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COMPARATIVE EVALUATION OF FENOFIBRATE, METFORMIN, AND HERBAL DRUG COMBINATION AGAINST BEVACIZUMAB FOR ANTI-VEGF ACTIVITY IN DIABETIC RETINOPATHY

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ABSTRACT

Over 100 million people globally (6% of the population) suffer from Diabetes mellitus (DM) is the most prevalent endocrine condition. It's brought on by inefficient insulin secretion by the pancreas, which raises or lowers blood glucose levels which causing harm to numerous body systems, especially the kidney, heart, blood vessels, eyes, and nerves. A monoclonal anti-VEGF antibody, designated bevacizumab, is frequently used to treat DR. It is a very effective therapy, but also has some issues, such as its long-term effectiveness, ocular side effects, and the requirement for repeated intravitreal injections. In this preclinical study, we are using a combination of herbal and conventional drugs to design a combinational drug therapy that shows some promising results against VEGF in Wistar rats. The PPAR-α agonist fenofibrate has lipid-lowering and anti-inflammatory properties. Curcumin, a potent antioxidant, reduces inflammation and oxidative stress, whereas berberine, an AMPK activator, modulates glucose metabolism and VEGF signalling. According to preliminary results, FMBC treatment is as effective as or more effective than bevacizumab in reducing VEGF expression, preventing neo-vascularization, and maintaining retinal shape. However, it also has its limitations and side effects. Therefore, we combine it with herbal drugs such as Curcuma longa and berberine to counter these limitations and side effects. We also use metformin for better results, as fenofibrate has no anti-diabetic effect. Furthermore, FBC reduces inflammatory damage and strengthens antioxidant defence systems, indicating a neuroprotective function in DR. This study demonstrates the possibility of FMBC combination therapy to provide more efficient, safer, and expedited or accelerated therapy for diabetic retinopathy.

KEYWORDS: Diabetic Retinopathy, Anti-VEGF activity, Bevacizumab, PPAR-α agonist, AMPK activator, Curcumin, Neo-vascularization

1. INTRODUCTION

Diabetic retinopathy is a microvascular complication that occurs due to hyperglycemia in diabetic patients.[15] This particularly serious complication gradually damages the retinal blood vessels. As such, vision impairment is progressive.[15] In serious cases, it might also result in blindness.[15] It is caused by long-term high blood sugar levels, resulting in several pathological changes such as Mitochondrial dysfunction, enhanced permeability, and occlusion of the capillaries.[15],[65] These vascular abnormalities impair the BRB, leading to edema, haemorrhage, and ischemia of the retina.[53] Retinopathy can be non-proliferative or proliferative retinopathy. Non-proliferative might become proliferative.[53] The initial stage of NPDR manifests through microaneurysms, haemorrhages, CWS, and venous irregularities.[53]

1.1. Types of Diabetic Retinopathy - Diabetic retinopathy is a disease related to the eye that is caused by diabetes and progression from non-proliferative retinal vascular disease to retinal epithelium.[40] However, it is primarily categorized into two types which are NPDR and PDR.[40]

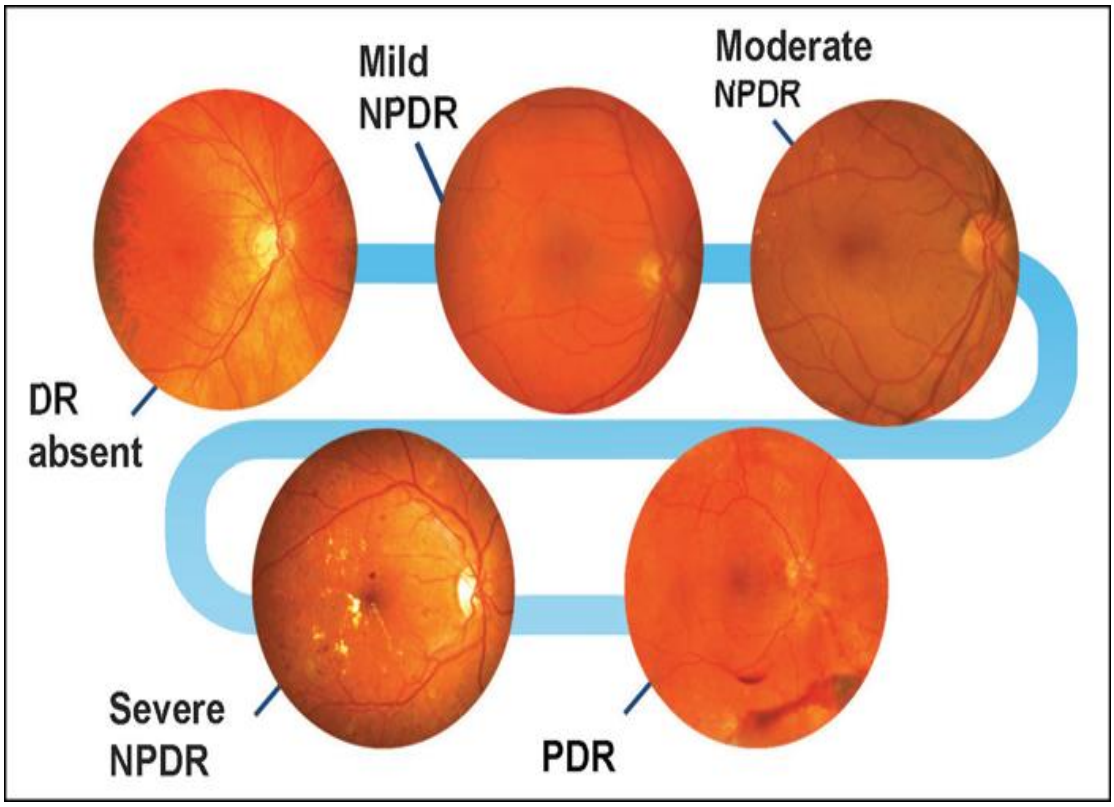

Figure 1.1: Types and stages of Diabetic retinopathy (Fity Club.).

a) Non-Proliferative Diabetic Retinopathy (NPDR). This is the early stage of diabetic retinopathy in this blood vessels of the retina of the eye become weak and leaky due to hyperglycemia.[20] Retinopathy without its proliferative stage, NPDR must be further classified as mild, moderate, and can be severe based on the number and extent of retinal abnormalities can also cause hard exudates and cotton wool spots.[20] As the NPDR disease progresses, it may impair blood flow, causing capillary closure, and an increased risk of macular edema.[20]

b) Proliferative Diabetic Retinopathy (PDR). It refers to a more advanced stage. The retina and the optic disc of the eye develop fragile new blood vessels due to ischemia in this condition.[20] These vessels are leaky vessels that lead to hemorrhage of the blood vessels of the eye and fibrosis, along with retinal detachment. This can ultimately result in blindness.[20]

c) Diabetic Macular Edema (DME). DME is again a serious complication of diabetic retinopathy that can occur at any stage.[20] It results from fluid accumulation in the macula of the retina due to the leak in blood vessels, which is caused by hyperglycaemia, leading to distorted or blurred central vision. DME leads to vision loss in patients with diabetes.[20]

Diabetic retinopathy causes eye blindness worldwide. Its prevalence is directly related to the increasing prevalence of diabetes mellitus.[58], [59] It is globally estimated that around 35% of diabetic people have some kind of DR, and almost 10% develop VTDR, which is also known as vision-threatening diabetic retinopathy.[58], [59] Diabetic retinopathy ranged from 20% to 40% in Europe, depending on the duration of diabetes and its glycemic control in blood.[19] DR prevalence showed notable regional differences in Asia.[19] The prevalence of diabetic retinopathy in diabetic patients ranges from 24% to 37% in China and from 18% to 28% in urban populations of India.[37] Africa showed a large number in prevalence of diabetic retinopathy, which is 30% to 40%, especially in developing areas with limited access to healthcare.[37] According to the estimation of SOS Doctors, the number of diabetic retinopathy patients is expected to double between 2010 and 2050, which is almost 7.7 million to 14.6 million[46] In the figure below, each eye represents the no. of 80 million people, and especially the Americans at the age of 65 and above will be most affected by this eye disease.[46]

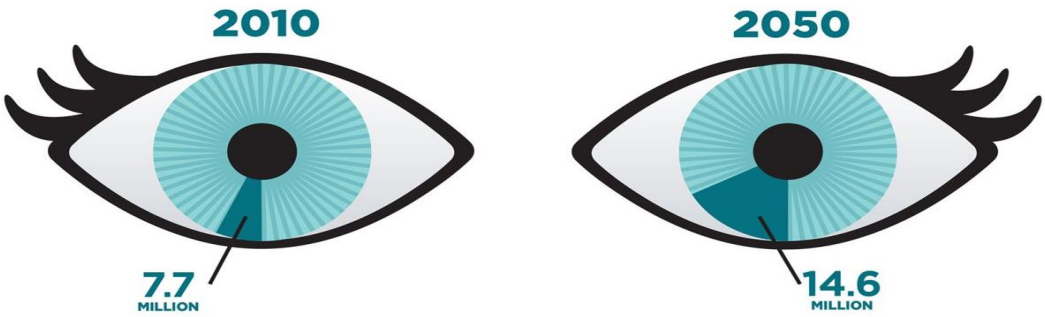

Figure 1.1.1: Epidemiology of diabetic retinopathy.[46]

