

Diabetic Retinopathy: Role of Traditional Medicinal Plants in its management and their molecular mechanism.

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Abstract: The objective of this review is Diabetic Retinopathy (DR) and Role of herbal medicines for the treatment of DR. Eye is unique structure of the body and its anatomical and physiological framework is said to be unique. Every organism has adaptive capacity to lead life on earth. Due to modern life style, the number of diseases increasing day by day. Diabetic Retinopathy is an ocular manifestation of the systemic disease and sight-threatening disease. The treatment of modern system of medicine, focal laser therapy, anti-vascular growth factor drugs. These treatment modalities have side effects. Various medicinal plants have been studied and shown to be effective in the management of DR. based on a various biomarkers present in them.

Keywords: Diabetic Retinopathy, Poloyl Pathway, Protein Kinase C- β , Vascular endothelial growth factor. Tumor Necrosis factor.

I. Introduction

Neurodegeneration leads to various disorders of central nervous system. It is also associated with the retinal disease like Glaucoma and Retinitis Pigmentosa. It is more prominently expressed in the case of microvascular neurodegeneration of eye in diabetes known as Diabetic Retinopathy, the legal cause of blindness in working age adults all over the world. ^{1,2} According to the report of world health organisation Diabetic Retinopathy cause 5% blindness globally ³ It is estimated that Diabetes mellitus affects the 4% population of the world out of which half of the population lives with Diabetic Retinopathy. Nearly all the patients with type I and 75% patients with type II develops Diabetic Retinopathy(DR) after a duration of 15 years. ⁴ Diabetic Retinopathy considered to be result of vascular changes in the retinal circulation. In the early stage, vascular occlusion and dilation occur which changed to Proliferative Retinopathy with the development of new blood vessels. The incidence of diabetes gradually increases. It is suggested that increase in the eye complications if not properly managed may lead to severe eye damage. ⁵ Risk factors for Diabetic Retinopathy comprise duration of diabetes, level of glycaemia, presence of high blood pressure, dependence on insulin, pregnancy, levels of selected serum lipids, nutritional and genetic factors. Medical interventions can decrease some of the risk of vision loss caused by diabetic retinopathy. ⁶ There are important differences seen over the past decades in the diagnosis, mitigation, cure, prevention and socioeconomic factors influencing the geographic distribution of Diabetic Retinopathy. ⁷ South East Asia and western pacific accounts for more than fifty percent of diabetic patients in world. China, India, Indonesia and Bangladesh alone contribute to global burden of 45%. A global meta-analysis report suggest that 1 in 3 (34.6%) had diabetic Retinopathy in US, Australia, Europe and Asia. It should be noted that 1 in 10 patients with diabetes have vision threatening Diabetic Retinopathy. ⁸

Classification of Diabetic Retinopathy:

Diabetic Retinopathy classified into two stages based on the severity and the progression of the diseases. Non-Proliferative Diabetic Retinopathy and Proliferative Diabetic Retinopathy. Proliferative Diabetic Retinopathy further classified into Mild, Moderate and Severe. Mild Non-Proliferative Diabetic Retinopathy (NPDR) the first clinical signs of DR. One of the earliest clinically detectable sign of diabetic retinopathy include the appearance of small and local lesions of microaneurysms, small retinal haemorrhage and distortions called Intra Retinal Microvascular Abnormalities(IRMA). Moderate NPDR is characterised by extensive Intra Retinal haemorrhage and a poorly perfused retina within which venous vascular wall may distend. Severe NPDR includes blockage of significant no of small blood vessels in the retina. As a result, the retina become deprived of oxygen. Vascular endothelial growth factor (VEGF) and protein kinase C beta (PKC- β) are highly responsible for this condition. ^{9,10,11}

Diabetic Retinopathy	Findings Observable on Dilated Ophthalmoscopy
No apparent DR	No abnormalities
Mild nonproliferative DR	Microaneurysms only
Moderate nonproliferative DR	Microaneurysms and other signs (e.g., dot and blot hemorrhages, hard exudates, cotton wool spots), but less than severe nonproliferative DR
Severe nonproliferative DR	Moderate nonproliferative DR with any of the following: <ul style="list-style-type: none"> • Intraretinal hemorrhages (≥20 in each quadrant); • Definite venous beading (in 2 quadrants); • Intraretinal microvascular abnormalities (in 1 quadrant); • and no signs of proliferative retinopathy
Proliferative DR	Severe nonproliferative DR and 1 or more of the following: <ul style="list-style-type: none"> • Neovascularization • Vitreous/preretinal hemorrhage

Table:1. international classification of Diabetic Retinopathy. (Source ICO Guidelines)

II. Mechanisms of Diabetic retinopathy:

1.Hyperglycaemia:

In last few decades extensive amount of research has been done to uncover the underlying molecular and biochemical mechanism associated with hyperglycaemia induced damage in diabetic retina. Hyperglycaemia a key metabolic abnormality observed in diabetes mellitus. If prevented early in the Progression of disease, it can help in preventing the onset or delaying the progression of microvascular disease. Endothelial cells lining the macrovasculature, including the retinal microcirculation, experience oxidative stress and get activated. This in turn promotes the adhesive interaction between circulatory inflammatory cells along with their activation. Cytokines derived from the activation of these cells augment an uncontrolled synthesis of inflammatory mediators by blood and endothelial cells. The resultant surge of inflammatory mediators into the systemic circulation may lead to flow disturbances and other physical factors towards the development of Diabetic Retinopathy. Inside the cell, high level of glucose may increase the flux through glycolytic pathways, stimulating protein kinase C and activation of the polyol 1-poly(ADP-ribose) polymerase (PARP) and hexosamine pathways. Further it increases the production of ROS, also high glucose level increases the non-enzymatic glycolation leads to high level of advanced glycation end product. The diabetes control and Complication trial demonstrated that the risk for development and progression of DR was significantly decreased in patient receiving intensive insulin therapy. However, the significant result was observed only after 6-7 years. It suggests that control over hyperglycaemia delays the progression of DR.^{12,13}

