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(RESEARCH ARTICLE)

TAMARINDUS INDICA SEED EXTRACT ATTENUATES RETINAL NEURODEGENERATION AND GLIOSIS IN DIABETIC RATS: COMPARISON WITH METFORMIN AND INSULIN

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ABSTRACT

Diabetes mellitus damages retinal neurons and glia as well as retinal vessels. This study compared ethanolic *Tamarindus indica* seed extract with metformin and insulin on retinal histology in streptozotocin (STZ)-induced diabetic rats. Sixty male Wistar rats were allocated to six groups (n = 10): normal control (NC), extract only (TO), and STZ-diabetic rats (70 mg/kg, intraperitoneally) given extract (DT; 200 mg/kg), metformin (DM; 100 mg/kg), insulin glargine (DI; 4 IU/day) and untreated diabetic control (DC) for six weeks. Retinal sections were stained with haematoxylin and eosin, phosphotungstic acid haematoxylin (PTAH), cresyl fast violet (CFV), glial fibrillary acidic protein (GFAP) immunohistochemistry and Masson's trichrome. The extract was non-toxic up to 3000 mg/kg. By week 6, fasting glucose was 41.3 mmol/L in DC but 5.1, 6.7 and 6.5 mmol/L in DT, DM and DI. DC retinas showed pyknotic nuclei, vacuolation, reduced cell density, marked thinning and disorganisation of the inner and outer nuclear layers, exaggerated PTAH-positive glial processes, intense GFAP immunoreactivity and extensive collagen in the inner plexiform and photoreceptor layers. All treated groups showed fewer pyknotic cells and less gliosis; DT retained near-normal thickness of the ganglion cell, inner nuclear and outer nuclear layers, whereas DM and DI showed residual thinning. Collagen deposition was prominent in DM and DI and reduced but present in DT. Ethanolic *T. indica* seed extract attenuated diabetic retinal neurodegeneration and gliosis at least as well as metformin and insulin, but did not prevent collagen deposition.

Keywords: Retinopathy; Müller cells; Glial fibrillary acidic protein; Phytotherapy; Hyperglycaemia; Histopathology

1 INTRODUCTION

Diabetic retinopathy remains a leading cause of preventable visual loss in working-age adults. Although it has traditionally been defined by microvascular lesions, the diabetic retina also undergoes early neurodegeneration: ganglion cells and neurons of the inner nuclear layer are lost, the nuclear layers thin, and glial cells become reactive, often before vascular changes are visible [1,2]. The retina is a layered extension of the central nervous system with a very high metabolic rate, which makes it especially sensitive to sustained hyperglycaemia [3,4].

Hyperglycaemia drives retinal injury through overproduction of reactive oxygen species, lipid peroxidation, depletion of antioxidant defences and activation of inflammatory and apoptotic pathways [5,6]. Müller cells and astrocytes respond by up-regulating glial fibrillary acidic protein (GFAP), a change that is regarded as one of the earliest cellular signs of diabetic retinal stress [7–9]. Chronic hyperglycaemia also promotes extracellular-matrix accumulation and basement-membrane thickening, which can be detected as collagen deposition [10].

Metformin and insulin are standard glucose-lowering therapies, and metformin has additional antioxidant and anti-inflammatory effects that have been linked to protection of the retina in experimental diabetes [11,12]. Medicinal plants are being explored as affordable complementary agents. *Tamarindus indica* L. seed is rich in polyphenols, tannins, flavonoids and alkaloids and shows antioxidant and antidiabetic activity [13,14], but its effects on the diabetic retina have not been compared directly with those of standard drugs.

This study therefore compared retinal histology in normal rats, extract-only rats, untreated streptozotocin (STZ)-diabetic rats and STZ-diabetic rats treated with ethanolic *T. indica* seed extract, metformin or insulin. We hypothesised that the extract would reduce diabetes-associated retinal neurodegeneration, gliosis and collagen deposition.

