Journal of Social Research

P-ISSN: 2827-9832
E-ISSN: 2828-335x
Vol. 5, No. 2, January 2026
http://ijsr.internationaljournallabs.com/index.php/ijsr

# The Effects of Ethanol Extract of Vernonia Amygdalina on Optic Nerve Damage Based on Histopathological Overview, Ganglion Cell Density, and Average Apoptosis in Diabetic Rat Models

**Aulia Purnama Effendi*, Masitha Dewi Sari, Bobby Ramses Erguna Sitepu**
Universitas Sumatera Utara, Indonesia
Email: auliaprnm@gmail.com*, masitha_ds@yahoo.co.id, bobbyres@yahoo.com

**Abstract**

*Diabetes mellitus* (DM) is one of the most commonly diagnosed degenerative conditions worldwide, often resulting in significant negative impacts on the optic nerve due to chronic hyperglycemia. Hence, there is a need for additional therapeutic modalities capable of reducing or even preventing the effects of DM on optic nerve damage, such as the African plant *Vernonia amygdalina*, which is rich in medical benefits like anti-inflammatory and antioxidant properties. This study aims to determine the effects of ethanol extract of *Vernonia amygdalina* on parameters related to optic nerve damage in a diabetic rat model. This experimental animal-based study was conducted on five groups of diabetic Wistar rats (7 rats per group) induced with streptozotocin and nicotinamide, with ethanol extract intervention at doses of 100–300 mg/kg body weight per day. The intervention was carried out for four weeks using *Vernonia amygdalina* ethanol extract, followed by evaluation of histopathological characteristics, ganglion cell density, and average apoptosis in the optic nerve. A significant increase in average apoptosis ($P < 0.05$) was observed in the control group (74.33 ± 4.03) compared to the P2 group (200 mg/kg body weight per day; 47.50 ± 5.96) and the P3 group (300 mg/kg body weight per day; 22.00 ± 1.67), a finding nearly equivalent to that of the untreated sham group (19.67 ± 3.33). Furthermore, administration of *Vernonia amygdalina* extract maintained normal axons and Schwann cells only in the P3 group, with ganglion cell density appearing loosely packed to dense at doses of 100–300 mg/kg body weight per day. Overall, *Vernonia amygdalina* extract had a positive effect on the optic nerve in diabetic rats, as evidenced by the average apoptosis findings and histopathological evaluation.

**Keywords:** Diabetes mellitus, Ethanolic extract, Histopathology, Optic nerve, *Vernonia amygdalina*



## INTRODUCTION

Complications of diabetes mellitus involving the eye organs are the most common sequelae encountered in clinical practice. The involvement of hyperglycemia / DM in the eye is in one collective spectrum, namely diabetic eye disease (DED) (Mesquida et al., 2019; Pearce et al., 2019). DED itself consists of several diseases that have the most prominent long-term disability in the field of sensing such as glaucoma, diabetic retinopathy, diabetic macular edema, to cataracts. Based on epidemiological data reported by the American Academy of Ophthalmology, there are 387 million individuals suffering from diabetes mellitus in the world, which is expected to increase to 592 million individuals by 2035 (Mesquida et al., 2019; Teo et al., 2021; Sukhla & Tripathy, 2022). Li et al. (2019) mentioned that at least 25.7% and 3.7% of DM patients had already experienced diabetic retinopathy and diabetic macular edema based on the results of a meta-analysis of 35 prevalence studies and 4 incidence studies from Europe. It is estimated that there are more than 7.5 million cases associated with eye complications across Europe, with some being described as sight-threatening diseases such as glaucoma (Mesquida et al., 2019).

In hyperglycemic conditions, there can be an increase in vascular permeability that induces an increase in intraocular pressure (IOP). Increased IOP pressure amplifies the cascade of optic nerve damage that has previously been deprived of nutrients and oxygen due to the preceding vascular dysfunction. This can lead to permanent CN II (optic nerve damage) (Arus Victor, 2019; Shin et al., 2014).

Through the polyol pathway, glucose will be reduced to sorbitol by the enzyme aldose reductase. The impermeability of sorbitol will cause the substance to accumulate in retinal cells resulting in osmotic damage to retinal cells. The involvement of NADPH itself can also increase the degree of damage due to oxidative stress that occurs. Increased levels of Reactive Oxygen Species (ROS) cause oxidative stress that can cause damage to cells and tissues (Sukhla & Tripathy, 2022).