2.Advanced glycation endproducts: High level of glucose in both type I and type II Diabetes leads to formation of reactive derivatives via non-enzymatic protein glycation which includes non-enzymatic condensation reaction between reducing glucose and amine residues of proteins, lipids and Nucleic acids. These derivatives lead to series of complex reaction to give an irreversible cross linked complex group of compound known as advanced glycation end products (AGEs). It is observed in the blood retinal barrier and endothelial cell injury.¹⁴ Retinal pericytes play an important role in the maintenance of the microvascular homeostasis and promote accumulation of AGEs during diabetes. Increase in the formation of AGEs themselves able to produce free radicals further leads to DR. Their acquisition has been found in the retinal blood vessels, serum and vitreous of diabetic patients and animals.¹⁵

3.Polyol Pathway: Polyol pathway is a metabolic pathway where a part of excess glucose gets metabolised to sorbitol and it is converted to fructose. Aldose reductase is a rate limiting enzyme in this pathway. Glucose and Galactose are substrate to this enzyme and are reduced to galactitol and sorbitol, respectively under diabetic condition. It is a two-step metabolic pathway in which glucose is reduced to sorbitol and further converted to fructose by sorbitol dehydrogenase using NAD⁺ as co factor. The unused glucose enters in polyol pathway where aldose reductase reduces it to sorbitol. This reaction oxidizes NADPH to NADP⁺. Hexokinase can return the molecule to glycolysis pathway by phosphorylating fructose to fructose-6-phosphate. However, in the case of uncontrolled diabetic hyperglycaemia the reaction ultimately favours the production of sorbitol. Excess sorbitol activates the polyol pathway results in the decrease of reduced NADPH and oxidised NAD⁺. These are necessary co-factors in redox throughout the body. Decrease in the level of these co-factors resulted into decrease in the level of glutathione (free radical scavenger) and increased oxidative stress. It is the major factor in retinal damage.¹⁶

4.Oxidative Stress In Diabetic Retina: Diabetes and Hyperglycaemia cause oxidative stress in the Retina and may play a pivotal role in the development of diabetic retinopathy by damaging the retinal cells. The antioxidant defence enzyme activities responsible for scavenging the free radicals and maintaining the redox homeostasis, such as SOD (Superoxidase dismutase), CAT (Catalase), and GSH (Glutathione) are decreased in diabetic retina. Cell is equipped with intracellular antioxidant, GSH probably the most important defence the cell is equipped with. Which act as a reactive oxygen species scavenger and modulate intracellular redox reaction.^{17,18} The level of this intracellular antioxidant(GSH,SOD,CAT) decreased in retina in diabetes. Finally, due to imbalance between pro-oxidant(Vitamin C and E) and ant- oxidant (GSH,SOD,CAT) insufficient neutralization of free radicals occurs and causes oxidative stress. It further leads to oxidation of cellular lipids, proteins and nucleic acids causing further damage.^{14.}

5.Angiogenic Mechanism in Diabetic Retinopathy

i.Vascular endothelial growth factor: A great deal of effort has been focused on VEGF because it is elevated in diabetic retinopathy and causes both proliferation and increases permeability in vascular endothelial cells. Vascular endothelial growth factor is an endothelial cell specific angiogenic and permeability inducing factor that has been implicated in the pathogenesis of diabetic retinopathy.¹⁹ VEGF act as survival factor for newly formed blood vessels in the retina and it also inhibits apoptosis caused by tumor necrosis factor. Inhibition of VEGF function may control the pathogenic neovascularisation such as diabetic retinopathy and tumour growth whereas enhancement of VEGF may stimulate new blood vessel growth in ischemic tissue.²⁰ VEGF is associated with breakdown of blood tissue barrier. Its expressions correlate with the development of new blood vessels. These data suggest that VEGF secretion by neural retina may have a dual role to reduce neuronal apoptosis and to encourage neo vascularisation hypoxia region of the tissues. It was reported in the study that VEGF is expressed prior to Proliferative Retinopathy or Retina without diabetes. Several studies reported the basal level of VEGF is secreted from normal retina and most of the body cells which is needed for the tropic function of Retinal Pigmented epithelium for the maintenance of choreocapillery. Vascular oozing and neovascularisation are only observed in the pathologic state. Additional studies required in this field for presence of VEGF in non-diabetic choroidal retina and in presence of VEGF absence of neovascularisation observed in normal Retina.²¹

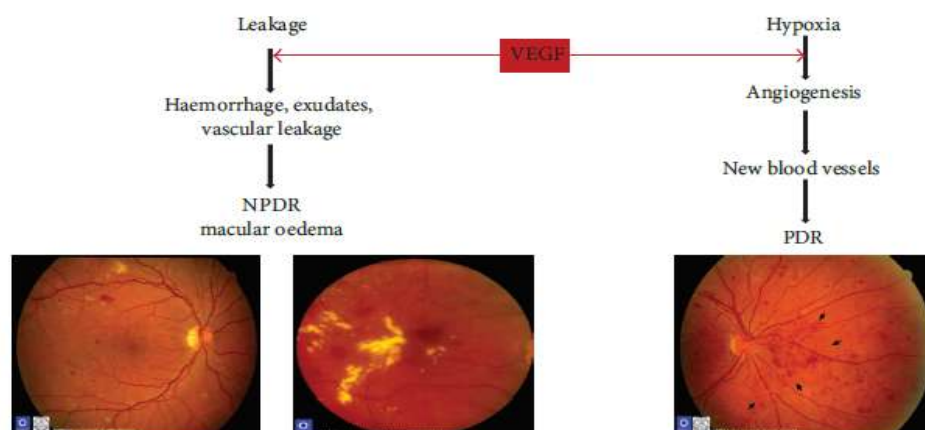


Fig:1. Vascular endothelial growth factor (VEGF) pathways in Non Proliferative Diabetic retinopathy (NPDR) and Proliferative Diabetic Retinopathy (PDR) and carbonic anhydrase (CA).Robinson et al⁹

ii. Protein Kinase C -beta (PKC- β)