2 MATERIALS AND METHODS

2.1 Study design and setting

This controlled experiment was carried out in the animal unit of the Department of Human Anatomy, School of Medicine and Health Sciences, Mulungushi University (Livingstone Campus), Zambia, between January and March 2026. The extract was characterised chemically at the Department of Chemistry, University of Lagos, Nigeria. The retinas reported here and the occipital cortex reported in a companion manuscript were obtained from the same animals; the extract characterisation, toxicity, blood glucose and body-weight data are therefore common to both reports. The individual rat was the experimental unit.

2.2 Chemicals and reagents

Streptozotocin (Calbiochem, Darmstadt, Germany), metformin hydrochloride (Sun Pharmaceutical Industries, Haryana, India), ketamine and xylazine (Supriya Lifescience, Mumbai, India) and long-acting insulin glargine were used. Phytochemical and antioxidant reagents, including Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine, thiobarbituric acid and ascorbic acid, were obtained from Sigma-Aldrich (St Louis, MO, USA). The primary antibody was polyclonal rabbit anti-GFAP (Dako Z0334, lot 00066091; Dako, Glostrup, Denmark). All other chemicals were of analytical grade.

2.3 Plant material and extraction

Mature *T. indica* pods were harvested from wild trees in the Ngwenya area of Livingstone, Zambia, in August–September 2025 and authenticated by the Department of Biological Sciences, University of Zambia (accession no. UZL 22534). Seeds freed from pulp were washed, dried at 40–50 °C and milled to give 200 g of powder, which was extracted with 95% ethanol in a Soxhlet apparatus at 60–70 °C for 6–8 h. The solvent was removed on a steam bath (≤50 °C) and the residue dried to constant mass; the yield was 12% (w/w). The extract was stored at 4 °C in a sealed, light-protected container and dissolved in physiological saline immediately before each daily dose.

2.4 Phytochemical analysis and acute toxicity

Eleven phytochemical classes were screened with standard colour and precipitation tests [13], seven constituents were quantified spectrophotometrically or gravimetrically [15], antioxidant activity was measured against ascorbic acid by ferric reducing antioxidant power (FRAP), DPPH, nitric oxide, hydrogen peroxide and lipid peroxidation assays [16], and constituents were profiled by gas chromatography–mass spectrometry (GC-MS; Agilent 6890A/5973C, HP-5 column, NIST 14 library) [13]. Acute oral toxicity was tested by Lorke's two-phase method [17]: after an overnight fast, three groups of three rats received 100, 200 or 500 mg/kg and, in the absence of toxicity, three further groups received 1000, 2000 or 3000 mg/kg. Rats were observed continuously for 4 h, intermittently for 24 h and daily for 14 days. These 18 animals were not used in the main study.

2.5 Animals

Sixty healthy male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 180–220 g, were obtained from the University of Zambia. They were housed at 20–24 °C and 45–65% relative humidity on a 12 h light/dark cycle with free access to standard chow and water, and acclimatised for two weeks. Group size was derived from the resource equation [18], with ten rats per group to allow for attrition. Only males were used, to avoid oestrous-cycle effects on glycaemia [19]. Animals that were ill before allocation, failed to become diabetic or did not complete treatment were to be excluded; all 60 rats completed the study.

2.6 Induction of diabetes and treatment

After an overnight fast, diabetes was induced by a single intraperitoneal injection of STZ (70 mg/kg) freshly dissolved in cold 0.1 M sodium citrate buffer (pH 4.5) [19–21]. Rats with fasting blood glucose above 7 mmol/L after 72 h were considered diabetic and were randomly allocated to the diabetic + extract (DT), diabetic + metformin (DM), diabetic + insulin (DI) and untreated diabetic control (DC) groups; non-diabetic rats were randomly allocated to the normal control (NC) and extract-only (TO) groups (Table 1). From 72 h after induction, treatments were given once daily between 09:00 and 10:00 h for six weeks: extract (200 mg/kg) [22] and metformin (100 mg/kg) by oro-gastric cannula, and insulin glargine (4 IU/day) by intraperitoneal injection.