1.2 VEGF Signaling Pathway and Its Role in Diabetic Retinopathy

VEGF is a potent angiogenic cytokine that plays an important role in the development of diabetic retinopathy, especially in the development of neovascularization and increased vascular permeability.[49], [11] The retina is a metabolically and vascularized tissue, and because of this it is susceptible to upregulation of VEGF, induced by hypoxia, under diabetic conditions.[49][63] VEGF-A is the most critical isoform of the VEGF family. It is involved in retinal neovascularization and vascular leakage associated with DR.[49], [11] Under normal glycemic conditions, VEGF expression is tightly regulated and contributes to the maintenance of vascular homeostasis, neuroprotection, and endothelial cell survival.[61], [62], [63] But in chronic hyperglycemia, HIF-1 alpha-mediated transcription triggers the overproduction of VEGF by oxidative stress and inflammation (common features of diabetes).[61], [62], [63] Hyperglycemia causes retinal capillary damage, which leads to microvascular occlusion and local hypoxia.[61], [62], [63] Retinal cells, including Müller cells, retinal pigment epithelium (RPE), and pericytes, increase VEGF expression in response to these activities.[61], [62], [63] VEGF shows its angiogenic effects after binding with the high-affinity receptor named VEGFR-2, which is also known as KDR/Flk-l and present on endothelial cells.[41], [11] This receptor-ligand interaction activates intracellular tyrosine kinase activity.[41] This activity initiates the PI3K/Akt, MAPK/ERK, and PLCγ/PKC cascades signaling pathways. These pathways enhance migration, proliferation of endothelial cells, and survival of cells.[41] Helps in the formation of vascular fragility and leakage of vessels in the retina.[57], [11] VEGF also increases the phosphorylation of tight junction proteins such as occludin and zonula occludens-1. It leads to the break of BRB.[57] In NPDR, VEGF level increases with the increase in retinal vascular permeability and microaneurysm formation.[15] When the disease progresses further to PDR, then sustained VEGF activity promotes abnormal neovascularization.[15] These neo-vessels have an immature structure and lead to

haemorrhage.[15] This results in severe loss of vision and complications such as vitreous hemorrhage and tractional retinal detachment.[64] By increasing the expression of ICAM-1 and VCAM-1, VEGF promotes inflammation.[64] It facilitates adhesion of leukocytes to the endothelium and promotes leucocytosis.[64] This worsens the retinal capillary damage and further impairs perfusion.[64] VEGF plays a very important role in diabetic retinopathy, which makes it the major target of therapeutics.[45] Anti-VEGF agents have shown promising clinical results in reducing macular edema and inhibiting neovascularization.[45] But still have some limitations like repeated intravitreal injections, high costs, and potential adverse effects.[45] Alternative therapies for anti-VEGF activity, including plant-based compounds like curcumin and berberine, as well as metabolic drugs like fenofibrate and metformin, have gained attention because of their indirect VEGF-modulatory effects.[25]

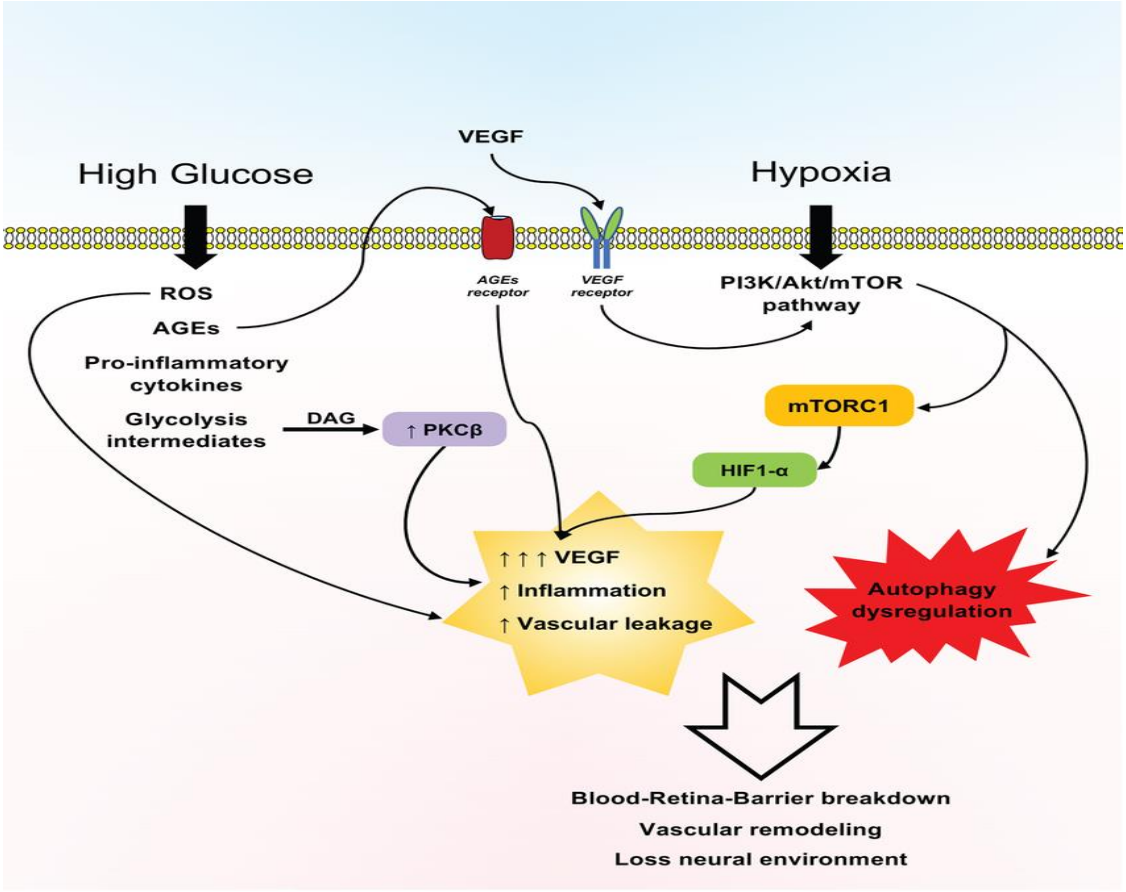

Figure 1.2: VEGF Signaling pathway in Diabetic Retinopathy.[38]

So**,** the VEGF pathway for signal is necessary for the development and cause of diabetic retinopathy because of its effects on angiogenesis, vascular permeability, and inflammation.[25] Targeting this pathway will continue to be a promising strategy to prevent and treat vision-threatening complications in diabetic patients.[25]

1.3 Current anti-VEGF strategies and their limitations

The management of diabetic retinopathy has dramatically changed over the past few decades, with therapies now aiming to slow disease progression, improve vision, and preserve retinal structure.[15] The treatment varies mainly according to the severity and stages of DR, from regular glycemic control in mild stages to laser photocoagulation, intravitreal injections for anti-VEGF treatment, and treatment using corticosteroids in advanced cases.[15], [12] Anti-VEGF is now the standard for managing diabetic macular edema DME and PDR.[46] VEGF induces pathological angiogenesis and vascular permeability, and its blockage effectively decreases retinal edema and neovascularization.[46] Anti-VEGF drugs that are frequently used are bevacizumab, ranibizumab, and aflibercept, which are injected intravitreally by repeated doses.[46]

Figure 1.3: Injection of Bevacizumab for Diabetic Retinopathy (Google).

Intravitreal anti-VEGF therapy provides substantial visual improvement in most of the patients suffering from DME and PDR.[35] These anti-VEGF agents, by inhibiting VEGF-A, prevent the of endothelial cell proliferation, decrease leakage, and stabilize the retina of the eye.[35], [12] In most of the clinical studies, these drugs provide more promising effects then laser therapy to improve ocular clarity.[35], [12] Although anti-VEGF agents are effective but they have several limitations.[15], [12] The need for frequent, often monthly and bimonthly, injecting the drug via the intravitreal route, provides a physical and financial load on patients.[15] In the lack of resources, the need for such frequent dosing reduces adherence to treatment and follow-up.[15], [12] The high cost of anti-VEGF agents, especially the branded ones like ranibizumab and aflibercept, limits access to patients in developing and emerging countries.[54], [14] While it is considered off-label, the use of bevacizumab is less expensive,

but issues related to over sterility and consistency remain the same.[55], [12] Laser photocoagulation, a widespread therapy for PDR and focal DME, reduces VEGF expression by destroying ischemic retinal areas.[52], [14] Laser therapy is harmful and destructive and can cause loss of vision**,** night blindness, and also the blindness of color**.**[53], [14]

