

**Neuroprotective Effect of *Azadirachta indica* Ethanol Extract on Retinal
Neurodegeneration in a Diabetic Rat Model**

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Natural and Applied Sciences, Lead City University, Ibadan, Oyo state, Nigeria**

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Biochemistry**

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Certification

This is to certify that I, **Ilegomah Anuoluwapo Eunice** with the Matriculation number **LCU/PG/006003** carried out this research project titled “**Neuroprotective Effect of *Azadirachta Indica* Ethanol Extract on Retinal Neurodegeneration in a Diabetic Rat Model**” in the Department of Chemical Science (Biochemistry), Faculty of Natural and Applied Science, Lead City University, Ibadan, Oyo State for the award of Master of Science (MSc.) in Biochemistry, and that this has not been previously submitted.

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(Supervisor)

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Date

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Prof. O.M. Ighodaro

(Head of Department)

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Date

Dedication

This research study is dedicated to God and to all researchers who continue to hold the line at the frontiers of science, striving tirelessly to discover solutions to diverse human health challenges.

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Acknowledgment

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While many contributed to the success of this work, I take full responsibility for any errors or omissions that may be found.

Abstract

Diabetic retinopathy is a leading cause of adult blindness, characterized by neurodegeneration, vascular dysfunction, and progressive loss of retinal integrity. This study investigated the neuroprotective effect of *Azadirachta indica* ethanol extract (AIEE) on retinal neurodegeneration, through bioactive compound profiling, molecular docking and streptozotocin-induced diabetic rat model. Forty adult male Wistar rats were randomly assigned to five groups (n=8): normal control, diabetic untreated, diabetic treated with 200 mg/kg AIEE, diabetic treated with 400 mg/kg AIEE, and diabetic treated with metformin (7 µg/kg). Treatments were administered orally, twice daily for 21 days. Retina post-mitochondrial fraction was analyzed for glial fibrillary acid protein, glutamate, vascular endothelial growth factor, brain derived neurotrophic factor, nerve growth factor, C-reactive protein, and nuclear factor-kappa B using enzyme-linked immunosorbent assay. Biomarkers of oxidative stress including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) were estimated using colorimetric methods. Retina, optic nerve, and visual cortex tissues were analyzed for morphometric characteristics, periodic acid schiff (PAS), and neuronal counts using histological techniques. Data were analyzed using a one-way ANOVA with Tukey's post hoc test. Findings showed significant levels of oxidative stress, neurotransmitter imbalance, neurotrophin depletion, and inflammation in the untreated experimental animals compared to the control group. Treatment with 200 and 400 mg/kg AIEE ameliorated these effects in a dose-dependent manner by enhancing antioxidant defenses, suppressing pro-inflammatory markers, up-regulating neurotrophic factors, and restoring neurotransmitter balance. 400 mg/kg AIEE notably preserved retinal structure, reduced gliosis, improved neuronal morphology, and decreased PAS-associated glycogen accumulation, closely resembling healthy controls, Metformin produced comparable but slightly lower neuroprotective effects. These findings indicate that AIEE confers substantial neuroprotection against diabetic retinal degeneration, likely through its antioxidant and anti-inflammatory phytoconstituents (Ethanone 1-[3-methyl-3-(4-methyl-3-pentenyl) oxiranyl]-, 1,2-Cyclopentanedione, 3,3,5,5-tetramethyl-, alpha.-D-Glucopyranoside, methyl). These compounds showed good binding affinities and interactions with key protein targets in neurodegeneration, highlighting *A.indica* potential as a therapeutic candidate for the management of diabetic retinopathy.

Keywords: *Azadirachta indica*, Diabetic retinopathy, Neuroprotection, Phytochemicals

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List of Acronyms

ADMET - Absorption, Distribution, Metabolism, Excretion, and Toxicity

AGE - Advanced Glycation End-Product

BDNF - Brain-Derived Neurotrophic Factor

BDNF - Brain-Derived Neurotrophic Factor

CRP - C-Reactive Protein

DME - Diabetic Macular Edema

DR - Diabetic Retinopathy

ECM - Extracellular Matrix

GABA - Gamma-Aminobutyric Acid

GCL - Ganglion Cell Layer

GC-MS - Gas Chromatography–Mass Spectrometry

GFAP - Glial Fibrillary Acidic Protein

INL - Inner Nuclear Layer

IPL - Inner Plexiform Layer

MDA - Malondialdehyde

NFL - Nerve Fiber Layer

NF- κ B - Nuclear Factor Kappa B

NGF - Nerve Growth Factor

NPDR - Non-Proliferative Diabetic Retinopathy

NVU - Neurovascular Unit

OPL - Outer Plexiform Layer

PDR - Proliferative Diabetic Retinopathy

PEDF - Pigment Epithelium–Derived Factor

PKC - Protein Kinase C

RGC - Retinal Ganglion Cell

VEGF – Vascular Endothelial Growth Factor

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