Table 1: Experimental groups and treatments

Group	Description	Treatment (once daily for six weeks)	n
NC	Normal control	None	10
TO	Non-diabetic, extract only	*T. indica* extract 200 mg/kg, oral	10
DT	Diabetic + extract	*T. indica* extract 200 mg/kg, oral	10
DM	Diabetic + metformin	Metformin 100 mg/kg, oral	10
DI	Diabetic + insulin	Insulin glargine 4 IU/day, intraperitoneal	10
DC	Diabetic control	None	10

Diabetes was induced in DT, DM, DI and DC with a single intraperitoneal injection of streptozotocin (70 mg/kg). Oral doses were given by oro-gastric cannula.

2.7 Blood glucose and body weight

Fasting blood glucose was measured weekly between 09:00 and 10:00 h on tail-vein blood with an Accu-Chek Compact Plus glucometer (Roche Diagnostics, Mannheim, Germany), and body weight was recorded weekly on a Venus VT 30 SL scale, from two weeks before induction to the end of week 6 [21].

2.8 Euthanasia and retinal tissue preparation

About 48 h after the last dose, overnight-fasted rats were deeply anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally and decapitated after loss of the pedal withdrawal reflex [23]. The eyes were enucleated, and ten eyes per group were immersion-fixed in freshly prepared 10% formal saline for 72 h, processed into paraffin and sectioned at 4 μm.

2.9 Histology and immunohistochemistry

Sections were stained with haematoxylin and eosin (H&E) for general cytoarchitecture, phosphotungstic acid haematoxylin (PTAH) for glial fibres, cresyl fast violet (CFV) for Nissl substance and neuronal density, and Masson's trichrome (MT) for collagen [24]. For GFAP immunohistochemistry, sections underwent heat-induced epitope retrieval and were incubated with anti-GFAP (1:500, 30 min, room temperature, as recommended by the manufacturer); binding was visualised with the Dako EnVision+ HRP-polymer anti-rabbit system and 3,3′-diaminobenzidine [25]. Sections were photographed at ×400 with a Swift Cam 20 MP camera on an Olympus BH2 microscope.

Each stain was assessed descriptively against predefined features: the organisation and relative thickness of the nerve fibre (NFL), ganglion cell (GCL), inner plexiform (IPL), inner nuclear (INL), outer nuclear (ONL) and photoreceptor (PRL) layers; nuclear pyknosis, vacuolation and cell density (H&E); the density of glial fibrillary processes (PTAH); Nissl staining and neuronal loss (CFV); the extent of GFAP-immunoreactive glial processes across layers (GFAP); and the presence and location of blue-stained collagen (MT). Sections were examined by an assessor blinded to group allocation;

the predominant pattern in each group was recorded and representative fields were photographed. No cell counts, layer measurements or scores were obtained, so histological differences were not tested statistically.

2.10 Statistical analysis

Blood glucose and body weight are expressed as mean ± standard error of the mean (SEM; n = 10 per group) and were analysed by two-way repeated-measures analysis of variance (group × week) with Tukey's post hoc test in GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered significant.

2.11 Ethical approval

The protocol was approved by the Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (SMHS-MU4-2024-39) and the National Health Research Authority of Zambia (NHRA-1835/28/12/2024). Animal care followed the guidance of the International Council for Laboratory Animal Science, the *Guide for the Care and Use of Laboratory Animals* and the 3Rs principles, and the study is reported according to the ARRIVE 2.0 guidelines [26]. Humane endpoints were weight loss above 20% of baseline, inability to reach food or water, severe lethargy, hunched posture or laboured breathing.