The activation of PKC may result in a number of effects on the vasculature that are characteristic of those seen in retinopathy. The enzyme protein kinase C in its beta isoform appears to increase the adverse effect of the hyperglycaemia tissue. There are generally thirteen different available isoforms of PKC- β family these are further classified into classical, novel, atypical forms depending upon the release of the intracellular calcium. The activation of the PKC resulted in the change in vasculatures which leads to Diabetic Retinopathy. These includes the thickening of the basement membrane endothelial permeability and angiogenesis. The activation of PKC also increases in cytokines containing vasoactive factor and transforming growth factor TGF- β and Vascular endothelial growth factor VEGF. In retinopathy, interestingly a large no of PKC inhibitors are available. Rottlerin is a delta isoform inhibitor of PKC with some selectivity however, it also inhibits the enzyme protein kinase A and Calmodulin. A compound Indocarbazole also inhibit the multiple PKC isoform. In addition, preliminary studies suggest that inhibition of PKC may benefit diabetes-induced neuropathy, nephropathy and cardiovascular disease. If proven effective for these indications in ongoing and future clinical

trials, the availability of PKC selective inhibitors such as LY333531 may add a powerful new therapeutic option to present armamentarium against the debilitating retinal disease of diabetes, and perhaps benefit for other complications of diabetes as well.²²



Fig.2. Diabetic induced activation of Protein Kinase C

6. Inflammation In Diabetic Retina:

Diabetic retinopathy is shown to share many similarities with chronic inflammatory disease. Levels of cytokines, including interleukin (IL)-1 β , -6, and -8 are increased in the vitreous fluid of patients with proliferative diabetic retinopathy. While the serum cytokine level remain intact. IL- 8 is a mediator of inflammation produced by various cells including blood monocytes, macrophages and endothelial cells. It is also associated with Sepsis syndrome a chronic condition. The high serum concentrations of Tumour Necrosis Factor alpha(TNF- α) also observed in the Diabetic Retinopathy. IL-6 also present in the variety of fluid in Diabetic Retinopathy. Infiltrating Monocytes as well as Retinal pigment epithelial cells are the sources of IL-6. Moreover, it is also reported that the non-inflammatory cytokines like IL-2, IL-10, IL-4 and Interferon- γ remains absence from Retinal Fluid of PDR. The macrophages obtained from Diabetic Retinopathy Patients Produced more TNF- α (Tumer necrosis factor alpha) compared to normal macrophages. However, the source of TNF- α remain uncertain in non diabetic humans. Identification of sources of TNF- α requires further studies.²³ According to the study of Demircan et al 2006 both the TNF - α and IL- 1 β are highly responsible for retinal angiogenesis activity and apoptosis.²⁴

- (i) **IL-1** \square : Progressive Diabetic retinopathy increases the adhesion of the leucocytes to the endothelial cells. Since diabetes is distinguished by systematic endothelial dysfunction with immune inflammatory events, it can be resulted that leucocytes and lymphocytes may be responsible for the complications associated with diabetes.²⁵ In hyperglycaemic condition IL-1 β speed up the natural cell death of the retinal capillaries via activation of NF-kappa B . IL-1 β actuate the expression of certain genes and the promoters of the genes are regulated by the NF-kappa B. It is reported in the study that the expression of the NF-Kappa B is the early event in the diabetic Retinopathy that is sustained by increased in the death of the retinal capillary cells. It is reported experimentally by administrating IL-1 β in the Vitreous fluid of Lewis Rat initiate the retinal inflammatory response further accompanied by breakdown of the blood retinal barrier. These pro-inflammatory changes are some of the earliest inflammatory changes observed in the Retina of Diabetic animals. Retinal capillaries cells undergo apoptosis one of its mechanism associated with IL-1 β . Administration of Aspirin in the Diabetic dog inhibit the development of Retinopathy. These studies suggest that the inflammatory pathway is the precursor for the Retinopathy in the presence of hyperglycaemia.²⁶
- (ii) **Tumer Necrosis Factor-** \square : TNF alpha is the prototype member of the family cytokine it includes Fas Ligand (FasL), CD40 ligand and TNF-alpha related apoptosis inducing ligand (TRAIL) and induce apoptosis, cell differentiation and activation. It is found normally in the extracellular matrix, Endothelial and vessel wall, of the proliferative diabetic Retinopathy. TNF - α directly involved in the inflammation of endothelial cell.²⁷ TNF - alpha can be used as the biomarkers for early detection of diabetic Retinopathy. Relation of TNF- α and vascular diabetic damage is reported in several studies. TNF - α levels in tears confirms a good markers in its association with diabetic micro complications.²⁸ The blood Retinal barrier plays a central role in maintaining the homeostasis in retinal microenvironment however its breakdown occurs in Diabetic Retinopathy. The expression of TNF- α occurs via a proinflammatory cytokine TNF- α , a potent mediator of leucostasis which is induced with other mediators like VEGF, IL-1 β , Platelet activating Factor in Retinal Vasculature. It is seen that early suppression of the level of TNF- α in the diabetic animals and patients save the damage of the blood retinal barriers. The study suggests that the retinal Leucostasis and apoptosis can be mediated with the help of management of expression of TNF- α .²⁸

