the normal control group (without STZ), which had normal architecture, intact lens fibres, well-organised retinal layers, and normal pancreatic islets. The group treated with STZ alone showed significant histological alterations in comparison to the normal control tissues. Retinal sections revealed degeneration of photoreceptor cells, weakening of the ganglion cell layer, and disarray of retinal layers. Lens sections showed increased opacity, protein aggregation, and lens fibre disruption, all of which were signs of cataract development. In addition to inflammatory cell infiltration, pancreatic tissues displayed β-cell degeneration, decreased islet size, and cellular necrosis.

On the other hand, tissues from the groups treated with *Flueggea virosa* (FV), especially at higher doses, demonstrated a noticeable improvement in histoarchitecture. Retinal layers were preserved in retinal slices with little cellular loss and degeneration. Reduced fibre disruption with near-normal architecture and lower opacity were found in lens tissues. Improved islet morphology with reduced cellular damage and partial β-cell repair was seen in pancreatic sections. With nearly normal retinal, lens, and pancreatic architecture, the higher dose FV-treated groups showed very minor structural changes that were similar to those of the conventional drug-treated group (Figure 4).

In Figure 4, Sections of the pancreas (A), retina (B), and lens (C) demonstrate morphological changes among the experimental groups. (1) The normal control group exhibits clear lens structure, normal retinal layers, and healthy pancreatic islets. (2) The STZ control group displayed substantial pathological changes, such as lens opacity/structural disruption, retinal disarray with cellular destruction, and pancreatic islet degeneration. (3) The standard (metformin) group has a moderate level of tissue architecture repair.

(4) The pancreatic, retina, and lens morphology of the *Flueggea virosa* low-dose group showed a slight improvement with partial recovery. (5) *Flueggea virosa* mid-dose group exhibiting enhanced structural integrity, decreased cellular damage, and modest restoration. (6) The *Flueggea virosa* high-dose group exhibits nearly normal architecture with clear lens structure, ordered retinal layers, and well-preserved pancreatic islets, suggesting notable protective effects.

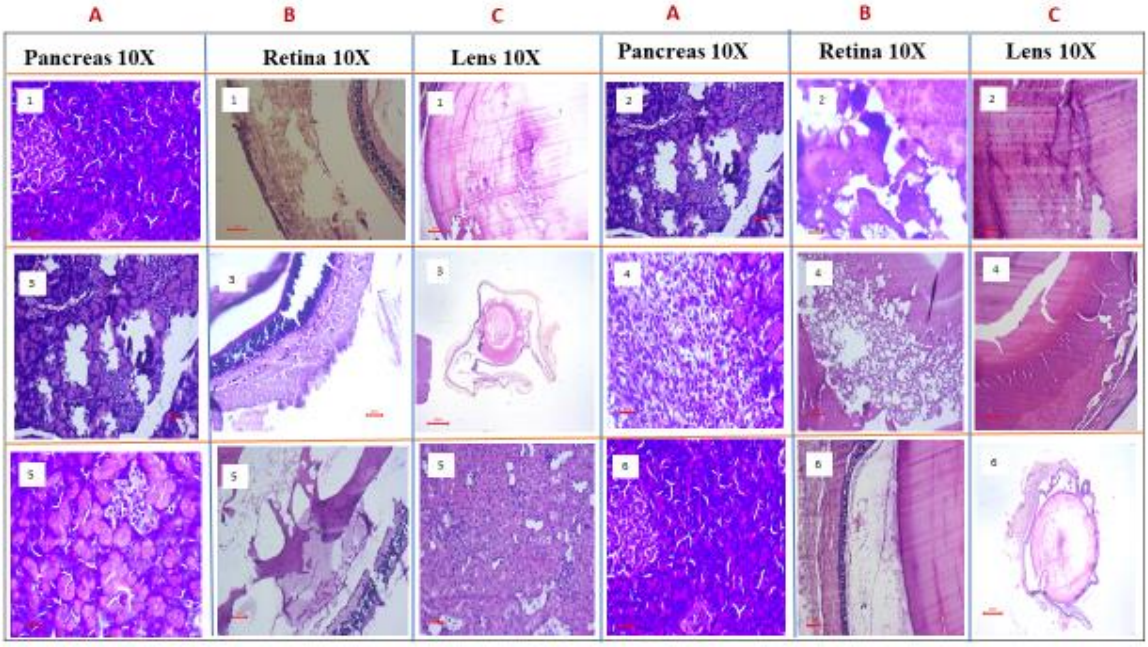


Figure 4: *Flueggea virosa* impact on histological alterations in the pancreas, retina, and lens of rats with streptozotocin-induced diabetes (H&E staining, 10×)