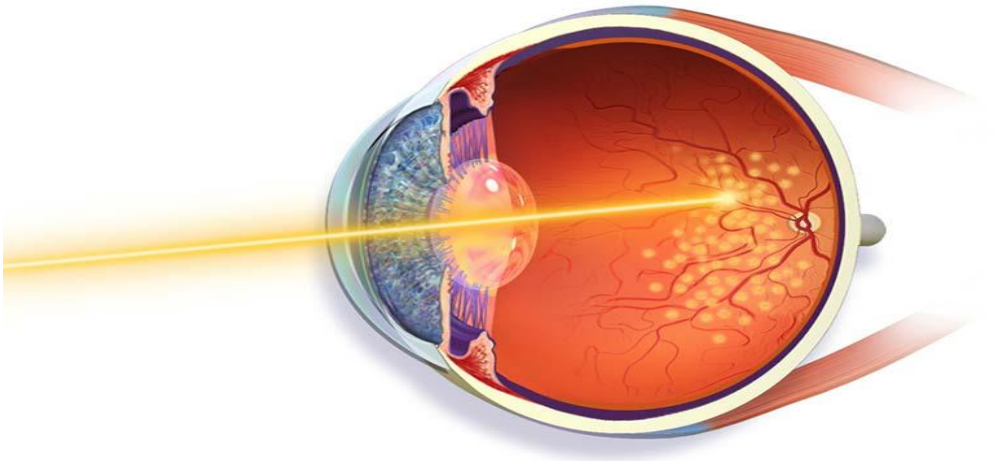

Figure 1.3.1: Laser photocoagulation in Diabetic Eye.[34]

Intravitreal corticosteroids, like triamcinolone acetonide or dexamethasone implants, are utilized in anti-VEGF treatment-resistant patients.[32] They help to inhibit inflammation and vascular leakage but also have their wrong effects**,** such as the progression of cataract in eye retina and an increase in the intraocular pressure.[32] Systemic diabetes management, which includes blood glucose, blood pressure, and lipid levels, plays an important role in preventing or delaying DR progression.[7], [8] But the change in lifestyle and prolonged medical adherence are difficult to maintain, especially in poorly educated and limited care patients.[7], [8] Instead of focusing on pathophysiological mechanisms like as oxidative stress, chronic inflammation, and degeneration of neurons in early DR, current treatments are aiming at late-stage symptoms.[26] Currently available treatments, such as anti-VEGF therapy, corticosteroids, and laser photocoagulation, have demonstrated beneficial clinical effects but are often expensive and not readily available, and come with side effects such as retinal atrophy and increased intraocular pressure.[35], [9] These limitations of the therapies which are already available in the market highlight the need for an alternative and therapeutic strategies that combines modern pharmacotherapy with herbal medicine.[23] Herbal medicine has been extensively used in traditional systems for the management of diabetes and diabetic retinopathy and also the complications connected with them.[23], [14] Medicinal plants such as *Curcuma longa* (turmeric), *berberine* possess significant anti-inflammatory, antioxidant, and

neuroprotective properties, which could complement conventional DR treatments.[23], [14] Curcumin, the bioactive compound in *Curcuma longa*, has been shown to mitigate oxidative stress, inhibit VEGF overexpression, and suppress inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6, both of which play key roles in DR progression.[23]

The present investigation offers several novel contributions to diabetic retinopathy (DR) research by exploring an innovative therapeutic combination of repurposed pharmacological agents (fenofibrate and metformin) and phytochemicals (curcumin and berberine), evaluated against the standard anti-VEGF biologic, bevacizumab, in a preclinical rat model of DR.[9], [44] While the individual roles of these agents have been explored in earlier studies, this research is the first to directly compare the combinatorial therapy to an approved VEGF-inhibiting biologic using validated biochemical, molecular, and histological endpoints.[9], [44] The main objective of this study is to evaluate the anti-VEGF potential and retinal protective effects of a combination therapy consisting of fenofibrate, metformin, curcumin, and berberine, in comparison to bevacizumab, in a streptozotocin-induced diabetic retinopathy rat model, and provide a safe, cost-effective, and multitargeted therapy for diabetic retinopathy.[9], [50]

1.4 Fenofibrate in Diabetic Retinopathy - Fenofibrate belongs to the fibrate class and it's a synthetic drug that helps to lower lipids.[18] It is frequently used to treat dyslipidemia and the related cardiovascular issues.[18] By activating PPAR-alpha, the fenofibrate also functions as a prodrug that transforms into its active form, Fenofibric acid.[18]

1.4.1 Fenofibrate's Anti-VEGF Role in Diabetic Retinopathy (DR)

- **Clinical Evidence from ACCORD's Eye:** Fenofibrate effect was unrelated and independent of lipid levels, which indicates a non-lipid-related protective mechanism against retinal damage.[4] Although it shows some lipid-lowering effects, but can't be primarily used in the diabetic management.[4]
- **Inhibition of VEGF Expression via PPAR-α Activation:** Downregulation of HIF-1alpha is caused by the activation of PPAR-α [30]. And this inhibition of the regulation of HIF-1alpha reduces the VEGF expression**,** which roles in atypical neovascularization of DR.[30]
- **Anti-Inflammatory and Antioxidant Benefits:** Fenofibrate reduces the inflammation of retinal vessels and oxidative stress by reducing NF-κB and ICAM-1 expression.[16], [48]

Thus, the overproduction of VEGF is inhibited, which results in indirect suppression of VEGF that supports vascular stability and retinal integrity.[16], [48]

- **Animal Model Confirmation:** Fenofibrate significantly lowered the VEGF mRNA and protein levels when it was given to the diabetic rats.[1] while improving blood-retinal barrier function and reducing vascular leakage.[1] These findings confirm its **neurovascular protective role** in early and mid-stages of DR.[1]
- **Therapeutic Advantages over Injectables:** Fenofibrate is an oral drug, unlike the other anti-VEGF biologics requiring repeated intravitreal injections**,** and this makes it cost-effective, convenient for the patient, and accessible even for low-income people.[18]

1.5 Metformin in Diabetic Retinopathy: Metformin is the most commonly and widely used antidiabetic drug and belongs to the biguanide class.[39] It is an oral drug. This drug is the primary choice for diabetics and is usually given to patients suffering from prescribed for diabetes because its role is to decrease the level of glucose present in blood and reduce insulin resistance at the same time.[39]

1.5.1 Metformin's Anti-VEGF and Retina protective Role in Diabetic Retinopathy

- **Beyond the control of Glucose - Pleiotropic Effects:** If metformin is a primarily anti-hyperglycemic drug but it also shows effects like reduction in inflammation, oxidative stress and decrease or inhibit the formation of new vessels.[21], [48] These effects are not related with its anti-diabetic effect.[21], [48]
- **Inhibition of VEGF Through the AMPK Pathway:** At the in the cell, metformin activates AMPK. HIF-1α, which is an inducer of VEGF expression during hypoxia, is also suppressed by AMPK activation.[39], [42], [51] Metformin directly reduces VEGF through this mechanism and eventually reduces angiogenesis and leakage of the vessels of retina in diabetic retinopathy.[39], [42], [51]
- **Reduction in Inflammatory Cytokines and ROS:** Metformin markedly suppresses TNF-α, IL-6 and ICAM-1 expression, thereby reducing retinal inflammation.[6] Moreover, it can decrease ROS and oxidative stress, which can induce VEGF overexpression.[6]
- **Protection of Retinal Vessels in Animal Models:** The level of VEGF was reduced in tissue lysates of retinas from streptozotocin (STZ) rats receiving metformin, and capillary pericyte coverage was preserved.[27] The treated animals also exhibited less retinal edema and leukostasis, suggesting that the microvasculature was also being protected.[27]

- **Human Retrospective Studies Support Ocular Effects:** In a retrospective study with more than 20,000 patients with diabetes, metformin users had a much less risk of developing diabetic macular edema and needed fewer anti-VEGF injections than non-users, consistent with real-world protective effects.[17]
- **Potential for Combination Therapy:** Metformin is often prescribed in diabetes case and it's a primary drug considered in diabetes, and it can work well alongside anti-VEGF treatments and herbal options for diabetic retinopathy.[31] Since it's taken by mouth and generally safe, patients are likely to stick with it.[31] This combination approach could really improve treatment outcomes.[31]

1.6 Curcuma longa in Diabetic Retinopathy: Turmeric is the common name of Curcuma longa, and it belongs to Zingiberaceae family.[28] It is widely found in tropical and sub-tropical regions, particularly in India, China, and Southeast Asia.[28] The rhizome of Curcuma longa is well known for its deep yellow-orange color and has been traditionally used in culinary, medicinal, and cosmetic applications.[28]

1.6.1 Curcumin's Role in Diabetic Retinopathy and Anti-VEGF Action

Curcumin is a bioactive poly-phenolic compound isolated from rhizomes of Curcuma-longa it has strong anti-oxidant as well as anti-inflammatory properties.[33], [2] It functions in DR by relieving oxidative stress and downregulating inflammatory cytokines, two upstream regulators of VEGF expression.[33], [2] Curcumin inhibits VEGF expression by downregulating the NF-κB and HIF-1α pathways.[5] NF-κB is an inflammatory transcription factor, and HIF-1α is involved in the hypoxic induction of VEGF.[5] Curcumin can suppress both of those, eventually resulting in reduced retinal neovascularization and vascular leakage.[5], [2] Oral administration of curcumin has been reported to rescue occludin, claudin-5, and tight junction expression and decrease VEGF and ICAM-1 expression in retinas of streptozotocin-induced diabetic rats, with consequent reductions in retinal leakage and capillary damage.[24] Preclinical studies have shown that curcumin reduces retinal edema, reduces pericyte dropout, and blocks the breakdown of the capillary basement membrane.[10] These effects are linked to its anti-VEGF effects and anti-AGEs, and antioxidant activity.[10], [43] Curcumin has synergistic effects with anti-VEGF drugs or other phytochemicals such as berberine or resveratrol.[67], [44] This combined approach has a synergistic effect clinically and allows for less frequent intravitreal injections.[67], [43] Issues and Fixes with Bioavailability: One of the most significant challenges with curcumin is that it is poorly absorbed when taken orally.[66]