7. Apoptosis In Diabetic Retina

(i) Bax/Bcl-2

Apoptosis has become a new focus in the field of Ophthalmic Research. Apoptosis is a physical and highly regulated process. It is a key feature of normal development, morphogenesis and tissue repair. The treatment remains not effective always since the involvement of apoptosis in the neurodegenerative eyedisease. Hence the removal of the apoptosis can be a cure of the disease. Bax and Bcl-2 are apoptotic factors. It is observed that Bax was more widely expressed in the conjunctiva of the patient then Bcl2 with and without diabetic retinopathy and further conductive to apoptosis in the presence of hyperglycaemia. Expression of pro-apoptotic factor Box plays a central role in the apoptosis of the retina. Which is tightly bound to aggravate the disease. It is also reported that the Bcl2 family genes positively or Negatively regulate the apoptosis and it is completely depending on the ratio of Bax /Bcl2 respectively. Exploitation of knowledge related to this mechanism leads to cure of the disease and also development of treatment for it.²⁹

(ii) Caspase – 3:

Cystine-aspartic proteases, cystine aspartates or cystine dependent aspartate- directed protease are a family of enzymes playing essential role in programmed cell death and inflammation. They are named as Caspases due to their specific cystine activity. The role of these enzymes in programmed cell death was first identified in 1993. H.Robert Hertz initially established the importance caspase in apoptosis. Caspase 3 was first cloned using the sequence of expressed sequence tag in a data base. It shares similarity with ICE(Interleukin-1 beta converting enzyme now known as caspase -1 at that time ICE was only known caspase) and named as CPP32.It plays an important role in execution and initiation of programmed cell death (apoptosis) The first incidence of caspase involved in photoreceptor death was found in drosophila in which mutation in rhodopsin gene also cause photoreceptor degeneration³⁰ The most studied cell death type in diabetic retinopathy is apoptosis. It has distinguishable features that makes it easily detectable with established techniques like TUNEL (Terminal dUTP Nick End Lebling) assay. Depending on cell types active caspase- 8 initiate one or more pathways first executioner of caspase 3. Another type of apoptosis called intrinsic apoptosis in which eventual execution of caspase 3. Intrinsic apoptosis initiated by intracellular stress both extrinsic and extrinsic pathways run near mitochondrion. Although general belief is that early treatment can regulate the apoptosis and can be a cure to disease. Hence it is necessary to start it early by the knowledge of various factors and pathways.³¹

(iii) Basement Membrane Thickening in Diabetic Retina

The vascular basement membrane thickening is the fundamental modification of small blood vessels in diabetes.Excess synthesis of basement membrane is responsible for such effect. Basement membrane is degraded by matrix metalloprotease. However thickness occurs increased production and decreased degradation of extracellular matrix protein.³² High level of glucose can increase mRNA expression of ECM(extra cellular matrix) proteins, collagen and fibronectin, in the retinal endothelial cells.³³ Alterations in the basement membrane in Diabetic Retinopathy cornea initiate respective changes in the expression of integrin. Integrins are heterodimerictransmembrane glycoproteins consisting of 1alpha subunit and 1beta subunit. They are cell adhesion molecules. Integrin alpha-3 is a protein that in humans is encoded by the ITGA3 gene.³⁴ ITGA3 is an integrin alpha subunit. Together with beta-1 subunit, it makes up half of the $\alpha3\beta1$ integrin duplex that plays a role in neural migration The suppression of $\alpha3\beta1$ integrin leads to degradation of basement membrane one of such mechanism leads to activation of matrix metalloproteinase that decrease the $\alpha3\beta1$ basement membrane ligands³⁵

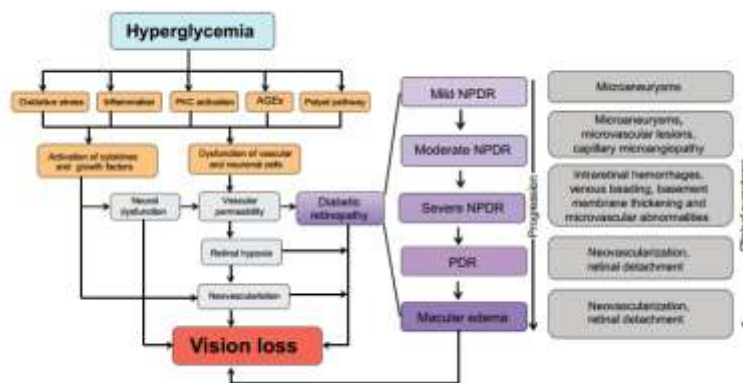


Fig.3 Showing the molecular mechanism of diabetic retinopathy and clinical symptoms and association with each stage.⁹

the preparation of pickles, vegetable dishes, daals, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhancement of flavour. In traditional medicine, fenugreek is thought to promote digestion, induce labour, and reduce blood sugar levels in diabetics, although the evidence for these effects is lacking.⁴² It is reported in rats that protective effect in Diabetic Retinopathy was seen when *Trigonella foenum-graecum* Linn. (fenugreek) treatment provided to STZ induced diabetic rats. Fenugreek treated retina have a marked decrease in the level of inflammation and angiogenic biomarkers. Retinal stress remain controlled. Lesser thickening of capillary of basement membrane reported in fenugreek treated rats. Recently, Gupta and associates examined the effects of fenugreek in STZ-induced diabetic complications in the rat retina. Injection of STZ to Wistar rats considerably elevated the expressions of retinal inflammatory (IL-1 β and TNF- α) and angiogenic (VEGF and PKC- β) molecular biomarkers in diabetic retinae in comparison to normal retinae. Treatment with fenugreek (100 and 200 mg/kg body weight) for 24 weeks inhibited the expression of inflammatory and angiogenic molecular biomarkers. Moreover, fenugreek reinstated normal superoxide dismutase and catalase levels in the diabetic retinae. Fluorescein angiograms and photographs of the fundus of diabetic retinae indicated retinal vascular leakage. However, this effect was reversed in fenugreek-treated retinae. Fenugreek also decreased the thickened basement membrane in STZ-induced diabetic retinae. Hence, fenugreek may prevent retina degeneration in diabetic patients.⁴³ It is also reported that characterization of a new insulinotropic compound, 4-hydroxyisoleucine. This amino acid has been extracted and purified from fenugreek seeds, which are known in traditional medicine for their antidiabetic properties. 4-Hydroxyisoleucine increases glucose-induced insulin release, in the concentration range of 100 μ mol/l to 1 mmol/l, through a direct effect on isolated islets of Langerhans obtained from both rats and humans. The stimulating effect of 4-hydroxyisoleucine was strictly glucose dependent; indeed, ineffective at low (3 mmol/l) or basal (5 mmol/l) glucose concentrations, the amino acid potentiated the insulin secretion induced by supranormal (6.6–16.7 mmol/l) concentrations of glucose.⁴⁴ 4-Hydroxyisoleucine increase the glucose stimulated release of insulin from pancreatic islet cells.⁴⁵