3.3. Effect of *Flueggea virosa* on Glucose & HbA1c levels

Flueggea virosa extract on blood glucose and HbA1c levels in rats with streptozotocin-induced diabetic retinopathy. When compared to the normal control group, the STZ control group's blood glucose and HbA1c levels were significantly higher ($p < 0.001$), indicating severe hyperglycemia and inadequate glycaemic management. Metformin treatment significantly lowered HbA1c ($p < 0.01$) and blood glucose ($p < 0.01$) levels when compared to the STZ control group (see Figure.4 (a&b)). The effects of *Flueggea virosa* extract were also dose-dependent. While the mid-dose group showed a considerable decrease in HbA1c ($p < 0.05$) with non-significant changes in blood glucose, the low-dose group showed non-significant (ns) changes in both parameters. Significantly lower blood glucose and HbA1c levels ($p < 0.001$) were observed in the high-dose group as compared to the STZ control group. Overall, lower blood glucose and HbA1c levels showed that *Flueggea virosa* extract, especially at the high dose, successfully improved glycaemic management in diabetic retinopathy rats.

Glycaemic control was assessed by measuring (A) blood glucose levels (mg/dL) and (B) HbA1c (%). Blood glucose and HbA1c levels were significantly higher in the STZ control group than in the normal control group (####$p < 0.001$). While Flueggea virosa treatment showed dose-dependent effects, metformin administration significantly decreased both parameters (**$p < 0.01$). The mid-dose group demonstrated a moderate reduction (*$p < 0.05$) in glucose and HbA1c levels, whereas the low-dose group displayed non-significant (ns) reductions. Notably, when compared to the STZ control group, the high-dose group's blood glucose and HbA1c levels significantly decreased (***$p < 0.001$). The mean ± SEM ($n = 14$) is used to express the results. ####$p < 0.001$ in comparison to the normal control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison to the STZ control; ns: not significant.

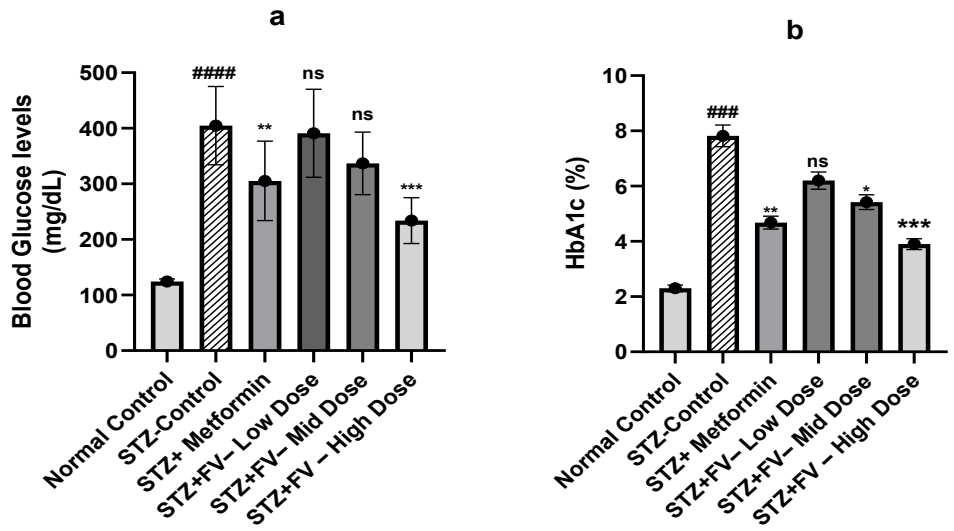
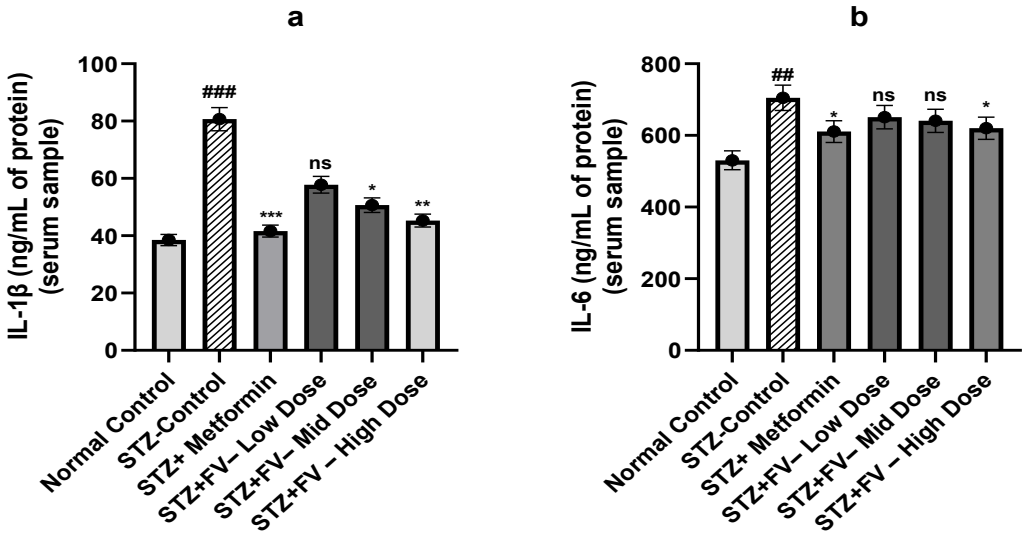