3 RESULTS

3.1 Acute toxicity and phytochemistry

No deaths or signs of toxicity occurred at any dose up to 3000 mg/kg (median lethal dose, LD_{50}, > 3000 mg/kg). All eleven phytochemical classes were detected; tannins (≈70 mg/100 g) and alkaloids (63 mg/100 g) predominated, followed by reducing sugars, phenols, flavonoids, cardiac glycosides and saponins. The extract showed concentration-dependent antioxidant activity that was slightly lower than that of ascorbic acid, and its main GC-MS constituents were 9-octadecenoic acid (23.2%), eugenol (20.4%), linoleic/linoelaidic acid (17.3%) and n-hexadecanoic acid (9.5%) (Supplementary Tables S1–S3).

3.2 Blood glucose and body weight

Baseline glucose was 4.2–4.9 mmol/L in all groups and rose to 30.8–33.4 mmol/L in the four STZ-injected groups 72 h after induction. Untreated diabetic rats remained hyperglycaemic (41.3 ± 0.18 mmol/L at week 6) and lost weight, whereas glucose fell progressively in DT, DM and DI to 5.1, 6.7 and 6.5 mmol/L, and these groups-maintained body weight ($p < 0.05$ vs DC; Table 2; complete weekly data in Supplementary Table S4). The extract had no effect on glucose or weight in non-diabetic rats.

Table 2: Fasting blood glucose (mmol/L) and body weight (g) before and after induction of diabetes

Week	NC	TO	DT	DM	DC	DI
Glucose						
−2	4.2 ± 0.01	4.6 ± 0.02	4.4 ± 0.01	4.2 ± 0.02	4.5 ± 0.02	4.9 ± 0.02
0	4.3 ± 0.02	4.4 ± 0.02	33.4 ± 0.09	31.4 ± 0.09	31.3 ± 0.91	30.8 ± 0.82
2	4.3 ± 0.02	4.2 ± 0.02	14.1 ± 0.03	21.5 ± 0.51	35.2 ± 0.99	20.5 ± 0.81
4	4.3 ± 0.02	3.9 ± 0.01	7.3 ± 0.02	12.6 ± 0.04	38.2 ± 0.14	11.7 ± 0.42
6	4.2 ± 0.02	3.7 ± 0.01	5.1 ± 0.02	6.7 ± 0.03	41.3 ± 0.18*	6.5 ± 0.27
Body weight						
−2	187.42 ± 3.88	188.30 ± 3.72	188.11 ± 3.52	187.54 ± 3.57	189.36 ± 3.91	188.25 ± 3.67
0	189.42 ± 3.48	190.56 ± 3.49	190.32 ± 3.74	189.71 ± 3.71	191.22 ± 3.54	190.88 ± 3.61
2	192.64 ± 3.38	193.28 ± 3.79	190.43 ± 3.79	189.19 ± 3.44	187.13 ± 3.31	190.03 ± 3.15
4	195.89 ± 3.58	196.77 ± 3.57	192.49 ± 3.14	189.66 ± 3.52	178.64 ± 3.48	191.23 ± 3.56
6	198.59 ± 3.91	199.81 ± 3.94	194.69 ± 3.76	191.31 ± 3.71	173.62 ± 3.41*	193.25 ± 3.74

Values are mean ± SEM (n = 10 per group). Week 0 = 72 h after streptozotocin. *$p < 0.05$ DC vs all other groups (two-way repeated-measures ANOVA with Tukey's test). NC, normal control; TO, extract only; DT, diabetic + *T. indica* extract; DM, diabetic + metformin; DC, diabetic control; DI, diabetic + insulin glargine.

3.3 Retinal histology

- H&E. NC and TO retinas showed normally arranged layers with healthy, densely packed nuclei. DT, DM and DI retinas contained some shrunken, pyknotic nuclei among normal cells, whereas DC retinas showed severe nuclear shrinkage, vacuolation and reduced cell density (Figure 1).