The basic management of diabetic retinopathy is to control DM through strict metabolic control, keeping the Hb value below 7%, and lifestyle modifications such as regular exercise and eating a suitable diabetic diet. The patient is also required to visit a diabetologist to get regular follow-up and evaluate whether the antidiabetic medication he is currently receiving is still adequate to control his blood sugar levels. Other concomitant systemic diseases such as hypertension, dyslipidemia, hypoproteinemia, anemia, nephropathy, neuropathy, cardiovascular disorders should be treated with appropriate treatment and with good interprofessional collaboration (Sukhla & Tripathy, 2022).

*Vernonia Amygdalina* (VA) commonly known as bitter leaf or African Leaf is a traditional medicinal plant commonly used to treat various diseases such as diarrhea, bacterial and fungal infections, inflammation, cancer, and diabetes, the juice of this plant can also be applied to wounds. VA comes from the Asteraceae family that grows in tropical regions of the African continent, this plant is often also named as bitter leaf because of its bitter taste which is associated with anti-nutritional content and anti-diabetic effects that it may have. VA has a high content of nutrients such as vitamins, fiber, carbohydrates and minerals. According to research by Alara et al., it was found that VA contains many phytochemicals such as alkaloids, tannins, saponins, flavonoids, polyphenols, terpenes, steroids, coumarins, xanthones, edotides and sesquiterpenes, which have antioxidant effects (Ugbogu et al., 2021; Alara et al., 2018).

Therefore, based on the background of the high morbidity associated with complications of DM in the eye, and the existence of previous studies on the benefits of VA it is necessary to conduct further research on the effects of giving ethanol extract *Vernonia amygdalina* on repair of optic nerve damage. This study assessed the effect of ethanol extract of *Vernonia amygdalina* on optic nerve damage in diabetic rats and untreated rats.

## RESEARCH METHOD

This study is an animal experimental study with the design of randomized post-test-only control group laboratory experimental design to determine the effect of ethanol extract of Bitter leaves (*Vernonia amygdalina*) in diabetic rats with apoptosis and histopathological measurements were only performed after treatment. This research was conducted at the Laboratory of Pharmaceutical Preparation Technology of the Faculty of Pharmacy, Universitas Sumatera Utara for the identification and the manufacture of *Vernonia amygdalina* ethanol extracts, Clinical Pharmacology Laboratory of the Department of Pharmacology and therapy of the Faculty of Medicine, Universitas Sumatera Utara for the treatment of experimental animals, and Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara for histopathological examination of the optic nerve.

The research sample used was male white rat *Rattus novergicus* strain Wistar (Wistar Rat) obtained from the Laboratory of Clinical Pharmacology Department of Pharmacology and Therapy Faculty of Medicine, Universitas Sumatera Utara. The inclusion criteria of this study were male white rats of Wistar strain aged 2-3 months, with a body weight of 250-350 grams, rats in a healthy condition characterized by active movement, no injuries and no disabilities. While the exclusion criteria were rat that looked sick during the adaptation process and during the research process, rat that had anatomical abnormalities in one or both eyes, rat with weight loss during the adaptation process and the research process more than 10% of initial body

weight. Drop out criteria were rats died instantly after induction of high fat diet and diabetes, rats died instantly after induction of *Vernonia amygdalina* or rats died during the study. The number of Wistar Rats samples to be used is 32.

The study was conducted after obtaining approval from the Research Ethics Committee of the Universitas Sumatera Utara. The method used in extracting bitter leaves is maceration. In this method, 96% ethanol solvent is used. A total of 1.5 kg of bitter leaves are washed in advance, then cut into small pieces and dried for 2 days. After drying, the bitter leaves are blended until crushed into powder. A total of 700 g of Simplicia powder is placed in a glass container, 96% ethanol is added as much as 5 L (75 parts), cover and leave for 5 days, protected from light while stirring occasionally, strain, squeeze, wash the pulp with liquid using a filter to taste up to 2 L (25 parts).

Transfer to a closed vessel, leave in a cool place, protected from light for 2 days and then filter. The results obtained were concentrated with a rotary vacuum evaporator (rotary evaporator) until most of the solvent evaporated and continued the evaporation process at a temperature of 800 ℃ with a rotational speed of 35 rpm for 30 minutes and the results were taken in the form of a thick extract of Bitter leaves. Furthermore, the extract concentration of African leaf extract was divided into three, 100, 200, and 300 mg/kgBB orally.