Figure 5: Flueggea virosa affects blood glucose and HbA1c levels in rats with diabetic retinopathy caused by streptozotocin.

3.4. Effect of treatments on inflammatory cytokines in diabetic rats

Flueggea virosa extract affects inflammatory cytokines in streptozotocin-induced diabetic rats. TNF-α, IL-1β, and IL-6 levels were significantly higher in the STZ control group ($p < 0.001$ and $p < 0.01$) than in the normal control group, suggesting an increased inflammatory response (see Figure.5 (a-c)). Metformin treatment significantly decreased levels of TNF-α ($p < 0.01$), IL-6 ($p < 0.05$), and IL-1β ($p < 0.001$). Comparing *Flueggea virosa* extract to the STZ control group, the mid- and high-dose groups showed significant reductions in IL-1β ($p < 0.05$, $p < 0.01$), IL-6 ($p < 0.05$), and TNF-α ($p < 0.01$), while the low-dose group showed non-significant (ns) changes in some parameters. *Flueggea virosa* showed anti-inflammatory efficacy overall, with greater effects shown at higher dosages.

Figure 6. *Flueggea virosa* affects inflammatory cytokines in rats with streptozotocin-induced diabetes. To assess the inflammatory response, serum samples were analysed for (A) IL-1β (ng/mL of protein), (B) IL-6 (ng/mL of protein), and (C) TNF-α (ng/mL of protein). IL-1β and TNF-α levels (###$p < 0.001$) and IL-6 levels (##$p < 0.01$) were significantly higher in the STZ control group than in the normal control group. Metformin treatment dramatically decreased levels of TNF-α (**$p < 0.01$), IL-1β (***$p < 0.001$), and IL-6 (*$p < 0.05$). Similarly, *Flueggea virosa* extract demonstrated dose-dependent anti-inflammatory effects. In comparison to the STZ control group, the mid- and high-dose groups demonstrated significant decreases in IL-1β (*$p < 0.05$, **$p < 0.01$), IL-6 (*$p < 0.05$), and TNF-α (**$p < 0.01$), while the low-dose group showed non-significant (ns) changes in a few cytokines. The mean ± SEM (n = 14) is used to express the results. ###$p < 0.001$, ##$p < 0.01$ versus normal control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus STZ control; ns: non-significant.



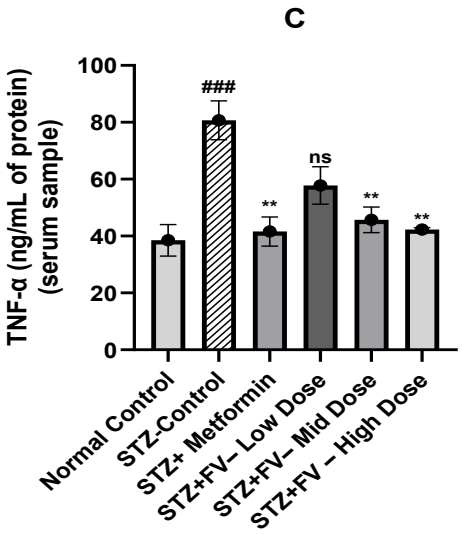


Figure 6: Flueggea virosa affects inflammatory cytokines in rats with streptozotocin-induced diabetes