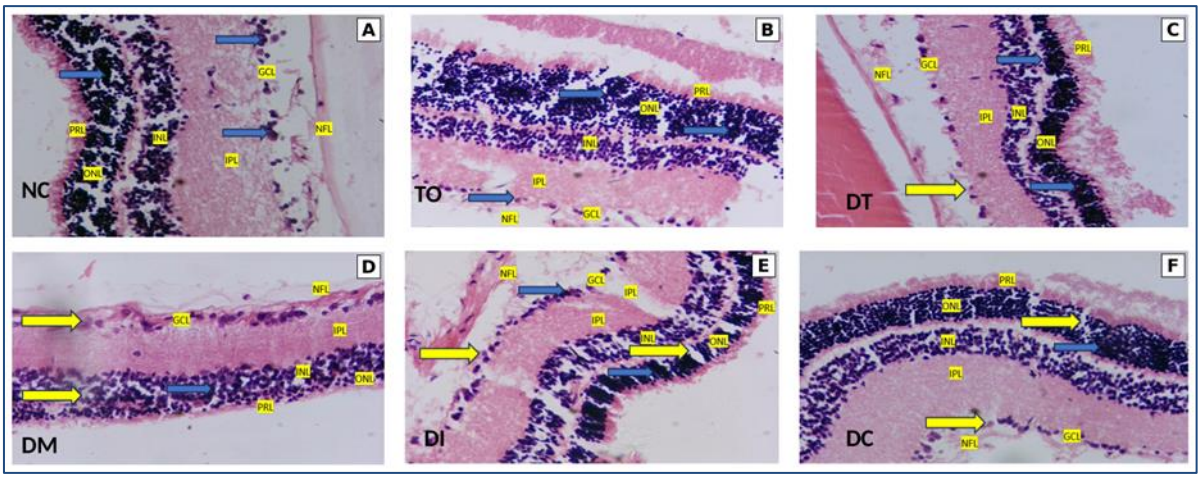


Figure 1: Retina at week 6, haematoxylin and eosin, ×400. (A) NC, (B) TO, (C) DT, (D) DM, (E) DI, (F) DC. Blue arrows, normal cells; yellow arrows, necrotic/abnormal cells. NFL, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; PRL, photoreceptor layer; BV, blood vessel.

- **PTAH.** Glial fibrillary processes were sparse and barely detectable in NC and TO. They were increased but close to normal density in DT, DM and DI, and markedly exaggerated in all layers of the DC retina (Figure 2).