3. Tulsi (*Ocimum Santum*)

The plant Tulsi or Holy Basil (Botanical name *Ocimum Sanctum*) belongs to family Lamiaceae. It is a tropical plant which grows as weed and also cultivated. Tulsi is worshipped by Hindus and is an important symbol of Hindu religion. It is a very common sight to find Tulsi Vrindavan (A special structure where tulsi is grown) in houses of Hindus. Texts of ayurveda describe the properties of tulsi as follows. It is light to digest and dries tissue secretions. Tulsi tastes hot and bitter. It can penetrate deep tissues and has anti-helmenthic properties. Due to these properties it normalizes kapha and vata. Leaves, flowers, seeds and roots of Tulsi are used in ayurvedic preparations. Apart from these researches have shown that tulsi is very effective in reducing blood sugar and blood cholesterol.⁴⁶ It is naturally available antioxidant. It is also a neuroprotective agent. Intraperitoneal administration of 5ml/kg to 10ml/kg to rats reported a protective role in selenite induced experimental cataract. A combination of Vitamin E and *Ocimum Santum* treatment also reported to reverse the change of Diabetic Retinopathy.⁴⁵ The aqueous extract of the leaves of the *Ocimum santum* decrease the level of blood glucose in both alloxan and STZ induced diabetes in rats.⁴⁷ Eugenol the active constituent of the plant is responsible for all the therapeutic action. Leaf extract increase the secretion of insulin by physiological pathway in animal model.⁴⁸

4. Quercetin:

Fruits and vegetables are the primary dietary sources of quercetin, particularly citrus fruits, apples, onions, parsley, sage, tea, and red wine. Olive oil, grapes, dark cherries, and dark berries such as blueberries, blackberries, and bilberries are also high in quercetin and other flavonoids.⁴⁹ Quercetin is a flavonoid that helps prevent blood clots by reducing histamine formation. It also slows the formation of insulin growth factors, as well as reducing high blood pressure resulting in the reduction of stress on the walls of blood vessels. It possesses antioxidant, antiapoptotic and anti-inflammatory property independent of glucose lowering effect. Diabetic retinopathy being a malnutrition disorder, dietary supplement can be helpful to check its progressive effects.⁵⁰ Gupta and associates examined the role of Quercetin in diabetic retina. Quercetin treated rat shows a decrease in the level of Caspase-3 in diabetic Retina. The basement Membrane thickness is also less in quercetin treated rat. It is widely reported for its antioxidant and anti-inflammatory properties. It also decreases the level of mediators of inflammation's. It shows that this bioflavonoid possesses retinal neuroprotective effect.

5. Green Tea (*Camellia sinensis*)

Green tea (leaves of *Camellia sinensis*, Theaceae) is a well known beverage among East Asia and it also possesses traditional medicinal value. Green tea (GT) possesses anti-inflammatory, antioxidative and anticarcinogenic properties. The catechins present in GT are commonly known as polyphenols and are

flavanols in nature. Tea polyphenols such as epigallocatechin gallate (EGCG) have cryoprotective properties such as inhibition of proinflammatory cytokines and inhibition of growth factors by inducing neovascularization. Hyperglycaemia if prevented early can help in decreasing the microvascular complications associated with diabetes. Experimentally by Gupta and associates it is reported that green tea helps in controlling the thickness of basement membrane. TNF- α level is also comparable to the normal group rat. Expression of VEGF was not reported in rat treated with green tea. It is also reported in literature that the polyphenol Epigallocatechin gallate from green tea inhibit VEGF mediated angiogenesis. The study further reported that the treatment with green tea restore the antioxidant defence mechanism of Retina back to the normal. Green tea have potential to save the diabetic Retina.⁵¹

6. Hesperetin:

Hesperetin is a common flavonoid found in the citrus fruit. It is reported to a powerful free radical scavenger. Since, diabetic retinopathy is a multiple metabolic vascular inflammatory defect. Uncontrolled hyperglycaemia further lead to the development of Diabetic Retinopathy. It is reported in experimental work done by Dr. Gupta and associates that Hesperetin treated rat shows a decrease in the level of TNF- α and IL-1 β . It is also supported by various studies that dietary supplement Hesperetin decrease the level of IL-1 β . Due to its potential effect of antiapoptotic, antioxidant and anti-inflammatory Hesperetin lesser edematous Müller cell end feet's in nerve fibre layer and normally organised inner-nuclear layer.⁵² Hesperetin treated rat Retina reports a marked inhibition of PKC- β and VEGF. The electron microscopy further reports that Basement Membrane thickness not observed in the Hesperetin treated rats. A dilated blood vessels are reported in Hesperetin treated rats. This vasoprotective effect of Hesperetin can be used in prevention of microvascular disorders of retina.⁵³

