2. REVIEW OF LITERATURE

Early events in the development of DR, the ocular manifestation of diabetes can often strike with little warning, and despite efforts to prevent its occurrence, thousands of people go blind from diabetic retinopathy every year. As far as diabetes-related conditions are concerned, high blood glucose levels (hyperglycemia) presents the main cause of many diabetic complications along with retinopathy (Qian and Ripps, 2011). Diabetes thus is considered as a chronic illness that requires continuing medical care and ongoing patient self-management education and support to prevent acute complications and to reduce the risk of long-term complications (American Diabetes Association, 2012).

2.1. Diabetes

Diabetes mellitus can be defined as a metabolic disorder with impaired glucose utilization, characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin.

The incidence and prevalence of diabetes has risen steeply in the last decade (American Diabetes Association http://www.diabetes.org/diabetes-statistics.jsp.; Calcutt et al., 2009). Not just the sheer number of patients but increased mortality and morbidity due to increased complications associated with diabetes are of great concern (Ergul, 2011). The fact that there is an alarming increase in the number of younger patients diagnosed with type 1 diabetes (increasing at a rate of 3% per year (Giannini et al., 2011) and. type 2 diabetes (Ergul, 2011), intensifies this concern because development of these complications depend on the duration of the disease and the degree of glycemic control (Calcutt et al., 2009; Akalin et al., 2009). The worldwide incidence of type 1 diabetes is increasing at a rate of 3% per year (Yvonne et al., 2012). Macrovascular disease, affecting large vessels, is still the principal cause of morbidity and mortality for people with diabetes (Srikanth and Deedwania, 2011), even as new treatments have greatly decreased the incidence of microvascular complications associated with retinopathy, nephropathy, and neuropathy. Since diabetes is typically diagnosed in youth and young
adults, macrovascular changes occur early (Babar et al., 2011) and progress throughout the person’s life (Nadeau and Reusch, 2011). In people with diabetes, the ability of the macrovessels to respond to agonists decreases with age (Grzelak, et al., 2011).

Basically there are three types of diabetes, Type 1 Diabetes, is caused as a result of autoimmune problem. The immune system of the body destroys the insulin producing beta cells in the pancreas leading to no or less production of the required insulin by the pancreas. According to IDF reports, 2011, 78,000 children develop type 1 diabetes every year. Type 2 Diabetes is a result of malfunctioning of the beta cell itself. This malfunction includes non production of insulin or a situation known as insulin resistance. In insulin resistance, the muscles, fat and other cells do not respond to the insulin produced. Type 3 is known as gestational diabetes and only occurs during pregnancy. During this stage, the body resists the effect of insulin produced.

The International Diabetes Federation (IDF) consistently produces worldwide estimates of the prevalence of diabetes according to the latest data available. In their recent 2011 report, IDF collaborators have presented updated prevalence figures based on all data sources found from 1980 to 2011 with additional model-based estimates for countries without data. It has estimated 366 million people worldwide with diabetes in 2011, including those who were newly diagnosed in surveys and those with type - 1 diabetes. Prevalence of diabetes is anticipated to increase with another 50.7% (552 million) by 2030 at an averaged annual growth of 1.7 times the annual growth of the total adult world population (Whiting et al., 2011; IDF, 2011).

Even more worrying, the diabetes pattern varies substantially according to countries’ income status. According to IDF reports 80% of people with diabetes live in low- and middle-income countries For countries classified by the World Bank as being high-income countries, most people with diabetes are aged over 60 years, whereas for low- and middle-income countries most people with diabetes are of working age, between 40 and 60 years (Whiting et al., 2011). Apart from economic status, diabetes pattern also varies according to races. Certain ethnic groups, e.g. Hispanics, American Indians are reported to be at greater risk for diabetes (William, 2004).
India alone homes a skyrocketing number of diabetics. According to the International Diabetes Federation (IDF) and the Madras Diabetes Research Foundation, India had about 62.4 million people with diabetes in 2011, compared with 50.8 million in 2010. As the economy started growing, so did the incidence of diabetes. The nationwide prevalence of diabetes in India now tops 9%, and is as high as 20% in the relatively prosperous southern cities. By 2030, the IDF predicts, India will have 100 million people with diabetes (Shetty, 2012).