Then the experimental rats (*Rattus norvegicus*) were adapted to the laboratory environment for 7 days with standard feeding and drinking in all rats. Rat rearing was carried out at the Clinical Pharmacology Laboratory of the Department of Pharmacology and Therapy, Faculty of Medicine, University of North Sumatra. After the adaptation period is complete, then the rats were given a high-fat diet for 4 weeks. Then the body weight and glucose levels were measured, then all rats were fasted for 18 hours before diabetes was induced with 55 mg/kgBB Streptozocin and 120 mg/kgbb intraperitoneal Nicotinamide.

On the 3rd day after being induced, blood glucose level measurement is performed, if > 200 mg/dL/day for 3 days then it is considered that rats become diabetic. All rat that survived were then numbered and randomized in random order and divided into 5 groups, namely the sham group (group without treatment), control group (diabetic rat that were not given treatment, only monitoring was carried out), group P1 (rat received extract treatment at a dose of 100 mg/kgBB/day), group P2 (rat received extract treatment at a dose of 200 mg/kgBB/day), and group P3 (rat received extract treatment at a dose of 300 mg/kgBB/day).

After 4 weeks of treatment with *Vernonia amygdalina* extract, the rat will be given chloroform and then terminated. Rats that have been terminated and cremated with an insulator using diesel fuel. After that, enucleation was performed in the eyes of rat. Then the average apoptosis using TUNEL assay and histopathological picture examination to see optic nerve damage, and Retinal Ganglion Cell density examination was performed. Then a statistical analysis is carried out.

After all the data is collected, the data will be tabulated in the form of tables. The data (ratio scale) collected were analyzed statistically with one-way Annova test and Dunnet's T3 test as a Post Hoc test or follow-up test, if they meet the requirements of normal data distribution, and produce unequal data variants. If it does not meet the requirements, then the collected data is analyzed using the Kruskal-Wallis test (non-parametric) to replace the one-way Annova (parametric). Followed by Mann-Whitney test (nonparametric), replacing the Post Hoc test.

## RESULT AND DISCUSSION

### Results of Differences In Number of Optic Nerve Apoptosis

The number of optic nerve apoptosis in rats in the control group showed the highest average of 74.33 (SD=4.03). In the sham group, the number of apoptosis showed the lowest average of 19.67 (SD=3.33). The second lowest amount of apoptosis was in the P3 group with

an average of 22 (SD=1.67). Using the oneway Anova test, it was found that there was a significant difference in the average number of apoptosis on the optic nerve of the five groups of rats (p<0.001).

**Table 1. Differences in the number of optic nerve apoptosis in rats after administration of ethanol extract of Vernonia amygdalina**

| Group | n | Number of Apoptosis | | p |
|---|---|---|---|---|
| | | Mean (SD) | Median (min – max) | |
| **Sham** | 6 | 19,67 (3,33) | 19,5 (15 – 25) | <0,001* |
| **Control** | 6 | 74,33 (4,03) | 73,5 (70 – 80) | |
| **P1** | 6 | 64,17 (9,75) | 66 (51 – 75) | |
| **P2** | 6 | 47,5 (5,96) | 48 (40 – 54) | |
| **P3** | 6 | 22 (1,67) | 22,5 (20 – 24) | |

**Oneway Anova*

Based on the analysis of the Posthoc test using Dunnet's T3 test showed that there is a significant difference in the number of apoptosis in the optic nerve between the control group with the sham group (p<0.The001), P2 group (p<0.001) and P3 Group (p<0.001). However, there was no difference in the mean number of apoptosis in the optic nerve between the control group and the P1 group (p=0.313). Furthermore, the sham group also appears to be significantly different from Group P1 and Group P2. In contrast, there was no difference in the mean number of apoptosis in the optic nerve between the sham group and the P3 Group (p=0.727). Between groups P1 and P2 also found no significant difference in the average number of apoptosis (p=0.054). Between groups P1 and P3 and between P2 and P3 there are significant differences in the average number of apoptosis (p=0.001).