7. Tinospora. Cardifolia

Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms. (Family: Menispermaceae) commonly known, as "Amrita" or "Guduchi" is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis. The starch obtained from the stem known as "Guduchi-satva" is highly nutritive and digestive and used in many diseases. During last two decades, the drug has been subjected to extensive phytochemical, pharmacological and clinical investigations and many interesting findings in the areas of immunomodulation, anticancer activity, liver disorders and hypoglycaemic are reported.⁵⁴ The action of this herbal drug is depending upon the inhibition of peripheral glucose uptake and endogenous secretion of insulin. It is reported experimentally by Dr. Gupta and associates that *Tinospora cardifolia* reduced the expression of PKC hence VEGF is also regulated. No sign of cataract development well reported in *Tinospora cardifolia* treated animal group. The animal treated with *Tinospora cardifolia* shows regulated retinal cytokine mediators. It can be observed by inhibit the expression of TNF- α and IL-1 β in *Tinospora cardifolia* treated group. A decreased level of CAT and GSH in the treatment group reports that it may possess microvascular complication protective effect.⁵⁵

III. Role of Chinese Traditional Medicines:

1. Panax notoginseng

Panax notoginseng (Family Araliaceae), commonly known by the name San Qi in Chinese traditional medicine, has already been reported to possess antidiabetic activities. But very little is known about its potential in management of retinopathy. According to the studies done on its antidiabetic activity, several saponins extracted obtained from the roots of this plant were found to lower fasting blood glucose levels, improve glucose tolerance and produce antihyperlipidemic effects. The major saponins eliciting these effects were identified as ginsenoside Re 14, ginsenoside Rd, ginsenoside Rg1, ginsenoside Rb1 and Notoginsenoside R1.⁵⁶ Recently, it has been found that these saponins also possess antioxidant and anti-inflammatory properties. The antioxidant activity of *P. notoginseng* saponins is attributed to their ability to scavenge hydroxyl and superoxide radicals (thus preventing the free radical-induced apoptosis of retinal pigment epithelium)⁵⁷.

2. Litsea japonica

Litsea japonica (L. japonica) is native Korean plant. This herb has been consumed as a vegetable food in Korea for long time, however its pharmacological activities remain unclear. The chemical constituents of this plant contain numerous lactones, alkaloids, essential oils, fatty acids, and terpenoids. It is reported that treatment with an extract of *L. japonica* inhibit diabetes-induced breakdown of BRB and lowered expression of retinal vascular endothelial growth factor (VEGF) in db/db mice. Extract of *L. japonica* also suppressed the degradation of occludin, an important tight junctional protein in BRB (Blood retinal barrier). These results suggest the potential therapeutic usefulness of *L. japonica* for retinal vascular permeability diseases. Kim and colleagues

reported about the ameliorative effect of an ethanolic extract of *L. japonica* on diabetes-induced neuronal apoptosis of the retina in db/db mice. Treatment with an ethanolic extract of *L. japonica* (100 or 250 mg/kg body weight) once per day orally for 12 weeks in db/db mice resulted in a trivial decrease in blood glucose levels without any significant effect on those of HbA1c. Immunoreactivity against advanced glycation end product (AGE) was evident only in the large and small retinal vessels of the normal mice, while AGE-positive signals were situated in the inner neural retina and retinal vessels in the vehicle-treated db/db mice. This suggests that retinal tissues exhibit significant accumulation of serum AGEs. However, treatment with ethanolic extract of *L. japonica* reduces the amount of AGE deposited in these regions. In addition, the ethanolic extract of *L. japonica* inhibits the stimulation of nuclear factor-kappa B (NF- κ B). These results suggest that the ethanolic extract of *L. japonica* may be advantageous for the treatment of diabetes-induced retinal neurodegeneration; moreover, its neuroprotective effect might be attributable in part to its effect on AGEs⁵⁸.

3. Fufang Xueshuantong

Fufang Xueshuantong (FXT) capsule was originally formulated twenty years ago and also the State Food and Drug Administration of China accepted it in 2003 for treatment of stable angina pectoris and retinal vein occlusion⁵⁹. Fufang Xueshuantong (FXT), also known as compound Xueshuantong in China. It is a Chinese herbal formula that comprises four traditional plants, namely *Astragalus membranaceus*, *Scrophularia ningpoensis*, *P. notoginseng* and *Salvia miltiorrhiza*⁶⁰. FXT is used clinically for the treatment of DR. Moreover, FXT reduces the progress of microvessel lesions in the retina by declining pericyte loss and reducing acellular capillaries. This effect was in agreement with downregulation of aldose reductase hyperactivity and expression of VEGF. Thus, FXT reported protective effects against STZ-induced retinal lesions in rats. (Constituent combination of FXT (cFXT) is composed of saponins of *P. notoginseng*, harpagoside, cryptotanshinone, tanshinone-I, and astragaloside-A. Although cFXT had no effects on the STZ-induced augmented blood glucose levels and body weight in rats, it inhibited the augmented erythrocyte aggregation, plasma viscosity, and pericyte and acellular vessel loss. This was accompanied by reversal of the increased expression of VEGF, endothelin-1, excessive activation of aldose reductase, and hypoeexpression of pigment epithelium-derived factor in the retinas of STZ-treated rats. Hence, the main constituents of Fufang Xueshuantong Capsule, namely saponins of *P. notoginseng*, harpagoside, cryptotanshinone, tanshinone-I, and astragaloside-A. These are responsible for its protective effects in STZ-induced retinal lesions in rats. These constituents will facilitate development of new treatment for DR, and can be used for quality control of Fufang Xueshuantong Capsules.⁶¹

4. Astragalus membranaceus

Astragalus membranaceus (Family Fabaceae), commonly known as Huang Qi in traditional Chinese medicine, is a herb whose roots have been used for decades for their antitumour, immunomodulatory, diuretic, antioxidant, anti-inflammatory, antithrombotic and antidiabetic properties. The major phytoconstituents exhibiting these properties include polysaccharides (astragalans I, II and III), saponins (astragalosides I–VIII and isoastragalosides I and II), flavonoids, isoflavonoids, sterols, amino acids, volatile oils and trace elements. A recent study reported its multiple protective effects of astragaloside IV in the animal models of diabetic retinopathy which included reduction in retinal ganglion cell apoptosis, inhibition of activation of NF- κ B and various cytokines (thus eliciting anti-inflammatory effects) and downregulation of the expression of enzyme aldose reductase (the enzyme involved in the polyol pathway).^{62,63}