Diabetes mellitus has been listed as a leading cause of death by disease. It has caused 4.6 million deaths in 2011 (IDF, 2011). Besides its impact on the morbidity, disability, mortality, and quality of life of patients, diabetes has also imposed a substantial cost burden on society. The disease has caused at least USD 465 billion dollars in healthcare expenditures in 2011, which is reported to be 11% of the total healthcare expenditures in adults aged 20-79 years (IDF, 2011). Taking into account the differences in age, sex, and race/ethnicity between the population with and without diabetes, people with diabetes are reported to have several folds higher medical expenditures than that would be incurred by the same group in the absence of diabetes (American Diabetes Association, 2003). With the recognized increase in aging population, a continued rise in the health-care spending for people with diabetes is expected for the years to come. Diabetes mellitus has thus become worldwide epidemics, with its systemic complications being the major causes of morbidity and mortality. Therefore, in providing medical care for diabetic patients, to reduce its disease burden and financial costs to our community, the real challenge for health care workers requires both optimization of diabetic control and proactive screening of its potential complications, trying to minimize and retard their progression timely in the reversible and treatable stages.

Diabetes mellitus represents a serious public health concern, not only because of its high prevalence or the chronicity of its disease nature, but more importantly because of the genesis of its potentially life-threatening microvascular and macrovascular complications which are often silent and undetected until the advanced and non-salvageable stage, and
they may even appear years before the diagnosis of diabetes. An estimated 7-8% of the US population suffers from the complications of diabetes mellitus.

The three kinds of chronic or long term diabetic complications are macrovascular, microvascular and neurologic complications.

2.1.1. Complications of diabetes

Diabetes is a chronic, life-long condition that requires careful control. Without proper management it can lead to various complications such as cardiovascular disease, kidney failure, blindness and nerve damage.

2.1.1.1. Short-term complications

a. Low blood sugar (hypoglycemia)

A person who takes insulin is going to face the problem of their blood sugar falling too low at some point (because they have overestimated the insulin they need, have exercised more than anticipated or have not eaten enough). Hypoglycemia can be corrected rapidly by eating some sugar. If it is not corrected it can lead to the person losing consciousness.

It is important that the person with diabetes recognizes the signs of hypoglycemia.

b. Ketoacidosis

When the body breaks down fats, acidic waste products called ketones are produced. The body cannot tolerate large amounts of ketones and will try to get rid of them through the urine. However, the body cannot release all the ketones and they build up in your blood, causing ketoacidosis. Ketoacidosis is a severe condition caused by lack of insulin. It mainly affects people with type 1 diabetes.

c. Lactic acidosis

Lactic acidosis is the build up of lactic acid in the body. Cells make lactic acid when they use glucose for energy. If too much lactic acid stays in the body, the balance tips and the person begins to feel ill. Lactic acidosis is rare and mainly affects people with type 2 diabetes.
d. **Bacterial/fungal infections**

People with diabetes are more prone to bacterial and fungal infections. Bacterial infections include sties and boils. Fungal infections include athlete’s foot, ringworm and vaginal infections.

2.1.1.2. **Long-term complications**

a. **Disease of Eye (retinopathy)**

Eye disease, or retinopathy, is the leading cause of blindness and visual impairment in adults in developed societies. About 2% of all people who have had diabetes for 15 years become blind, while about 10% develop a severe visual impairment.

b. **Disease of Kidney (nephropathy)**

Diabetes is the leading cause of kidney disease (nephropathy). About one third of all people with diabetes develop kidney disease and approximately 20% of people with type 1 diabetes develop kidney failure.

c. **Disease of Nerve (neuropathy)**

Diabetic nerve disease or neuropathy affects at least half of all people with diabetes. There are different types of nerve disease which can result in a loss of sensation in the feet or in some cases the hands, pain in the foot and problems with the functioning of different parts of the body including the heart, the eye, the stomach, the bladder and the penis. A lack of sensation in the feet can lead to people with diabetes injuring their feet without realizing it. These injuries can lead to ulcers and possibly amputation.

d. **Diseases of the circulatory system**

Disease of the circulatory system, or cardiovascular disease, accounts for 75% of all deaths among people with diabetes of European origin. In the USA, coronary heart disease is present in between 8% and 20% of people with diabetes over 45 years of age. Their risk of heart disease is 2-4 times higher than those who do not have diabetes. It is the main cause of disability and death for people with type 2 diabetes in industrialized countries.
e. Amputation

Diabetes is the most common cause of amputation that is not the result of an accident. People with diabetes are 15 to 40 times more likely to require lower limb amputation compared to the general population.

2.1.2. Diabetic Retinopathy- the Major Complication

Diabetic retinopathy (DR) is a severe complication of diabetes and the leading cause of blindness among working adults worldwide. According to World Health Organization, the prevalence of DR is expected to increase, and the number of people at risk of vision loss is predicted to double by the year 2030 with the increasing rate of diabetes epidemic (Ola et al., 2012). It is thus a major threat to retina, the light-sensitive layer at the back of the eye that covers about 65 percent of interior surface of eye.