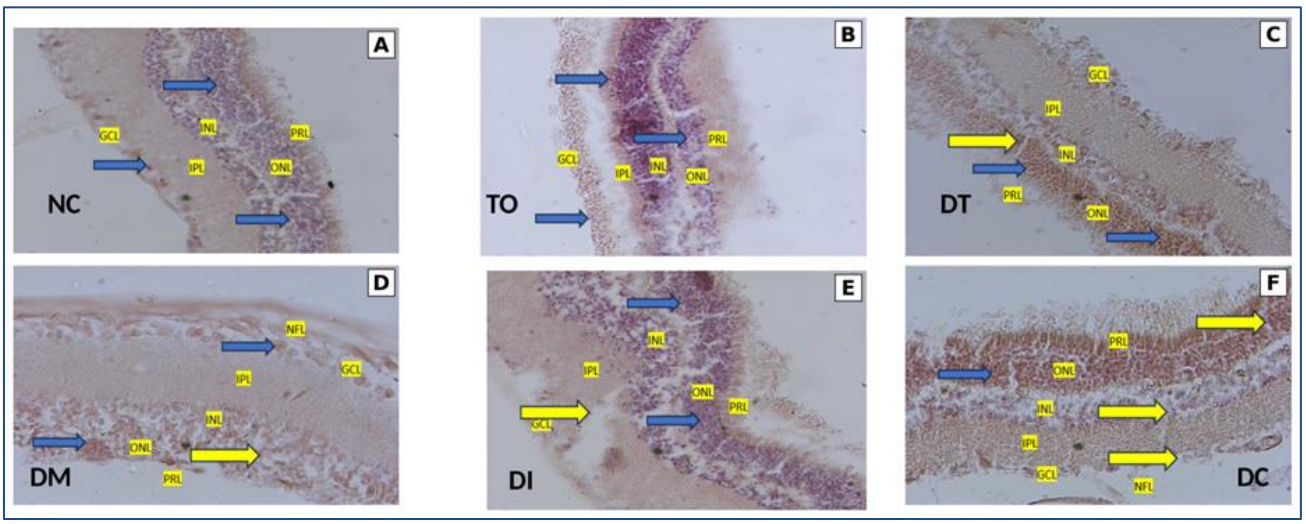


Figure 2: Retina at week 6, phosphotungstic acid haematoxylin, ×400. (A) NC, (B) TO, (C) DT, (D) DM, (E) DI, (F) DC. Blue arrows, normal cells; yellow arrows, necrotic/abnormal cells. NFL, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; PRL, photoreceptor layer; BV, blood vessel.

- **CFV.** NC and TO retinas showed normal neuronal density and thickness of the GCL, INL and ONL. DC retinas showed massive neuronal loss with extensive thinning and disorganisation of the INL and ONL. DM and DI retinas contained some pyknotic neurons with residual thinning of the INL and ONL, whereas DT retinas retained almost normal thickness of all three neuronal layers (Figure 3).

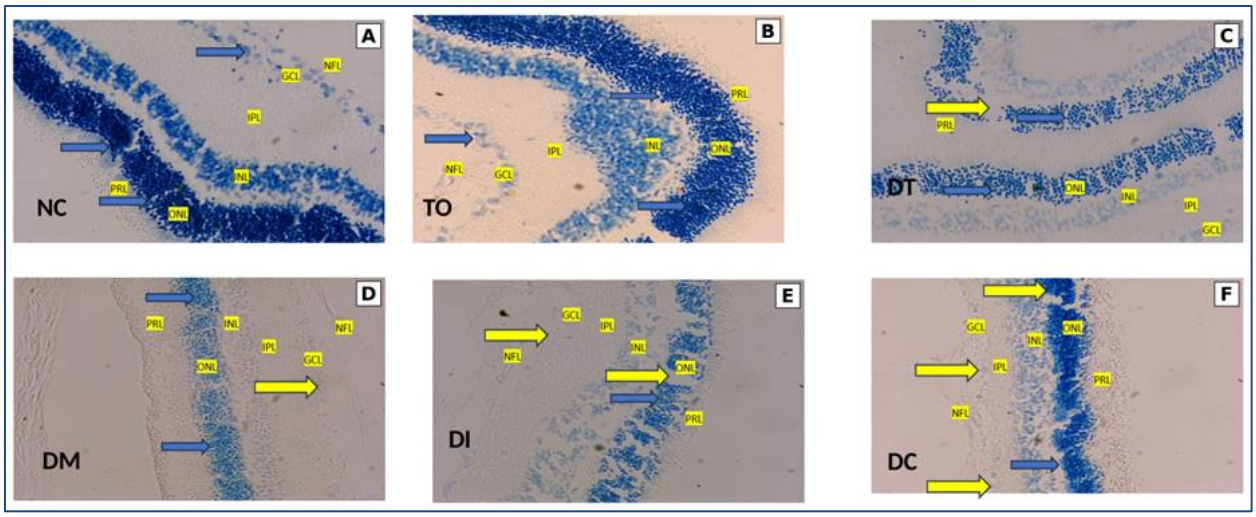


Figure 3: Retina at week 6, cresyl fast violet, ×400. (A) NC, (B) TO, (C) DT, (D) DM, (E) DI, (F) DC. Blue arrows, normal cells; yellow arrows, necrotic/abnormal cells. NFL, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; PRL, photoreceptor layer; BV, blood vessel.

- **GFAP.** NC and TO retinas showed intact neurons in the GCL, INL and ONL. DT, DM and DI retinas showed a few damaged GCL neurons with intact INL and ONL, whereas DC retinas showed intense GFAP immunoreactivity throughout the retinal layers (Figure 4).