5. Ginkgo biloba

Ginkgo biloba, also known as maiden hair tree, one of the oldest surviving tree species and is known for its utility in food and medicine since centuries. The various pharmacological activities of *G. biloba* include enhancement in cerebral blood flow, neuroprotection and antiapoptotic actions against oxidative stress-induced damage, toning of vasculature and inhibition of platelet activation. Due to these properties, it has been commonly used in the Chinese traditional medicine for the treatment of asthma, tinnitus, vertigo, diabetes and several circulatory disorders such as peripheral vascular diseases, but most importantly dementia-related disorders, such as Alzheimer's disease. Even today, *G. biloba* extracts are being clinically used to improve the learning, cognitive and motor activities in dementia patients and as an adjunctive therapy in schizophrenia. The chemical constituents responsible for these activities are reported as flavones, terpenoid tetraterpenes (ginkgolides A, B, C, J, P and Q, and bilobalides), flavanol glycosides (quercetin, catechin) and proanthocyanins. Its antioxidant, neuroprotective and blood flow-improving properties make it a potential candidate for its utilization in the prevention of diabetic retinopathy. Indeed, some studies have reported beneficial effects of *G. biloba* extract in retina during hyperglycaemia. Treatment with *G. biloba* extract in diabetic animal models greatly reduces the nitric oxide-induced oxidative stress by acting as nitric oxide scavenger, besides decreasing its production.^{64,65}

6. Puerariae lobata

Puerariae lobata (Family Fabaceae), popularly Known as Kudzu root, is one of the most used herbs in the traditional Chinese medicine due to the wide range of pharmacological activities produced by its active phytochemical constituent puerarin, an isoflavonoid. Puerarin, also known as Gegen in China, isolated from the dried root (*Puerariae radix*) of *P. lobata*, possesses a wide range of beneficial properties such as vasodilatory, cardioprotective, neuroprotective, hepatoprotective, analgesic, antipyretic, antioxidant, anti-angiogenic, anti-inflammatory, antinociceptive, hypoglycaemic, hypocholesterolaemic, antithrombotic and osteoblastic, owing to the virtues of which it has been used both as dietary supplement and medicines. Apart from puerarin, two other isoflavones present in *P. lobata*, daidzein and genistein, are also responsible for some pharmacological actions. *P. lobata* is one of the few Chinese herbs whose antidiabetic retinopathy potential is well reported and acknowledged. Generally, it protects the retinal epithelial and neuronal cells from apoptosis by its antioxidant mechanisms. It prevents peroxynitrite-induced cellular apoptosis, by primarily reducing mRNA expression of iNOS (thus suppressing nitric oxide production) and enhancing SOD (super oxide dismutase) activity (thus scavenging superoxide radical), thereby inhibiting peroxynitrite radical formation. puerarin also attenuates AGE-induced oxidative stress by downregulating receptor for advanced glycation end product mitigate its expression.^{66,67}

8. Miscellaneous:

There are certain herbs that will strengthen blood vessels and prevent the deterioration and leakage of blood into the eye. Bilberry is rich in vitamins A and C which protect and strengthen the eye. Ginkgo Biloba increases the diameter of blood vessels and allows the flow of more blood to nourish the retina. Grape seed extract is effective in preventing blood leakage from damaged vessels. Other herbs include parsley, It has strong anti-oxidants, vitamin C, and lutein. This helps protect the macula from oxidation damage. Shark Cartilage stops the progression of the condition, as it slows down the growth of the tiny blood vessels that could potentially affect the eyesight. Prevention can be the best Diabetic Retinopathy Treatment. Leading a healthy lifestyle, reducing stress, managing diabetic blood levels and controlling high blood pressure are key to controlling the severity of the degeneration on retinopathy.⁶⁸

Table: II mechanism of action of Major Herbal drugs in Diabetic Retinopathy (Source Behl et al 2017.)⁷⁰

S. No	source of Herbal Drugs	Major Constituent	Mechanism of action
1.	<i>Curcuma longa</i>	Curcumene, Curcumenone, Curcone, Curdione, Cineole, Curzerenone, epiprocurmenol, eugenol, Camphene, Camphor, Bornel, Procurcumadiol, Procurcumenol, Curcumins, unkonan A, B, & D, B- sitosterol	anti-oxidant, possess antiangiogenic properties against SDF-1 α .
2.	<i>Trigonella foenum-graecum</i>	4-hydroxyisoleucine	Anti-inflammatory, anti angiogenic
3.	<i>Ocimum Santum</i>	Eugenol	antioxidant
4.	citrus fruits, apples, onions, parsley, sage, tea, and red wine. Olive oil, grapes, dark cherries, and dark berries such as blueberries, blackberries, and bilberries	Quercetin	antioxidant, antiapoptotic and anti-inflammatory property
5.	<i>Camellia sinensis</i>	epigallocatechin	anti-inflammatory, antioxidative and anticarcinogenic
6.	citrus fruit	Hesperetin	free radical scavenger, antiapoptotic, antioxidant and anti-inflammatory
7.	<i>Tinospora Cardifolia</i>	(-)Epicatechin, Tinosporin, Isocolumbin, Palmatine,	Antioxidant, Furanolactone, Tinosporin, Tinosporide, Jateorine, Columbin, Clerodane derivatives, Berberine, choline, Tembetarine, Palamtine, Jatrorrhizine
8.	<i>Panax notoginsen</i>	ginsenoside Re 14, ginsenoside Rd, ginsenoside Rg1, ginsenoside Rb1 and Notoginsenoside R1	antioxident
9.	<i>Litsea japonica</i>	lactones, alkaloids, essential oils, fatty acids,	Antioxidant and antiapoptotic