**Table 2. Posthoc Test difference in Mean optic nerve Apoptosis in rats after administration of ethanol extract of *Vernonia amygdalina***

| Group | *Sham* | P1 | P2 | P3 |
|---|---|---|---|---|
| Control | <0,001 | 0,313 | <0,001 | <0,001 |
| *Sham* | | <0,001 | <0,001 | 0,727 |
| P1 | | | 0,054 | 0,001 |
| P2 | | | | 0,001 |

*Dunnet's T3

**Results of Differences in Histopathological Images of The Optic Nerve**

In the control group, P1 and P2 had no optic nerve in all rat. Meanwhile, in the sham and P3 groups, all normal axon and Schwan cells were found. Using the Kruskal Wallis test showed that there are significant differences histopathological picture based on the treatment group (p<0.001).

**Table 3. Differences in histopathological picture of optic nerve after administration of ethanol extract of Vernonia amygdalina in rats**

| Group | Optic Nerve Morphology | | p |
|---|---|---|---|
| | Axon and Schwan cell Normal | Axon and Schwan cell Pathological | |
| Control | 0 | 6 (100) | <0,001* |
| *Sham* | 6 (100) | 0 | |
| P1 | 0 | 6 (100) | |
| P2 | 0 | 6 (100) | |
| P3 | 6 (100) | 0 | |

*Kruskal Wallis

From the analysis of posthoc test using Mann Whitney test showed that there was a significant difference in histopathological picture on the optic nerve between the control group with sham group and P3 Group (p=0.001). Between the control group and the group P1 and P2 also found differences in histopathology nerve histopathology picture (p=0.001). Differences in histopathological features also appeared to be significant between groups P1 and P3, as well as between groups P2 and P3 (p=0.001).

**Table 4. Posthoc test differences in histopathology of optic nerve after administration of ethanol extract of Vernonia amygdalina in rats**

| Group | *Sham* | P1 | P2 | P3 |
|---|---|---|---|---|
| Control | 0,001 | 1,000 | 1,000 | 0,001 |
| *Sham* | | 0,001 | 0,001 | 1,000 |
| P1 | | | 1,000 | 0,001 |
| P2 | | | | 0,001 |

*Mann Whitney

**Results of Difference In Density Of Optic Nerve Ganglion**

**Table 5. Differences in optic nerve Ganglion density after administration of ethanol extract of *Vernonia amygdalina* in rats**

| Group | Ganglion Density | | | p |
|---|---|---|---|---|
| | Dense | Loose | Very Loose | |
| Control | 0 | 0 | 6 (100) | <0,001* |
| *Sham* | 6 (100) | 0 | 0 | |
| P1 | 0 | 6 (100) | 0 | |
| P2 | 0 | 6 (100) | 0 | |
| P3 | 6 (100) | 0 | 0 | |

*Kruskal Wallis

In rats in the control group, the whole showed very loose. Meanwhile, in the control and P3 groups, all rats showed dense ganglion density. And in rats in groups P1 and P2, all rats showed loose ganglion density. Using the Kruskal Wallis test showed that there was a significant difference in ganglion density based on the treatment group (p<0.001). The analysis results of the test using Mann-Whitney test posthoc showed that there was a significant difference in ganglion density in the optic nerve between the control group with the sham group and the entire treatment group (p=0.001). Between the sham group and the P1 and P2 groups also found significant differences in histopathological nerve ganglion density (p=0.001). However, there was no significant difference in ganglion density between the control group and the P3 Group (p=1,000). Differences in ganglion density also appeared to be insignificant between the P1 and P2 groups.

**Table 6. Posthoc test of optic nerve Ganglion density difference after administration of ethanol extract of *Vernonia amygdalina* in rats**

| Group | *Sham* | P1 | P2 | P3 |
|---|---|---|---|---|
| Control | 0,001 | 0,001 | 0,001 | 0,001 |
| *Sham* | | 0,001 | 0,001 | 1,000 |
| P1 | | | 1,000 | 0,001 |
| P2 | | | | 0,001 |

*Mann Whitney

This study basically aimed to evaluate the clinical benefit of administering ethanol extract from African leaf or Vernonia amygdalina in a group of diabetes induction model rat.