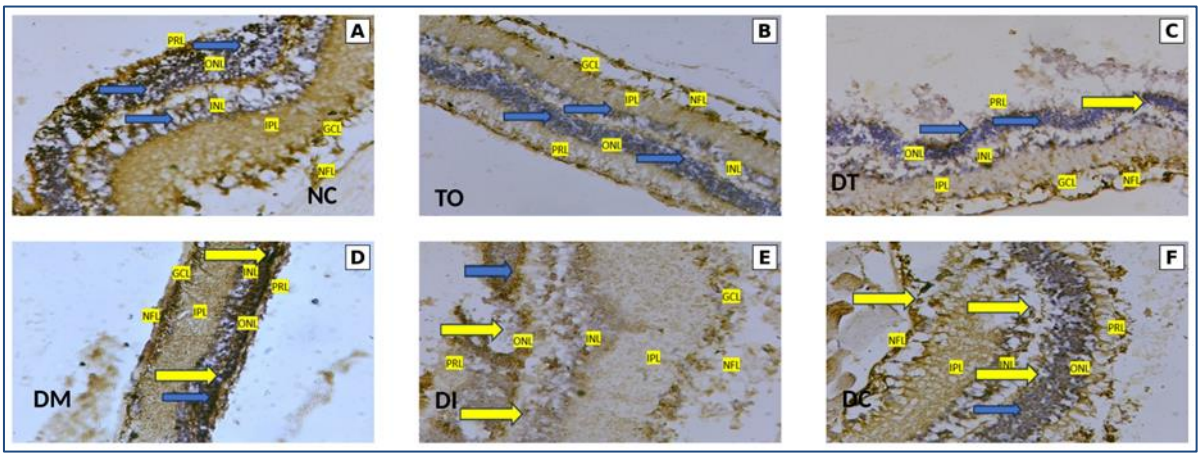


Figure 4: Retina at week 6, GFAP immunohistochemistry, ×400. (A) NC, (B) TO, (C) DT, (D) DM, (E) DI, (F) DC. Blue arrows, normal cells; yellow arrows, necrotic/abnormal cells. NFL, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; PRL, photoreceptor layer; BV, blood vessel.

- **MT.** No abnormal collagen was seen in NC or TO. Collagen deposition was extensive in DC, particularly in the IPL and PRL, prominent in DM and DI, and present but less extensive in DT (Figure 5).

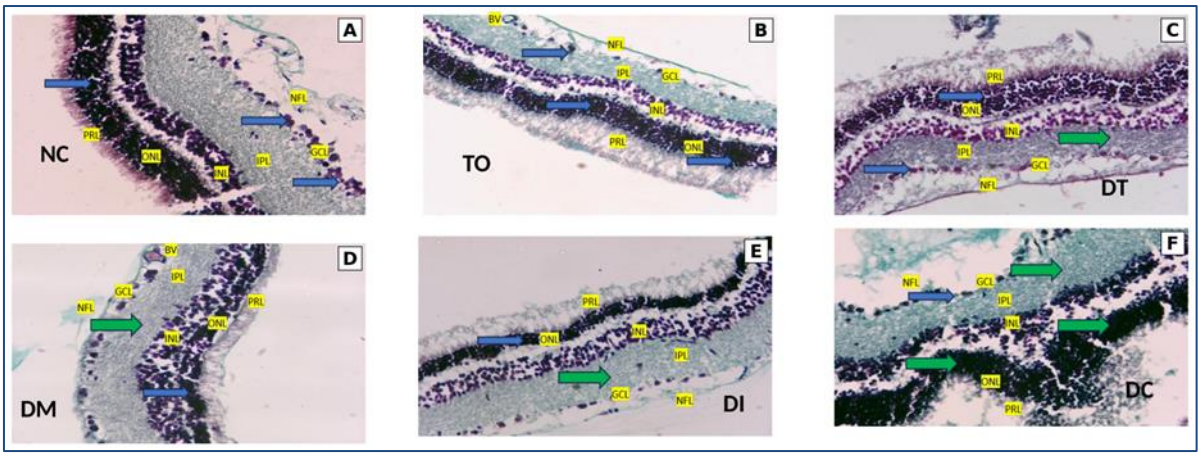


Figure 5: Retina at week 6, Masson's trichrome, ×400. (A) NC, (B) TO, (C) DT, (D) DM, (E) DI, (F) DC. Blue arrows, normal cells; green arrows, collagen deposition. NFL, nerve fibre layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; PRL, photoreceptor layer; BV, blood vessel.