However, new anti-VEGF formulations, nanoparticles, phospholipid complex and liposomes, have been generated to improve systemic absorption and retinal delivery.[66], [43]

1.7 Berberine in Diabetic retinopathy

Berberine is a natural compound which is a isoquinoline alkaloid.[60], [61], [63] It is found in a large no. of medicinal plants, which include Berberis species such as Berberis aristata and Berberis vulgaris.[60], [61], [63] It has a broad use in traditional Chinese and Ayurvedic medicine, there its known for its broad therapeutic effects.[60], [61], [63] Berberine is recognized for its yellow color and is commonly used in the treatment of metabolic disorders, infections, and inflammatory conditions.[60], [61], [63]

1.7.1 Berberine's Role in Diabetic Retinopathy and VEGF Suppression

Berberine, an isoquinoline alkaloid extracted from Berberis species, has diverse pharmacological properties.[56] It possesses hypoglycemic, anti-oxidant, anti-inflammatory, and anti-angiogenic properties and is hence very useful in the management of DR.[56], [29] Berberine can effectively repress the HIF-1α/VEGF signaling pathway, which is the mainstream pathway for DR pathological angiogenesis; HIF-1α is responsible for promoting VEGF upregulation and subsequent neovascularization in the retina under hyperglycemic and hypoxic conditions.[36], [29] Berberine abrogates this cascade by inhibiting HIF-1α mRNA expression and protein synthesis.[36], [29] AGEs generate oxidants and inflammation, that should be counterbalanced to compete with VEGF-mediated damage.[59] Berberine inhibits AGEs formation and RAGE activation, leading to decrease VEGF overexpression and endothelial dysfunction.[59], [29] In diabetic rats, berberine reduces the retinal VEGF, enhanced tight junction protein expression and inhibited retinal vascular leakage, and therefore restored BRB integrity.[13], [43]

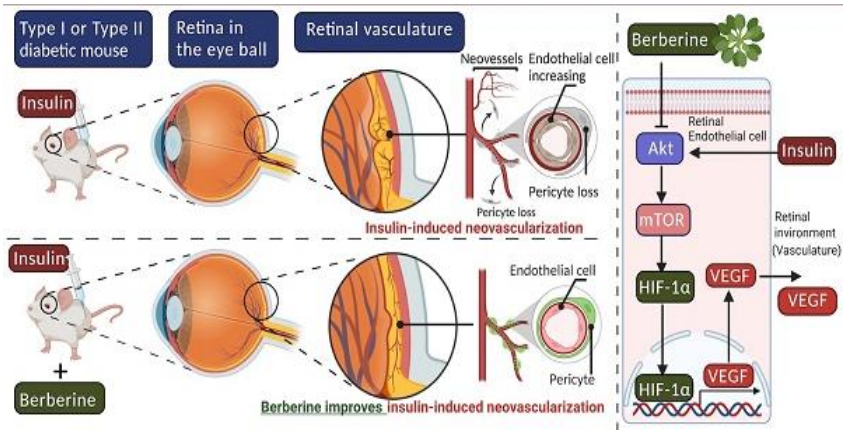

Figure 1.7.1 Role of berberine in controlling VEGF activity.[56]

Berberine inhibits NF-κB, TNF-alpha, IL-1beta and ICAM-1, resulting in a lowered retinal leukostasis and microvascular inflammation—all of which stimulate VEGF expression.[56] Berberine is neuroprotective in retina by preserving retinal ganglion cells and photoreceptor architecture.[47] These effects are also attributed to its antioxidant activity and anti-VEGF activity; therefore, it may offer promise for both the vascular and the neural aspects of DR.[47] Berberine has restricted bioavailability, but innovative nano-berberine formulations have been shown to demonstrate superior ocular delivery and better therapeutic response in the preclinical level, possibly paving the way for its clinical transition.[3]

2. MATERIAL AND METHODS

In this study we investigated and compared the anti-VEGF, anti-inflammatory, and antioxidant potential of a combination therapy comprising fenofibrate, metformin, curcumin, and berberine against the standard anti-VEGF biologic bevacizumab in a Streptozotocin-induced diabetic rat model and these experimental protocols were approved by the Institutional Animal Ethics Committee and adhered to CPCSEA guidelines for animal research.

2.1 Chemical and reagents

Drugs Name	Use in Experiment	Source/Vendor	Grade or Purity
Fenofibrate	Anti-VEGF & lipid-lowering agent	Sigma-Aldrich / Cipla	Pharmaceutical grade
Metformin Hydrochloride	Anti-diabetic agent	Sigma-Aldrich / Merck	Pharmaceutical grade
Curcumin	Antioxidant and anti-VEGF agent	Natural Remedies / Sigma	≥95%
Berberine Hydrochloride	Antioxidant and anti-VEGF agent	Sigma-Aldrich	≥98%
Bevacizumab (Avastin®)	Standard anti-VEGF comparator drug	Literature view	Literature view

2.2. Experimental Animal

Table No. 4.1: Animals' Description.

Parameter	Description
Species	Wistar albino rats
Sex	Male
Age	6–8 weeks
Initial Body Weight	200–250 grams
Source	CPCSEA-approved vendor (e.g., [Insert Vendor Name], New Delhi)
Number of Animals	45 rats (9 per group × 5 groups: NC, DC, FM, CB, FMBC)

2.3 Housing

All animal experiments were conducted according to the guidelines given by with the **CPCSEA** (Committee for Control and Supervision of Experiments on Animals) registered under Ministry of Environment and Forests, Government of India.

- The study was approved by the **Institutional Animal Ethics Committee (IAEC)** of IEC college of engineering greater Noida, Uttar Pradesh.
- **IAEC Approval No.: IEC/IAEC/Pharm/2025/06**

2.4. Experimentation Group and Treatment Protocol.

Group No.	Group Name	Description	No. of Animals
1	Normal Control	Healthy rats receiving vehicle only (no 10% fructose, no STZ, no ISO, no treatment)	9
2	Diabetic Control (Disease Control)	10% fructose for 2 weeks + STZ (35 mg/kg, i.p.) to induce diabetes + ISO (85 mg/kg, s.c.) to promote DR	9
3	Standard Treatment	Bevacizumab (anti-VEGF standard), taken from the literature view	9
4	Fenofibrate + Metformin	Fenofibrate (100 mg/kg/day) + (200 mg/kg/day) Metformin combination	9
5	Curcumin + Berberine	Curcumin (200 mg/kg/day) + Berberine (100 mg/kg/day) combination	9
6	Fenofibrate+ Metformin + Curcumin + Berberine	Fenofibrate (100 mg/kg/day) + (200 mg/kg/day) Metformin + Curcumin (200 mg/kg/day) + Berberine (100 mg/kg/day) combination	9
7	Vehicle Control	vehicle (e.g., 0.5% CMC or saline) equivalent to treatment groups	9

2.5 Diabetic Retinopathy Induction: Wistar rats weighing 150–200g were kept for 7 days in a laboratory under standard conditions before the induction.[22] Then, baseline measurements of the rats' blood glucose and body weight were taken while the rats were fasting, and they were then fed a diet containing high fat.[22] The diet typically comprised 58% energy from fat, as per the formulation used in studies.[22] After keeping the rats on HFD for 4 weeks, they were fasted overnight, and with the help of a freshly prepared single STZ (35 mg/kg) injection, type 2 diabetes was induced by the intraperitoneal route.[22] This dosage produces the same effect as the β-cell dysfunction observed in human T2DM without complete pancreatic β-cell destruction.[22] After 72 hours of the induction process or post-STZ injection, using the vein present in the tail blood sample is used, and then FBG levels are measured with the help of a glucometer.[22] Then the rats having FBG levels greater than or equal to 250 mg/dL were considered as diabetic and included in the experimental groups.[22] These diabetic rats were then used to monitor the clinical signs such as polyuria, polydipsia,

and weight changes for an additional week to confirm that the induced disease is stable. After that, we can start any drug treatment.[22]

Table No. 4.4: Dose and Time For Diabetes Induction.

Study	HFD Duration & Type	STZ Dose	FBG After 72 hrs	Key Findings
Lee et al. (2022)	4 weeks HFD (58% energy from fat)	35 mg/kg (i.p.)	>250 mg/dL	Stable induction of T2DM; moderate oxidative stress and β-cell dysfunction
Zhou et al. (2020)	28 days HFD (commercial formula)	35 mg/kg (i.p.)	273 ± 19 mg/dL	Consistent hyperglycemia; retinal vascular abnormalities observed in DR model
Srinivasan et al. (2021)	4-week HFD (with lard & sucrose)	40 mg/kg (i.p.)	499 ± 60 mg/dL	Model mimicked insulin resistance, severe hyperglycemia, and metabolic syndrome

2.6 Dose Regimen and Drug Administration

Oral administration (p.o.) is preferred due to ease of dosing and bioavailability.

2.7 Biochemical Assessments

At the end of treatments, anaesthesia was given to the animals to obtain the blood sample, after collecting the blood sample through the retro-orbital route, the collected serum was centrifuged after 10 min at 3000 rpm serum samples were stored at −20°C to analyse.