4. Discussion

Diabetic retinopathy and cataractogenesis are characterised by oxidative stress and inflammatory processes involving multiple cellular and molecular pathways in retinal and lens tissues, although the precise mechanisms underlying these complications remain unclear [29]. Along with the activation of inflammatory mediators and cytokines, a variety of cell types, such as retinal endothelial cells, pericytes, ganglion cells, and lens epithelial cells, actively contribute to the development of these disorders. Furthermore, an important factor in the development of disease is oxidative stress, which is typified by an overabundance of reactive oxygen species (ROS), reactive nitrogen species (RNS), and elevated lipid peroxidation. Dysregulation of pro-inflammatory cytokines and an imbalance in endogenous antioxidant defence mechanisms are two aspects of diabetic ocular problems that are complicated in nature and necessitate multi-targeted treatment methods. The streptozotocin (STZ)-induced diabetic rat model, which is characterised by persistent hyperglycemia, oxidative stress, and progressive tissue damage in the ocular and pancreatic systems, is a well-known experimental model that closely resembles the pathological features of human diabetes and its associated complications [30,31]. Chronic oxidative stress and inflammation, which cause structural and functional changes in retinal and lens tissues, are the main causes of diabetic retinopathy and cataractogenesis [32]. Numerous natural antioxidants have been shown to reduce oxidative stress and block pro-inflammatory cytokines, hence controlling diabetic complications [33]. There is minimal data on how different plant-derived chemicals affect diabetic ocular problems, despite earlier research showing their antioxidant and antidiabetic potential. *Flueggea virosa* showed considerable anti-inflammatory potential in the current study. These results are consistent with previous research indicating that phytochemicals protect cells by scavenging free radicals [34]. A growing body of research indicates that *Flueggea virosa* has a variety of pharmacological properties, including as cytoprotective, anti-inflammatory, and antioxidant actions, without having a major negative impact [35,19].

However, a brief research has been done on its protective function in halting the long-term development of diabetic retinopathy and cataractogenesis. In this work, we assessed the preventative and preventive effects of *Flueggea virosa* at various doses against diabetic sequelae caused by STZ, with a special focus on oxidative stress-mediated tissue damage in the retina and lens. It is evident from the observed improvement in tissue architecture and decrease pro inflammatory markers that *Flueggea virosa* protects against diabetic complications by modifying important pathological pathways. *Flueggea virosa* showed notable antihyperglycemic activity in addition to its antioxidant and anti-inflammatory properties, as shown by the decrease in increased blood glucose levels and glycated haemoglobin (HbA1c) in STZ-induced diabetic rats. The improvement in glycaemic management also helps to mitigate retinal and lens degeneration because prolonged hyperglycemia is a major cause of oxidative stress and consequent tissue damage. Long-term control of glucose homeostasis, which is essential in halting the development of diabetic complications, is reflected in the drop in HbA1c levels. Thus, *Flueggea virosa* therapeutic promise in treating diabetic retinopathy and cataractogenesis is highly supported by the combined benefits of better glycaemic management, decreased oxidative stress, and suppression of inflammation [36].

Retinal and lens homeostasis has been demonstrated to be damaged by increased production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as disruption of cellular signalling pathways throughout the pathogenesis of diabetic retinopathy and cataractogenesis. Chronic hyperglycemia causes oxidative stress that alters the structure and function of ocular tissues, destroying lens epithelial cells, retinal endothelial cells, and pericytes [37]. By scavenging reactive oxygen species (ROS) and triggering the Nrf2/HO-1 signalling pathway to regenerate antioxidant defence mechanisms including glutathione (GSH), catalase, and superoxide

dismutase (SOD), a number of phytochemicals and their structural counterparts have been demonstrated to prevent oxidative stress [38]. By promoting the induction of phase II detoxifying enzymes and antioxidant proteins, activation of Nrf2, a crucial transcription factor controlling antioxidant responses, lessens oxidative cellular damage. According to earlier research, Nrf2 activation reduces inflammation by suppressing the expression of pro-inflammatory cytokines through redox regulation and increasing antioxidant enzyme levels [39]. Significantly, oxidative stress and inflammatory mediators such as cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS) are strongly correlated in the pathophysiology of diabetic complications, resulting in increased production of reactive nitrogen species (RNS), lipid peroxidation (LPO/TBARS), and tissue damage in retinal and lens tissues [40]. Furthermore, the observed reduction in inflammatory markers suggests that *Flueggea virosa* has a protective impact by modifying oxidative and inflammatory pathways. The generation of reactive species, such as ROS and RNS, is crucial to the onset and development of diabetic ocular disorders because it damages membrane-bound enzyme systems and causes lipid peroxidation. Tissue damage is accelerated under hyperglycaemic situations due to increased susceptibility of cellular membranes and enzymes. *Flueggea virosa* therapy, on the other hand, successfully reduced these degenerative alterations, indicating its function in maintaining enzyme activity and stabilising cellular membranes. Overall, the study's findings show that *Flueggea virosa* protects against STZ-induced diabetic retinopathy and cataractogenesis by lowering oxidative stress, blocking pro-inflammatory mediators, and re-establishing tissue homeostasis through its strong antioxidant and anti-inflammatory qualities.