		and terpenoids	
10.	Astragalus membranaceus, Scrophularia ningpoensis, P. notoginseng and Salvia miltiorrhiza (Fufang Xueshuantong)	P. notoginseng, harpagoside, cryptotanshinone, tanshinone-I, and astragaloside-A .	Anti angiogenesis, anti- oxident
11.	Astragalus membranaceus	Polysaccharides (astragalans I, II and III), saponins (astragalosides I–VIII and isoastragalosides I and II), flavonoids, isoflavonoids, sterols, amino acids, volatile oils and trace elements	Reduce retinal ganglion cell apoptosis Decrease phosphorylation of ERK1/2 Inhibit activation of NF-κB and various cytokines Downregulate the expression of enzyme aldose reductase
12.	Anisodus tanguticus	Anisodamine, anisodine, hyoscyamine, scopolamine, tropine, apatropine, trichlorophenyl butyryloxytropine and cuscohygrine	Prevent retinal lipid peroxidation Downregulating the expression of plasminogen activator inhibitor-1 (PAI-1) and tissue factor Inhibit the production of TNF-α Activate α7nAChR Downregulate VPO-1
13.	Ginkgo biloba	Biflavones, terpene trilactones (ginkgolides A, B, C, J, P and Q, and bilobalides), flavonol glycosides (quercetin, catechin) and proanthocyanidins	Downregulate the expression of PAF Reduce the transcriptional expressions of HIF-1α and VEGF
14.	Puerariae lobata	Puerarin, genistein and daidzein	Prevent peroxynitrite-induced cellular apoptosis Attenuate AGE-induced oxidative stress Suppress the activation of NADPH oxidase, VEGF and HIF-1α Inhibit tyrosine kinase Prevent leucocyte–endothelial interaction, vascular dysfunction, leakage and oedema
15.	Salvia miltiorrhiza	Salvianolic acid A, rosmarinic acid	Upregulate endogenous antioxidant enzymes Inhibition of Ang-II-induced NADPH oxidase-4 (Nox4) Preventing endothelial cell proliferation and angiogenesis
16.	Lycium barbarum	Polysaccharides, zeaxanthin, carotene, betaine, cerebroside, beta-sitosterol, p-coumaric, and various vitamins	Upregulation of the expression of anti-apoptotic gene Bcl-2. Downregulate the expression of pro-apoptotic gene Bax Inhibit the activation of cytochrome c/caspase-3-mediated apoptotic pathway

IV. Conclusion

Being a complex multifactorial disorder the management of Diabetic Retinopathy is also not fixed. It requires multidisciplinary approach individualised according to severity of the disease. The primary preventive measure is good glycaemic control, and it is beneficial in reducing the incidence and progression of DR. Progression of Retinopathy in Diabetescan be reduced by 25% in the case of Diabetes Type I and 39% in the case of Type II with each 10% decrease in the level of Glycated Haemoglobin. There is no proper treatment available for Diabetic Retinopathy, prevention is the best approach to reduce blindness⁶⁹.

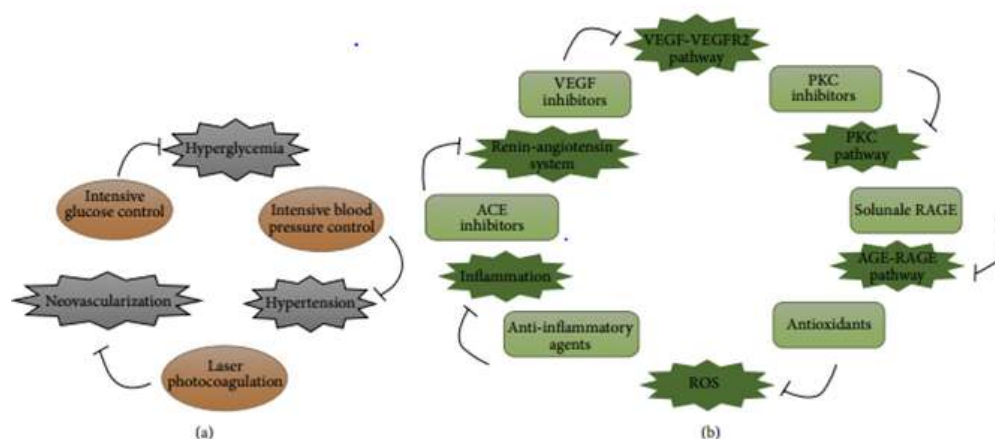


Fig:3 The established preventive measures include the general, Primary and the secondary preventive strategies. The novel and emerging therapeutic targets covers PKC inhibitors, VEGF inhibitors, ACE inhibitors and antioxidant drugs.⁷¹

Diabetic retinopathy is a vision-threatening complication associated with diabetes mellitus. Occurs by a result of several hyperglycaemia-induced pathological changes including increased oxidative stress-induced apoptosis of retinal endothelial and neuronal cells, angiogenesis and inflammatory responses. There is an urgent demand to develop a natural herbal therapy which could prevent the progression of this complication or cure the already existing pathological states in retina without causing any harm to the retinal tissue and free from any major side effects. In lieu of finding safer alternative treatments of diabetic retinopathy in plants, herbal drugs are being currently explored. Indeed, most of these herbs have reported positive results. Various pharmacological actions including antioxidant, anti-angiogenic, anti-inflammatory, PAF antagonism, aldose reductase inhibition, PPAR- γ agonism, among various others have been reported by these herbs. It indicates that they might mollify all the hyperglycaemia-induced pathological conditions. Preclinical and clinical studies performed so far on these herbs need to be acknowledged. The development of these herbs into clinical therapies should be accelerated to provide a safer treatment to the patients of diabetic retinopathy. Herbal drugs can potentially be used for the treatment or prevention of diabetic retinopathy. They alleviate several hyperglycaemia-induced pathological conditions in retina. The therapy for retinopathy currently, clinically limited to destructive techniques like laser photocoagulation and vitrectomy. This would provide a natural and safe therapy for diabetic retinopathy.

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