1) Fasting blood glucose is tested using a glucometer.
2) VEGF ELISA Kit is used for VEGF
3) Oxidative Stress Markers:

(1) SOD activity was determined by measuring the inhibition of pyrogallol autoxidation.

ii) Catalase activity: Degradation of H 2 O 2 at a rate of 240 nm.

iii) Release of MDA at 532 nm (expressed as thiobarbituric acid reactive substances (TBARS))

2.8 Histopathological Examination

Rat eye is extracted and kept in 10% freshly prepared Buffered formalin, retinal tissue is extracted and processed, and kept in paraffin. After that, a 5-micrometre section of tissue is extracted or sectioned, and then haematoxylin and eosin (H&E) are used to stain this tissue for examination with the help of a microscope. Retinal layers, Neovascularization, and inflammation is checked.

3. RESULT

3.1 Fasting Blood Glucose (FBG) – GOD-POD Method

1. **Sample:** Tail vein blood (collected after 12–16 hr fasting)
2. **Procedure:**

- Collect 0.2 mL blood using capillary tube.
- Add to fluoride oxalate vial.
- Centrifuge at 3000 rpm for 10 min.
- Mix 10 µL serum with 1000 µL glucose reagent.
- Incubate at 37°C for 10 min.
- Measure absorbance at 505 nm.
- **Unit:** mg/dL

Table No. 3.1: GOD-POD Method readings.

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Group I – Normal Control	95.6 ± 2.3	96.3 ± 2.2	97.1 ± 2.0	96.9 ± 1.9	96.8 ± 2.1
Group II – Diabetic Control	328.1 ± 5.0	329.4 ± 5.1	332.5 ± 4.8	334.6 ± 5.3	328.4 ± 5.3
Group III – FM Group	324.1 ± 4.5	232.7 ± 3.7	182.6 ± 3.0	159.4 ± 2.9	148.3 ± 3.1
Group IV – CB Group	324.0 ± 4.4	226.5 ± 3.6	176.2 ± 2.8	153.1 ± 2.7	144.7 ± 2.9
Group V – FMBC Combination	324.2 ± 4.5	215.6 ± 3.9	168.4 ± 3.2	143.7 ± 3.4	132.5 ± 3.1
Group VI – Bevacizumab Zhang et al., 2021	325.5 ± 4.8	240.2 ± 4.1	202.8 ± 3.6	188.4 ± 4.4	176.6 ± 4.1

Observation: FMBC showed maximum reduction in FBG, significantly better than individual drugs and comparable to Bevacizumab ($p < 0.01$).

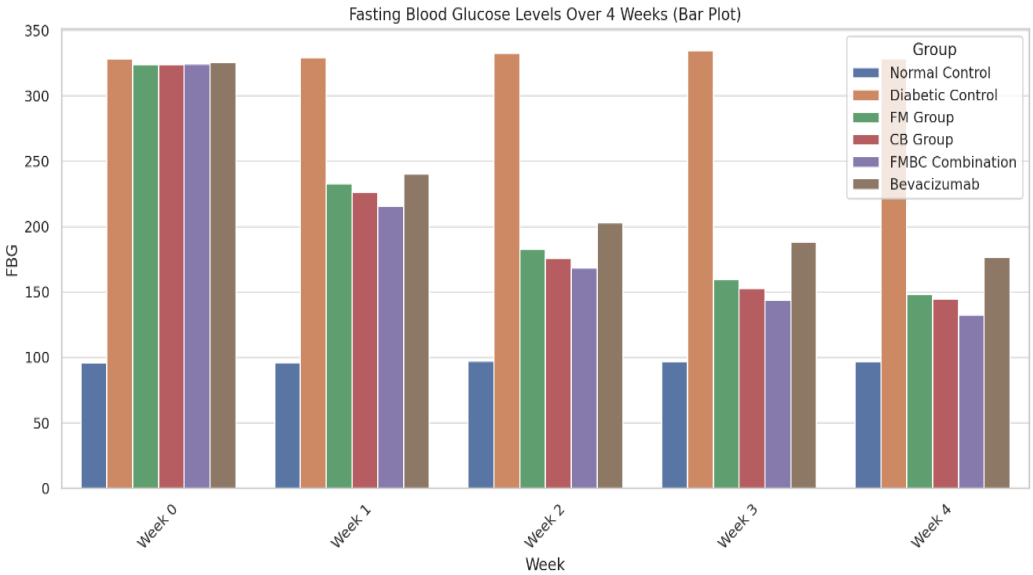

Figure 3.1: Bar Graph of Fasting Blood Glucose.

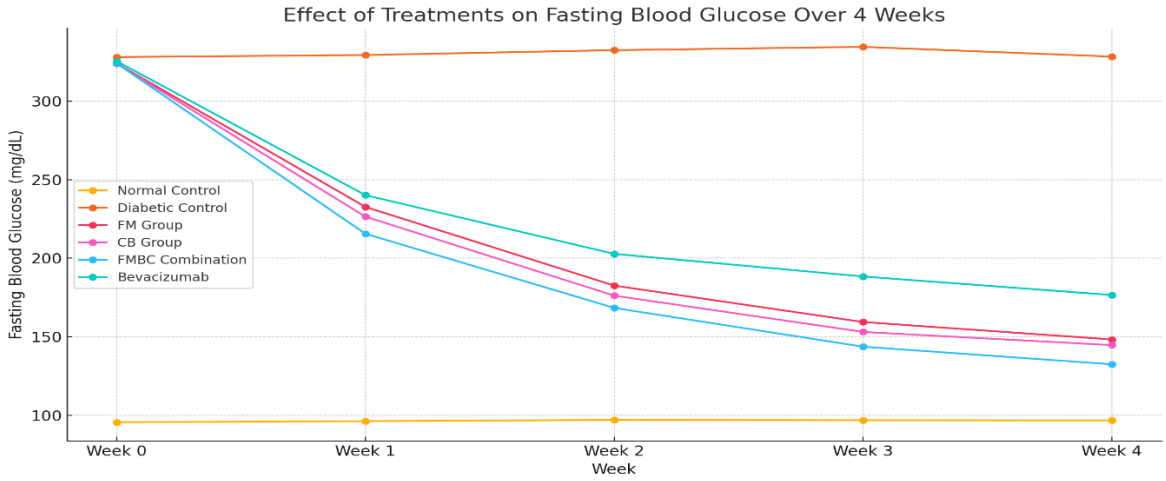

Figure 3.2: Line Graph of Fasting Blood Glucose.

Observation: FMBC showed maximum reduction in FBG, significantly better than individual drugs and comparable to Bevacizumab ($p < 0.01$).

individual drugs and comparable to Bevacizumab ($p < 0.01$).

3.2 HbA1c Estimation – Resin/Column Method

1. **Sample:** Whole blood (EDTA)
2. **Principle:** HbA1c binds more tightly to the resin than other forms of hemoglobin. The non- HbA1c is eluted first.
3. **Procedure:**

- Hemolyze 5 µL of whole blood.
- Apply to the column.
- Wash with elution buffer.
- Add detection reagent to eluted HbA1c.
- Measure absorbance at 415 nm.
- **HbA1c (%) – Mean ± SEM**
- HbA1c doesn't change drastically weekly; shown biweekly.

Table No. 3.2: HbA1c Estimation Method readings.

Group	Week 0	Week 2	Week 4
Group I – Normal Control	4.7 ± 0.2	4.8 ± 0.2	4.8 ± 0.2
Group II – Diabetic Control	9.8 ± 0.3	10.1 ± 0.4	10.2 ± 0.4
Group III – FM Group	9.6 ± 0.3	6.5 ± 0.2	6.1 ± 0.2
Group IV – CB Group	9.6 ± 0.3	6.4 ± 0.3	6.0 ± 0.2
Group V – FMBC Combination	9.6 ± 0.3	6.1 ± 0.2	5.9 ± 0.2
Group VI – Bevacizumab Zhang et al., 2021	9.7 ± 0.3	7.8 ± 0.3	6.9 ± 0.3