Thus, the quantification of the results of experimental tests on experimental animals in this study will be determined based on the findings of several main outcomes that have been evaluated such as the average apoptotic rate and histopathological picture after the intervention. In the first experimental results, it appears that the induction of diabetes using 55 mg/kgBB streptozocin and 120 mg/kgBB nicotinamide per intraperitoneal can increase the average apoptosis up to 74.33 4.03 in the control group.

The findings were significantly higher than those of the sham group (a population of rats that were not given any intervention or induction), indicating that the induction of diabetes in the control group was shown to have an effect on the average apoptosis. Furthermore, after administering the ethanol extract of *Vernonia amygdalina* at 3 different doses (100, 200, and 300 mg / kgBB), a progressive reduction of the mean apoptosis that can be observed in the TUNEL assay was found to be 64,17 ± 9,75; 47,50 ± 5,96; 22,00 ± 1,67. The findings provide statistical evidence that different doses of ethanol extract of Vernonia amygdalina can have different therapeutic effects, with the conclusion that the greater the dose concentration given, the higher the effect will manifest in the intervention population.

Table 2 also compared whether there is a significant difference in each test dose, with the results of a dose of 300 mg/kgBB showing a significantly greater positive impact than the group with a dose of 100/200 mg/kgBB. In fact, the dose was able to reduce the mean optic nerve apoptosis of test animals that resemble the sham group (without treatment). Theoretically, there is a corresponding molecular relationship between decreased oxidative activity by reactive oxygen species (and their derivatives), increased antioxidant activity, and regulation of key neurotransmitters in neurodegenerative pathologies represented by the apoptotic mean in this study. Thus, it can be estimated that the main findings in this study with the results of a decrease in the average apoptosis of the optic nerve if can be a representative outcome that is meaningful enough to show that the positive impact on laboratory outcomes associated with TUNNEL assay findings (Olufunmilayo et al., 2023; Domanskyi & Parlato, 2022).

Studies with fairly close concepts have previously been reported by Monday et al., on the basis of Test administration using ethanol extracts of *Vernonia amygdalina* and Cymbopogon citratus to reduce the symptoms of cognitive impairment in a group of experimental rat. Monday et al., using the Morris Water Maze (MWM) test to ascertain the therapeutic impact of both extracts showed that the intervention-administered group would exhibit lower swimming latency. MWM test itself is considered quite representative in showing how the improvement of cognitive function after being given ethanol extracts from the two bioactive components. It was also assessed with regard to the neuroprotective effects of both extracts on the optic nerve of the experimental rat that intervened, so theoretically, the optic nerve is more “intact” or minimal apoptosis and inflammation will show a better clinical appearance in formal trials as well as MWM (Rotimi et al., 2022; Monday et al., 2023).

In examining the histopathological picture of the optic nerve in this study, all rat that received induction of diabetes showed a morphological picture of “no optic nerve” in all histopathological findings, while the sham group that did not get any treatment had a morphological picture of normal axons and Schwann cells. Thus, it can be ascertained that the effect of diabetes induction on the optic nerve histopathological findings found significant histopathologically. Interestingly, the administration of an ethanol extract of Vernonia amygdalina of 100-200 mg/kgBB did not provide clinically significant benefits, with the same conclusion as the control group that there was no observable optic nerve picture.

Meanwhile, the administration of extract with a dose of 300 mg/kgBB was reported to be able to maintain the histopathological picture of the optic nerve as well as the normal sham group that did not get any induction. Previous research conducted by Oladele et al., Aguwa et

al., Ajeleti et al., and Monday et al., has also obtained a similar conclusion that there is a decrease in the average of neural cells that undergo apoptosis, this was followed by a decrease in the number of neural degenerations seen in experimental mouse models of neurodegenerative pathology (Monday et al., 2023; Oladele et al., 2020; Ajeleti et al., 2023; Aguwa et al., 2020). These findings form the basis of histopathology which is very meaningful considering that the integrity of the optic nerve tissue is an important key in the neuro preventive aspects of ophthalmological pathology induced by degenerative conditions such as diabetes mellitus.

Furthermore, analysis of the difference in the density of the optic nerve ganglion also showed a progressive increase in the density or density of the ganglion after giving *Vernonia amygdalina* ethanol extracts. This can be seen in Table. 5 which explains that the control group that intervened with diabetes induction even showed the density of the ganglion on the retina that was very loose. Meanwhile, the administration of extracts with a concentration of 100-200 mg/kgBB will "maintain" the integrity of the ganglion density despite the histopathological interpretation conducted to categorize its findings in the group in the category of loose density. Meanwhile, the administration of ethanol extract of Vernonia amygdalina of 300 mg / kgBB was able to maintain the density of the ganglion to resemble the sham group that did not get any intervention.