The results of this study showed that *Flueggea virosa* administered at different doses showed improved clinical outcomes, including restoration of dropped body weight, decreased cataract score, and decreased retinal damage score (as shown in relevant images (Figure 3 a-c-d)). *Flueggea virosa* therapy effectively prevented the onset of diabetic complications and maintained ocular integrity when compared to the group treated with STZ alone. Histological analyses further demonstrated that *Flueggea virosa* reduced microscopically visible damage to pancreatic, lens, and retinal tissues, including improved pancreatic islet architecture, less disruption of lens fibres, and preservation of retinal layers. These results suggest defence against hyperglycemia-induced structural damage and cellular deterioration. Furthermore, oxidative and inflammatory indicators were considerably lower in the treatment groups, according to biochemical analysis, indicating a reduction in cellular stress and inflammatory response. This finding suggests that *Flueggea virosa* protects retinal and lens tissues by reducing oxidative stress, inflammation, and tissue damage at varying dosages. Its capacity to control cellular reactions, lessen oxidative damage, and maintain tissue architecture against STZ-induced diabetic problems may be responsible for the protective benefits.

Furthermore, inhibiting inflammatory signalling pathways like JNK and NF-κB is essential for lowering inflammation and averting cellular damage. Together, these processes support the cytoprotective benefits of chemicals derived from plants in diabetes patients [41]. Due to elevated oxidative stress and pro-inflammatory mediator activation, the STZ-treated groups in the current study showed significant retinal and lens damage. Overall, the findings show that *Flueggea virosa* has strong cytoprotective and antioxidant properties that prevent cataractogenesis and diabetic retinopathy by lowering oxidative stress, inhibiting inflammatory signalling pathways, and increasing antioxidant enzyme activity.

In the current investigation, we discovered that *Flueggea virosa* therapy significantly decreased the levels of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in all dose groups as compared to the group that received STZ alone. This finding suggests that *Flueggea virosa* reduces inflammation linked to diabetic ocular complications by suppressing pro-inflammatory mediators (IL-1β, IL-6, and TNF-α), blood glucose levels and glycated haemoglobin (HbA1c) were markedly elevated in STZ-induced diabetic rats, suggesting inadequate glycaemic management. When compared to the diabetic control group, treatment with *Flueggea virosa* significantly lowered blood glucose levels and HbA1c values, indicating its antihyperglycemic action. The improvement in glycemic parameters further supports its role in preventing hyperglycemia-induced oxidative stress and inflammation.

5. Conclusion

In conclusion, oral *Flueggea virosa* administration markedly reduced the pathogenesis of diabetic retinopathy and cataractogenesis in the STZ-induced diabetic rat model. Reduced blood glucose and HbA1c levels, as well as reduction of oxidative stress and inflammatory indicators, demonstrated how well the medication improved glycaemic management. Additionally, *Flueggea virosa* showed protective benefits on pancreatic, lens, and retinal tissues by minimising cellular damage and maintaining structural integrity. Crucially, it was discovered that the benefits were dose-dependent, with the high-dose group showing better efficacy across biochemical, histological, and clinical criteria than the low and mid doses. This suggests that *Flueggea virosa* offers more therapeutic benefits in reducing diabetes complications at higher dosages. According to the study's findings, *Flueggea virosa* has strong anti-inflammatory and antihyperglycemic qualities that all work together to protect against diabetes complications. *Flueggea virosa* may therefore be a viable Siddha-based medicinal agent for the treatment of cataractogenesis and diabetic retinopathy. To precisely understand the molecular processes behind its protective properties, more research is necessary, especially with regard to important signalling pathways as Nrf2/HO-1 and NF-κB. Furthermore, it is required to isolate and characterise the active phytoconstituents that are responsible for the pharmacological activities that have been observed. To determine safety and efficacy profiles, long-term toxicology and pharmacokinetic studies should be carried out. Furthermore, to confirm these results in human subjects and investigate its potential as a standardised medicinal formulation, carefully planned clinical trials are crucial.

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Conflict of interest

The authors declare that they have no conflicts of interest in this manuscript.

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