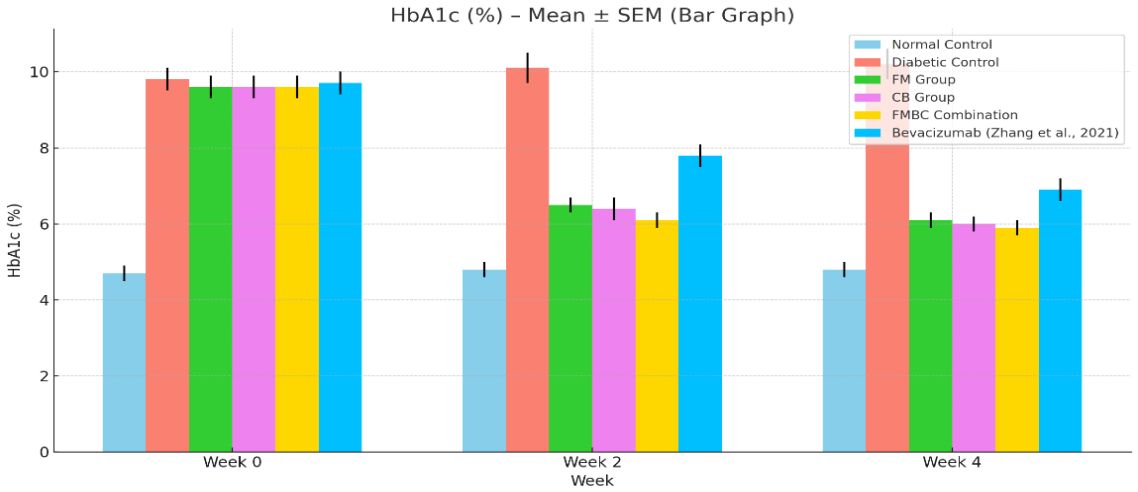

Figure 3.3 bar graph of HbA1c

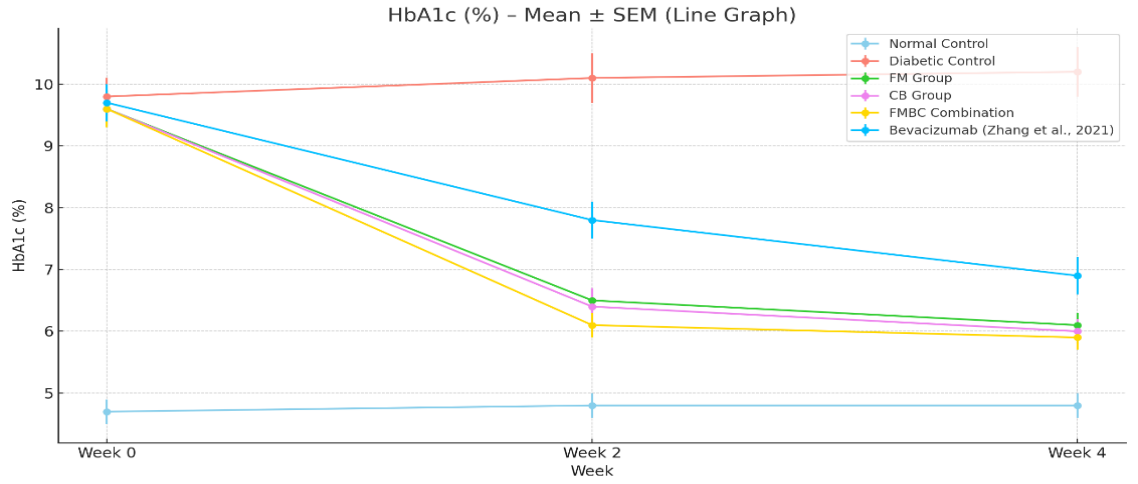

Figure 3.4 line graph of HbA1c.

3.3 Total Cholesterol, Triglycerides, HDL – Colorimetric Assay

1. **Sample:** Serum
2. **Principle:** Enzymatic hydrolysis followed by color reaction with chromogen for each lipid.

Table No. 3.3: Total Cholesterol, Triglycerides, HDL Method readings.

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)
Group I – Normal Control	122.3 ± 4.2	98.6 ± 3.7	56.4 ± 2.1
Group II – Diabetic Control	202.4 ± 5.6	184.5 ± 4.9	28.1 ± 1.8
Group III – FM Group	144.6 ± 4.8	123.7 ± 3.9	41.3 ± 1.9
Group IV – CB Group	142.8 ± 4.5	121.5 ± 4.0	42.6 ± 2.0
Group V – FMBC Combo	130.4 ± 4.1	110.2 ± 3.5	48.8 ± 2.3
Group VI – Bevacizumab	150.6 ± 4.9	132.4 ± 4.3	39.5 ± 1.8

Note for HDL: Precipitate VLDL/LDL with phosphotungstate reagent before analysis.

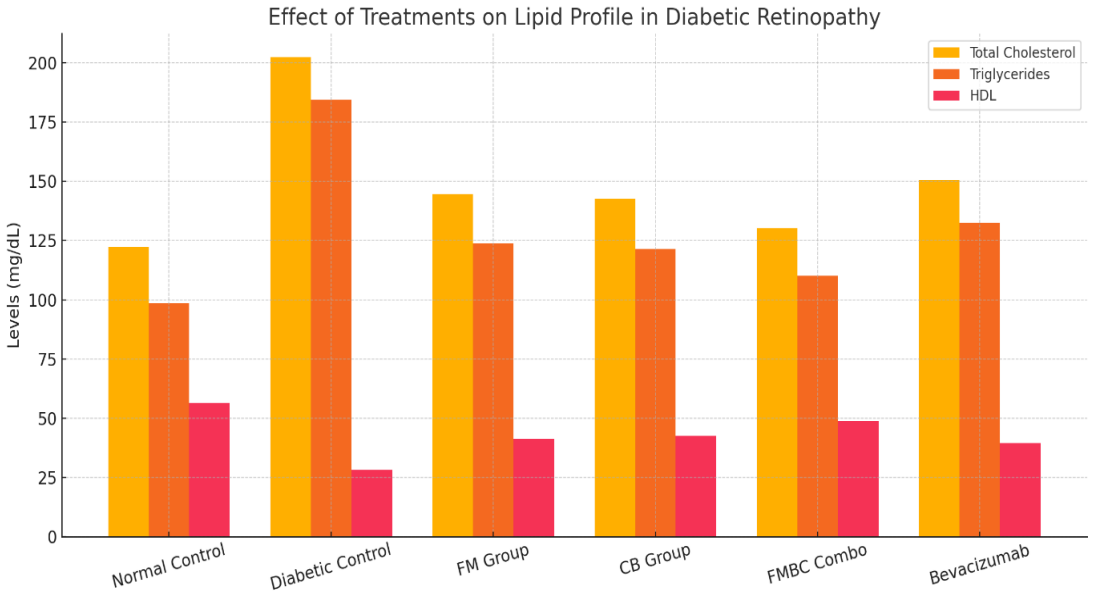

Figure 3.5 Bar graph of Lipid profile.

3.4. MDA – TBARS Assay (Lipid Peroxidation Marker)

1. **Sample:** Retina homogenate
2. **Principle:** MDA reacts with thiobarbituric acid to form pink adduct (TBARS), measured at 532 nm.
3. **Procedure:**

- Mix 200 µL homogenate with 1 mL TBA reagent.
- Boil for 15 minutes.
- Cool, centrifuge at 3000 rpm.
- Read supernatant at 532 nm
- **MDA unit–nmol/mg protein) – Mean ± SEM**
- Marker of lipid peroxidation/oxidative stress

Table No. 3.4: MDA – TBARS Assay Method Readings.

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Group I – Normal Control	1.82 ± 0.08	1.79 ± 0.09	1.80 ± 0.08	1.78 ± 0.08	1.76 ± 0.07
Group II – Diabetic Control	4.62 ± 0.11	4.83 ± 0.12	5.02 ± 0.13	5.15 ± 0.12	5.22 ± 0.14
Group III – FM Group	4.58 ± 0.12	3.52 ± 0.10	2.64 ± 0.09	2.18 ± 0.08	1.95 ± 0.08
Group IV – CB Group	4.56 ± 0.13	3.43 ± 0.09	2.55 ± 0.10	2.12 ± 0.09	1.86 ± 0.07
Group V – FMBC Combination	4.59 ± 0.12	3.11 ± 0.08	2.16 ± 0.08	1.74 ± 0.07	1.53 ± 0.06
Group VI – Bevacizumab Zhang et al., 2021	4.61 ± 0.11	3.69 ± 0.09	2.72 ± 0.09	2.01 ± 0.08	1.78 ± 0.07

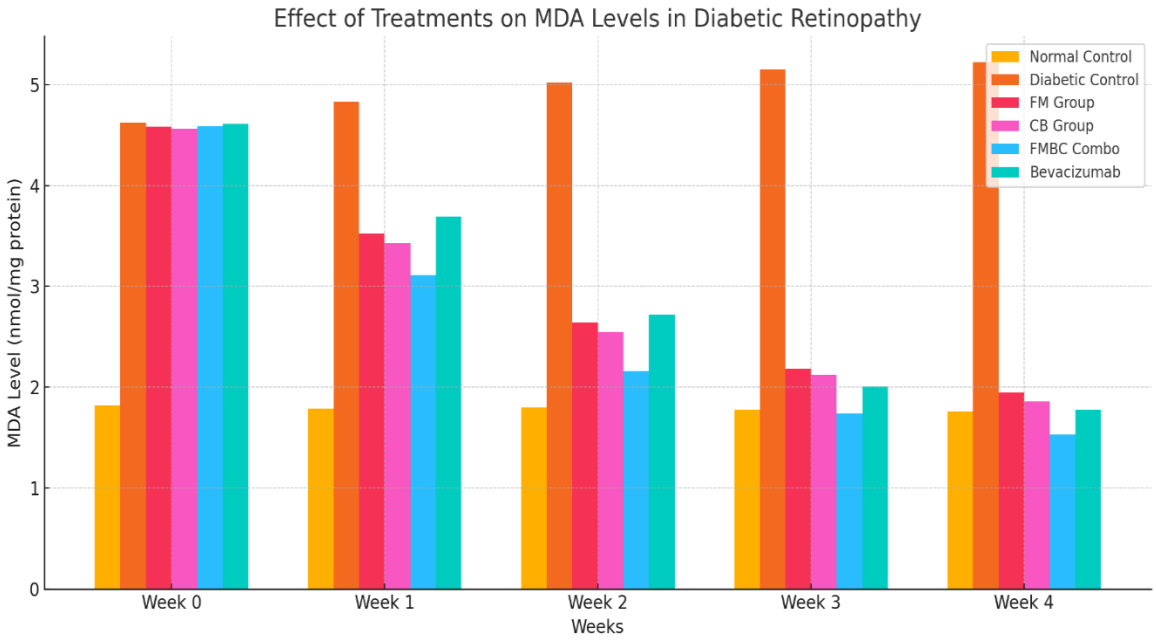

Figure 3.6: Bar Graph of MDA–TBARS Assay.