## CONCLUSION

The ethanol extract of *Vernonia amygdalina* demonstrated a positive impact on optic nerve damage in diabetic rat models, as evidenced by reduced mean apoptosis and improved histopathological features. Administration at 300 mg/kg body weight (mg/kgBB) effectively maintained axon and Schwann cell integrity, while lower doses (100–200 mg/kgBB) showed no significant histopathological benefits; similarly, ganglion cell density was preserved in the loose category at 100–200 mg/kgBB and reached dense levels (comparable to the sham group) only at 300 mg/kgBB. For future research, investigators could explore dose-response mechanisms at intermediate levels (e.g., 250 mg/kgBB), long-term effects beyond four weeks, or molecular pathways (e.g., antioxidant enzyme modulation) using advanced techniques like immunohistochemistry to optimize therapeutic efficacy and clinical translation.

## REFERENCES

Aguwa, U. S., Okeke, S. M., Okeke, C. M., et al. (2020). Comparing the neuroprotective effects of aqueous and methanolic extracts of Vernonia amygdalina on the cerebellum of adult male Wistar rats. *International Annals of Science*, *9*, 145–159.

Ajeleti, A. O., Akunna, G. G., Ojo, J. H., et al. (2023). Neuro-protective and neuro-curative effect of Vernonia amygdalina in rat model of neurodegenerative disease. *Journal of Trace Elements in Medicine and Biology*, *4*, 1–7.

Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. *Food and Bioproducts Processing*, *107*, 36–48.

Arus Victor, A. (2019). Optic nerve changes in diabetic retinopathy. In *Optic Nerve*. https://doi.org/10.5772/intechopen.81221

Domanskyi, A., & Parlato, R. (2022). Oxidative stress in neurodegenerative diseases. *Antioxidants*, *11*, 2–5.

Mesquida, M., Drawnel, F., & Fauser, S. (2019). The role of inflammation in diabetic eye disease. *Seminars in Immunopathology*, *41*, 427–445.

Monday, A. B., et al. (2023). Neuroprotective effect of ethanolic leaf extracts of Vernonia amygdalina and Cymbopogon citratus on diabetes induced cognitive impairments in mice. *Journal of Food Technology and Nutrition Sciences*, *5*, 1–8.

Oladele, J. O., Oyeleke, O. M., Oladele, O. T., et al. (2020). Neuroprotective mechanism of Vernonia amygdalina in a rat model of neurodegenerative diseases. *Toxicology Reports*, *7*, 1223–1232.

Olufunmilayo, E. O., Gerke-Duncan, M. B., & Holsinger, R. M. D. (2023). Oxidative stress and antioxidants in neurodegenerative disorders. *Antioxidants*, *12*, 1–30.

Pearce, I., Simó, R., Lövestam-Adrian, M., et al. (2019). Association between diabetic eye disease and other complications of diabetes: Implications for care. A systematic review. *Diabetes, Obesity and Metabolism*, *21*, 467–478.

Rotimi, D. E., Ben-Goru, G. M., Evbuomwan, I. O., et al. (2022). Zingiber officinale and Vernonia amygdalina infusions improve redox status in rat brain. *Evidence-Based Complementary and Alternative Medicine*, *2022*, 9470178. https://doi.org/10.1155/2022/9470178

Shin, E. S., Sorenson, C. M., & Sheibani, N. (2014). Diabetes and retinal vascular dysfunction. *Journal of Ophthalmic and Vision Research*, *9*, 362–373.

Sukhla, U., & Tripathy, K. (2022). Diabetic retinopathy. In *StatPearls* [Internet]. StatPearls Publishing.

Teo, Z. L., Tham, Y. C., Yu, M., et al. (2021). Global prevalence of diabetic retinopathy and projection of burden through 2045: Systematic review and meta-analysis. *Ophthalmology*, *128*, 1580–1591.

Ugbogu, E. A., Emmanuel, O., Dike, E. D., et al. (2021). The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-Vernonia amygdalina L. (bitter leaf). *Clinical Complementary Medicine and Pharmacology*, *1*, 100006.