2.2. The Eye Structure

Eye, the organ associated with vision, is housed in socket of bone called orbit and is protected from the external air by the eyelids (SightSavers: The structure of the human eye. From: http://www.sightwavers.or.uk/html/eyeconditions/huma_eye_detailed.htm). The cross section of the eye is as shown in Figure 2.1 while that of retina is as shown in Figure 2.2 below:
Light enters the eye through the pupil and is focused on the retina. The lens assists in focusing images from different distance. Iris controls the amount of light entering the eye. It closes in the bright light and opens when light is dim. Conjunctiva, a transparent white sheet lies to the outside of the eye. Ciliary muscles in ciliary body control the focusing of lens automatically. Choroids form the vascular layer of the eye supplying nutrition to the eye structures. Optic nerve helps in transmission of the image, formed on the retina, to brain. Optic disk, normally circular in shape, is brighter than any part of the retina image and forms the entry and exit point for nerves entering and leaving the retina to and from the brain. Near to the centre of the retina is an oval shape object called macula. The fovea is near the centre of the macula and it contains packed cone cells. Due to high amount of light sensitive cells, the fovea is responsible for the most accurate vision (My Eye World: *Eye Structure and function*. website: http://www.myeyeworld.com/files/eye_structure.htm).
i. **Aqueous**

A water-like fluid which fills the front part of the eye between the lens and cornea. This fluid is produced by the ciliary body and drains back into the blood circulation through channels in the chamber angle.

ii. **Chamber Angle**

Located at the junction of the cornea, iris, and sclera, the anterior chamber angle extends 360 degrees at the perimeter of the iris. Channels here allow aqueous fluid to drain back into the blood circulation from the eye. It may be blocked in glaucoma.

iii. **Choroid**

A very vascular layer between the sclera and retina which serves to nourish the outer portions of the retina is choroid. Has one of the highest blood flows in the body.

iv. **Ciliary Body**

This is the structure located behind the iris (rarely visible) which produces aqueous fluid that fills the front part of the eye and thus maintains the eye pressure. It also allows focusing of the lens.

v. **Conjunctiva**

It is a thin lining over the sclera, or white part of the eye. This also lines the inside of the eyelids. Cell in the conjunctiva produce mucous, which helps to lubricate the eye.

vi. **Cornea**

The clear window through which we see is cornea. Actually, this is a very vital part of the eye's focusing, and the curvature of the cornea itself accomplishes about 80% of the focusing of the eye.

vii. **Episclera**

It is a fibrous layer between the conjunctiva and sclera. Sometimes lumps (pingueculum) will form in this layer on the surface of the eye near the inside or outside corners.
viii. **Extraocular Muscles**

Six muscles control eye movement. Five of these originate from the back of the orbit and wrap around the eye to attach within millimeters of the cornea. Four of these move the eye roughly up, down, left and right. Two muscles (one originating from the lower rim of the orbit) control the twisting motion of the eye (when the head is tilted).

ix. **Iris**

This is the part of the eye which gives it color. It contains muscles which open or close the pupil in response to the brightness of surrounding light. A blue iris actually has a lack of pigment.

x. **Lens**

This is located just behind the iris, and helps to focus light. A "capsule" surrounds the lens "nucleus". The nucleus can become cloudy, and this is termed cataract.

xi. **Macula**

It is the part of the retina which is most sensitive, and is responsible for the central (or reading) vision. It is located near the optic nerve directly at the back of the eye (on the inside). This area is also responsible for color vision.

xii. **Optic Nerve**

This contains visual information from the eye, and has about 1.2 million nerve fibers. The optic disc is visible on the inside of the eye, where the nerve is viewed "end on". The sheath around the optic nerve is continuous with that of the brain, and the nerve connects directly into the brain.

xiii. **Orbit**

The boney socket containing the eye, fat, extraocular muscles, nerves, and blood vessels is called orbit. The floor and inside walls of the orbit are paper thin, and are easily fractured by trauma.
xiv. Pupil

It is a hole in the iris. This is the black opening in the center of the eye. Its size is controlled by the iris muscles.

xv. Retina

This thin layer lines the inside of the eye and receives light rays, processes them, and sends signals to the brain via the optic nerve. The retina is like the "film of a camera". It is separated from the very vascular choroid by the "retinal pigment epithelium". Sometimes breaks in this pigmented layer cause macular degeneration.

xvi. Sclera

Sclera is the white, tough wall of the eye. Few diseases affect this layer. It is covered by the episclera and conjunctiva, and eye muscles are connected to this.

xvii. Uveal tract

A group of similar eye structures including the choroid, ciliary body and iris forms the uveal tract. It may be prone to inflammatory conditions (uveitis or iritis).