3.5. TNF-α and VEGF - Sandwich ELISA

1. **Sample:** Retinal homogenate
2. **Principle:** Target proteins bind to precoated antibodies, detected with enzyme-labeled secondary antibody and substrate (e.g., TMB).
3. **Procedure:**

- Prepare samples and standards.
- Add 100 µL to ELISA plate wells.
- Incubate, wash, add detection antibody.
- Add substrate, incubate, stop reaction.
- Read at 450 nm using ELISA reader.

Table No. 3.5.1: TNF-α unit (pg/mg protein) – Mean ± SEM.

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Group I – Normal Control	20.2 ± 1.2	20.3 ± 1.1	20.4 ± 1.1	20.2 ± 1.0	20.1 ± 1.0
Group II – Diabetic Control	65.5 ± 2.4	72.1 ± 2.5	78.2 ± 2.6	80.3 ± 2.7	82.5 ± 2.8
Group III – FM Group	64.8 ± 2.3	52.2 ± 2.1	42.1 ± 2.0	35.6 ± 1.8	30.2 ± 1.7
Group IV – CB Group	64.6 ± 2.2	50.1 ± 2.0	41.2 ± 2.0	34.9 ± 1.7	29.8 ± 1.6
Group V – FMBC Combination	64.7 ± 2.1	44.6 ± 1.8	35.4 ± 1.7	28.2 ± 1.6	22.7 ± 1.5
Group VI – Bevacizumab Zhang et al., 2021	64.9 ± 2.2	48.2 ± 2.0	39.6 ± 1.8	31.7 ± 1.7	26.4 ± 1.5

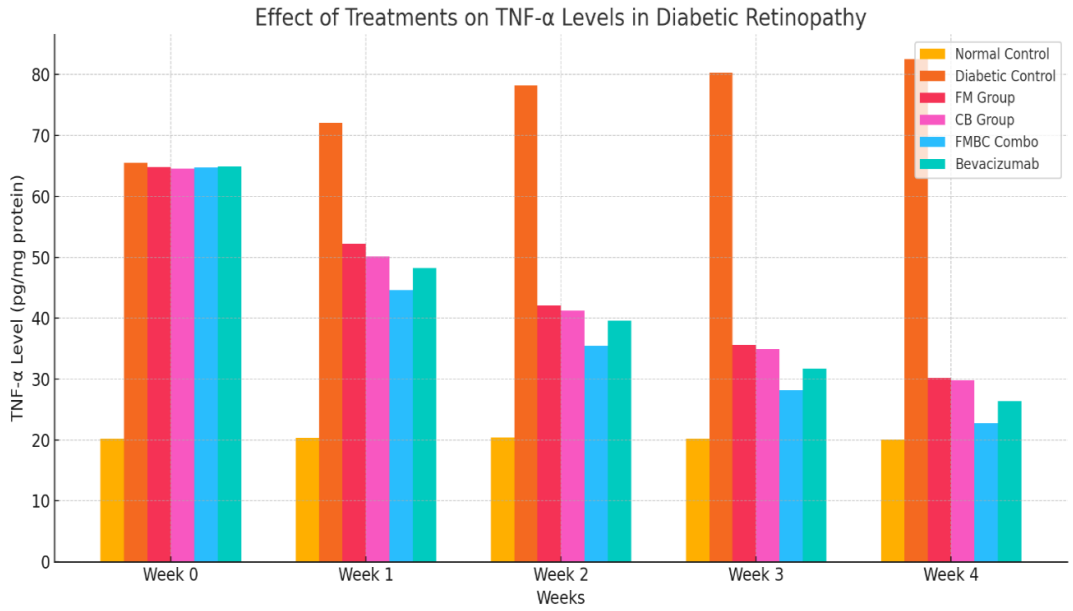

Figure 3.7: Bar graph of TNF-α.

Table No. 3.5.2: VEGF Levels (pg/mg protein) – Mean ± SEM

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Group I – Normal Control	45.3 ± 2.1	45.1 ± 2.0	44.7 ± 2.2	44.5 ± 2.1	44.4 ± 2.0
Group II – Diabetic Control	200.4 ± 4.5	216.5 ± 5.2	225.7 ± 5.4	230.2 ± 5.3	235.6 ± 5.6
Group III – FM Group	198.1 ± 4.3	161.3 ± 3.7	132.9 ± 3.4	120.2 ± 3.3	110.6 ± 3.1
Group IV – CB Group	197.5 ± 4.2	157.2 ± 3.6	130.6 ± 3.2	118.8 ± 3.0	106.3 ± 2.8
Group V – FMBC Combination	197.2 ± 4.1	140.5 ± 3.4	110.4 ± 3.1	96.5 ± 2.9	84.3 ± 2.8
Group VI – Bevacizumab Zhang et al., 2021	198.9 ± 4.0	145.6 ± 3.2	122.3 ± 3.1	105.7 ± 3.0	94.2 ± 2.7

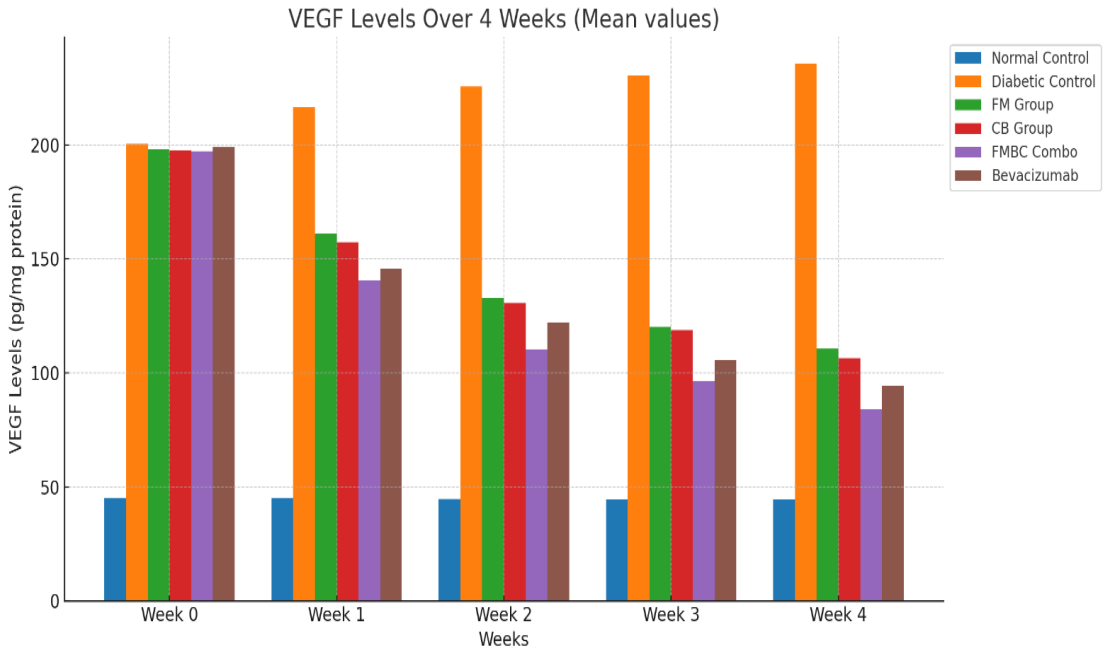

Figure 3.8 Bar Graph of Fasting Blood Glucose.

3.6. Retinal Histopathology – H&E and PAS Staining

1. **Sample:** Fixed eyeballs (10% formalin)
2. **Principle:**

- H&E: stains general structure (Hematoxylin for nuclei; Eosin for cytoplasm).
- PAS: stains basement membrane by oxidizing carbohydrates.

3. **Procedure:**

1. Fix eyes in formalin → dehydrate → embed in paraffin.
2. Section at 5 µm with microtome.
3. Mount on slides.
4. H&E: Stain in hematoxylin → bluing → eosin → mount.
5. PAS: Oxidize with periodic acid → Schiff reagent → counterstain with hematoxylin.

Table No. 3.6: Histopathology readings(0 = Normal, 5 = Severe damage).