4 DISCUSSION

This study shows that six weeks of untreated STZ diabetes produced widespread retinal neurodegeneration, gliosis and collagen deposition, and that ethanolic *T. indica* seed extract, metformin and insulin each limited these changes. The pattern of injury in DC – pyknotic nuclei, loss of Nissl-stained neurons and thinning of the INL and ONL – agrees with the neuronal loss described in experimental diabetic retinopathy and supports the view that the disease is neurodegenerative as well as vascular [1,2].

The intense PTAH and GFAP staining in DC indicates reactive gliosis. In the diabetic retina, GFAP is up-regulated in Müller cells and astrocytes in response to hyperglycaemia, oxidative stress and nuclear factor-κB (NF-κB)-driven inflammation, and this glial switch precedes overt vascular pathology [7,8]. Gliosis was much less marked in all treated groups, consistent with reports that glucose-lowering and anti-inflammatory interventions reduce retinal GFAP expression [8,9].

The clearest difference between treatments was in neuronal layer thickness: DT retained almost normal GCL, INL and ONL thickness, whereas DM and DI showed residual thinning. DT also had the lowest glucose at week 6, so tighter glycaemic control is the most likely explanation. The extract's phenols, tannins and eugenol, and its in vitro antioxidant activity, may add protection against the oxidative damage that drives retinal neuronal loss [5,13,14]; metformin's retinal benefits have likewise been attributed to both glycaemic and antioxidant actions [11,12]. Because neither oxidative markers nor layer thickness were measured in the retina, these mechanisms remain to be confirmed.

Collagen deposition was the finding least affected by treatment: it was reduced but still present in DT and prominent in DM and DI. Collagen accumulation in diabetic tissue reflects extracellular-matrix remodelling driven by pathways such as transforming growth factor-β signalling, which may need longer treatment, or treatment started earlier, to reverse [10].

Limitations

Histology was descriptive, although blinded, without layer measurements or cell counts; oxidative and inflammatory markers were not measured in retinal tissue; a single dose and time point were studied; and the STZ model does not fully represent human diabetic retinopathy. Quantitative morphometry, retinal biochemistry and electroretinography would strengthen these findings.

5 CONCLUSION

Daily oral treatment with ethanolic *T. indica* seed extract protected the retina of STZ-diabetic rats against neuronal loss and glial activation to a degree comparable to, and for neuronal layer preservation greater than standard metformin or insulin therapy, although it did not completely prevent collagen accumulation. These findings support further study of the extract as an affordable adjunct for preventing diabetic retinal neurodegeneration, ideally with quantitative morphometry and functional outcomes.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Statement of ethical approval

All animal procedures were approved by the Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (approval no. SMHS-MU4-2024-39) and the National Health Research Authority of Zambia (approval no. NHRA-1835/28/12/2024) before the study began, and complied with institutional and national guidelines, the *Guide for the Care and Use of Laboratory Animals* and the ARRIVE 2.0 guidelines.

Author Contributions

AN: conceptualisation, investigation, formal analysis, writing of original draft; INL: data collection, writing, review and editing; JM: data collection, writing, review and editing; FM: data collection, writing, review and editing; UAY: supervision, methodology, writing, review and editing. All authors read and approved the final manuscript and take responsibility for the integrity of the work.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence

An AI assistant (Claude, Anthropic) was used for language editing and manuscript formatting. The authors reviewed and verified all content and take full responsibility for the manuscript.

Related Manuscript

The occipital cortex of the same animals is reported in a separate manuscript submitted to the *Journal of Medical Sciences and Health*; extract characterisation, toxicity, blood glucose and body-weight data are common to both.

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