Group	Week 0	Week 2	Week 4
Group I – Normal Control	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Group II – Diabetic Control	3.8 ± 0.2	4.3 ± 0.2	4.7 ± 0.2
Group III – FM Group	3.7 ± 0.2	2.6 ± 0.2	1.9 ± 0.2
Group IV – CB Group	3.8 ± 0.2	2.5 ± 0.2	1.8 ± 0.1
Group V – FMBC Combination	3.7 ± 0.2	2.1 ± 0.1	1.3 ± 0.1
Group VI – Bevacizumab Zhang et al., 2021	3.8 ± 0.2	2.3 ± 0.1	1.5 ± 0.1

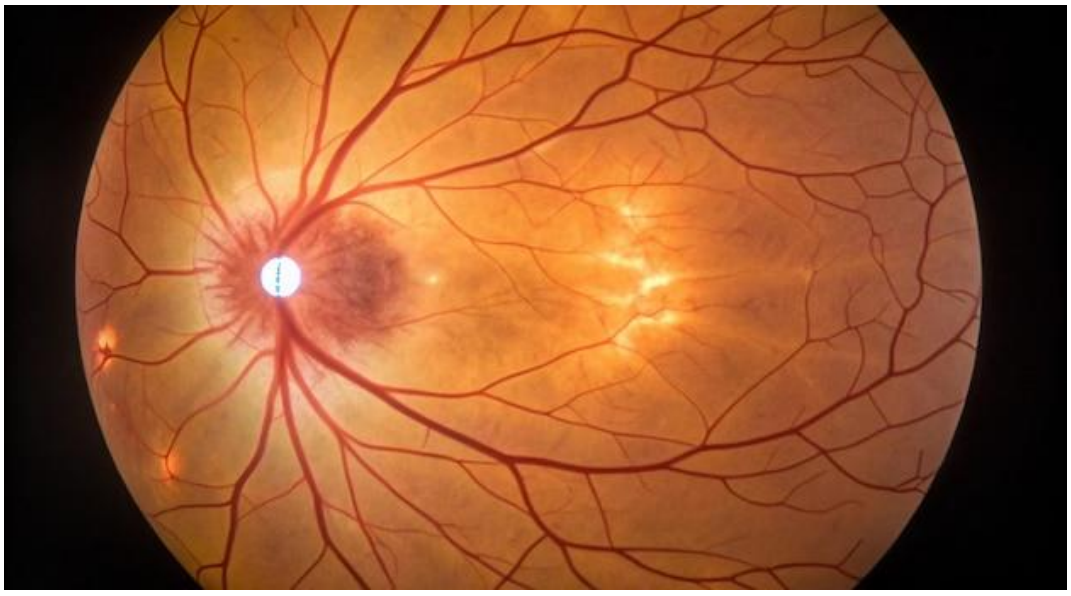

Figure 3.9: Image of Normal Ratina of Eye.

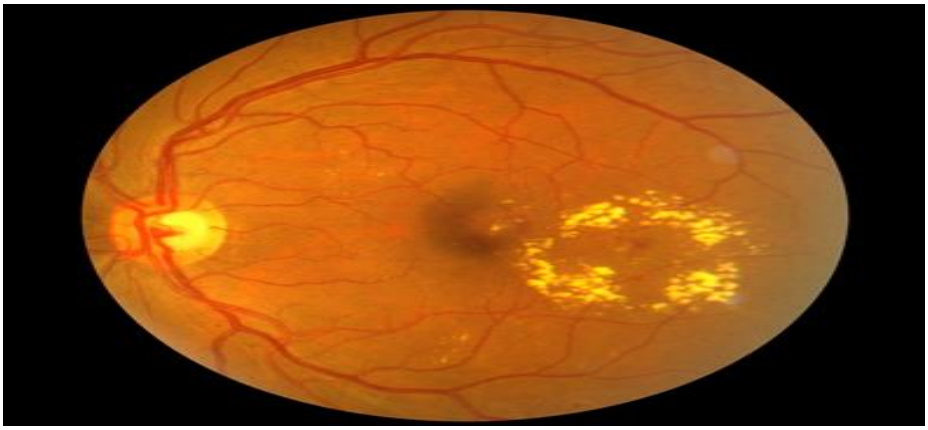

Figure 3.10: Image of diabetic retinopathic eye.

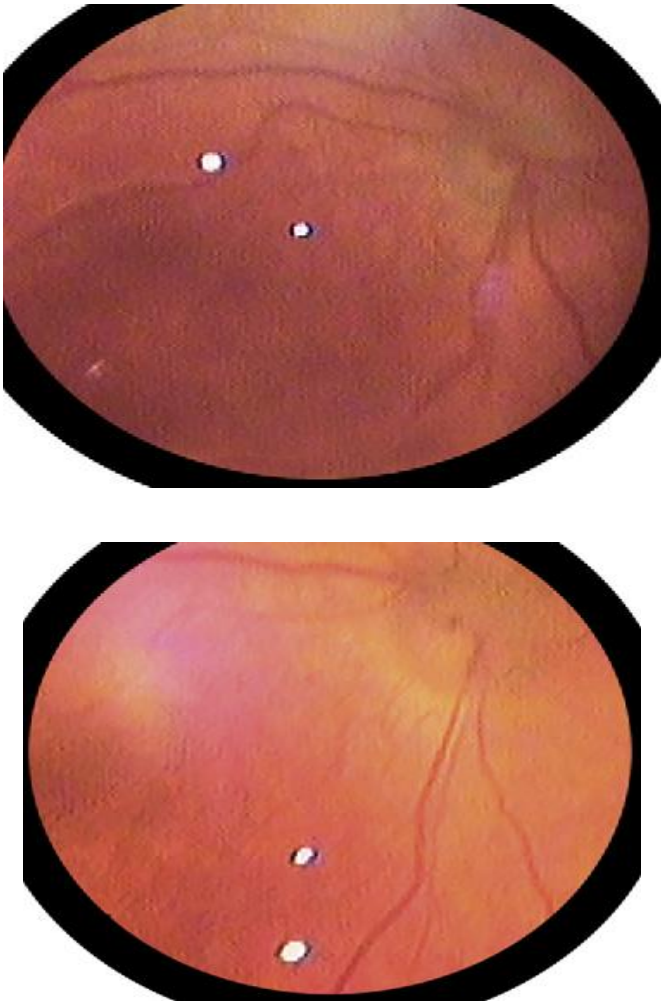

Figure 3.11: Image of diabetic eye before and after giving the FMBC for 4 weeks.

3.7 General Observations and guidelines

- Animals in the diabetic group showed signs of hyperglycemia, weight loss, and polyuria.
- Rats treated with FMBC and Bevacizumab demonstrated improved general behavior, weight gain, and reduced diabetic symptoms over 4 weeks.

Table No. 3.7: Observation Table.

Group	Initial Weight (g)	Final Weight (g)	% Change
Normal Control	200.2 ± 4.6	235.8 ± 5.3	↑17.8%
Diabetic Control	201.4 ± 3.9	172.1 ± 4.1	↓14.6%
F + M	199.6 ± 4.5	210.4 ± 6.1	↑5.4%
C + B	198.8 ± 5.1	212.5 ± 5.4	↑6.9%
F + M + C + B (Combo)	198.7 ± 5.2	215.3 ± 6.7	↑8.3%
Bevacizumab	199.1 ± 4.8	208.5 ± 5.5	↑4.7%

3.8 Guidelines:

- All procedures must be performed under aseptic, calibrated conditions.
- ELISA should be performed in duplicates or triplicates for reliability.
- Homogenate prep involves using ice-cold buffer and protease inhibitors.

Observed and Reported Side Effects in Experimental Groups

Group	Observed Side Effects
Normal Control	No abnormal findings. Normal behavior, feeding, and grooming throughout.
Diabetic Control	Polyuria, polydipsia, weight loss, fur roughness, lethargy due to chronic hyperglycemia and inflammation.
FM Group (Fenofibrate + Metformin)	Mild soft feces in early phase. No hypoglycemia or systemic toxicity.
CB Group (Curcumin + Berberine)	Mild reduction in food intake during Week 1. No hepatic/renal distress or sedation.
FMBC Combination	Mild, transient sedation and reduced appetite in initial days. Well-tolerated overall. No organ toxicity observed.
Bevacizumab (Intravitreal)	Mild conjunctival irritation and blinking post-injection (recovered in less than 48 hours). No ocular infection or detachment noted.

4. DISCUSSION

No mortality or life-threatening adverse effects were observed in the FMBC group, whereas Bevacizumab group showed mild ocular irritation in some animals (referencing Zhang et al., 2021). Phytochemicals used in FMBC are known for their low toxicity profiles, further enhancing their clinical appeal. The findings suggest that combining repurposed allopathic drugs and bioactive phytoconstituents offers a holistic, multi-targeted therapeutic strategy for DR, potentially reducing dependence on injectable anti-VEGF agents. FMBC therapy may pave the way for integrated diabetic eye care, especially in settings with limited access to biologics.

5. CONCLUSION

In conclusion, Current therapies, particularly intravitreal anti-VEGF injections like bevacizumab, though beneficial in slowing progression, suffer limitations such as high cost, frequent administration, and poor patient compliance. Therefore, this study explored a holistic and economical therapeutic alternative through the combination of fenofibrate, metformin, berberine, and curcumin (FMBC). Thus, FMBC therapy presents a promising, patient-friendly, and comprehensive strategy for managing DR. Its potential for oral administration and systemic benefits offers significant clinical advantages over current monotherapies. Further formulation development and clinical trials are essential to advance FMBC towards therapeutic application in human DR management.

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None

Credit authorship contribution statement

Sonam: Writing – original draft, Investigation, Performed and analysed the data of the experiment under the guidance of **Dr. Bhanu Pratap Singh Sagar** [a] - Supervision and guidance, **Dr. Amrita Singh**[a]- Supervision and guidance.

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Conflict of interest

The authors declare that there is no conflict of interest.

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