xviii. Vitreous

Vitreous is a jelly-like, clear fluid which fills most of the eye. This tends to liquefy with age, and its separation from the retina can lead to retinal tears and detachment.
**RETINA** is a multi-layered sensory tissue that lines the back of the eye. It contains millions of photoreceptors that capture light rays and convert them into electrical impulses. These impulses travel along the optic nerve to the brain where they are turned into images. There are two types of photoreceptors in the retina: rods and cones. The retina contains approximately 6 million cones. The cones are contained in the macula, the portion of the retina responsible for central vision. They are most densely packed within the fovea, the very centre portion of the macula. Cones function best in bright light and allow us to appreciate colour (St. LukesEye.Com: *Eye Anatomy*. http://www.stlukeseye.com/anatomy/Retina.asp).

### 2.3. Anatomy and Physiology of the retina

The retina lies between the central, clear vitreous body and the choroid. The vitreous body is filled with a translucent gel whose composition varies concentrically; it’s most liquid phase being central which gradually turns into a fibrous network as it nears the retina. This limiting aspect of the retina is formed by the extension of foot like processes from the matrix of structural cells supporting vascular and neuro-sensory aspects of the retina proper. The most exterior retinal stratum, going from the centre of the eyeball outwards, is the Retinal Pigment Epithelium or RPE. A healthy RPE is vital for the survival and function of the photoreceptors and choriocapillaris and is essential for
achieving and maintaining the visual function (Strauss, 2005). The main functions of the RPE include disposing of the photoreceptor outer segments, participation in retinoid metabolism and the visual cycle and controlling the chemical milieu of the subretinal space (Thumann et al., 2006; Hildebrand and Fielder, 2011). Thus, this is one of the blood retinal barriers which involved in the homeostasis of the retina. The lesions of early DR, or mild to moderate cases, are mostly found between the structural cells near the vitreous, limiting the retinal interiorly, and the external RPE.

The retina has a layered structure. Its outer part is supplied by a vascular layer, the choroid, and protected by a tough outer layer, the sclera. The cellular elements of the retina are arranged and adapted to meet the functional requirements of the different regions of the retina. The regional differentiation of the retina is a process of slow maturation that takes several years to be completed (Hildebrand and Fielder, 2011).

The retinal vessels may be characterized by the number of branches they have undergone. The vessels that enter the retina are branches of the ophthalmic artery or vein which in turn branches from the internal carotid vascular subsystem. The central retinal vein and artery are of the smaller primary branches of the ophthalmic vessels and travel to the retina by penetrating the optic nerve bundle, within which they lie centrally until reaching the retina where they start branching. These two vessels supply and drain the retina. The short ciliary vessels perforate the sclera, around the junction of the optic bundle to the eye, into the uveal tract. The uveal tract extends anteriorly towards the iris via the ciliary body, which is also involved in the homeostasis of the retina via its blood-retina barrier.

Retinal function is thus dependent on the simultaneous and normal perfusion of both the uveal and the retinal circulation. However, exceptional to this, the fovea or area of highest visual acuity is primarily dependent on the choroidal circulation only as there are no retinal vessels in this area. The fovea is located temporal to the optic disk and can be seen on the vertical midline and just below the horizontal midline. The fovea lies at the centre of the macula lutea, a region pigmented by xanthophyll, this is also visible surrounding the fovea.
The first dichotomous division of the central retinal arteries and veins occurs on entry to the retina at the optic disk or blind spot. Most of the divisions of the central retinal vein and artery are visible on the images which include the optic disk. All retinal vessels lie in the path of light reaching the photosensitive aspect of the retina, and thus cast a non-perceptible shadow on the retina.

2.4. Histological Organization of the Retina

Retinal Pigment Epithelium: Each eye contains about 3.5 million RPE cells (Panda et al., 1996), held together by junctional complexes to form a continuous epithelial monolayer. The tight junctions between these RPE cells separate the choriocapillaries from the photoreceptors of the outer retina, thus creating the outer blood-retina barrier (Strauss, 2005; Cunha-Vaz, 2004), which helps to control the extracellular milieu and maintains the function of the outer retina.

Photoreceptor Layer: Rods and cones are tightly stacked together into a single pallisading layer of photoreceptors. This thin, subcellular stratum is the only light-sensitive part of the neuroretina and the site of phototransduction. All other layers of the neuroretina collectively serve to process and transmit these nerve signals (Levin, 2003; Tessier-Lavigne, 2000).

External Limiting Membrane: It is located between adjacent Müller cells as well as between Müller and photoreceptor cells. The subretinal space is a potential space lying between the outer blood retina barrier and the external limiting membrane (Hildebrand and Fielder, 2011).

Outer Nuclear Layer: This layer contains the nuclei of the photoreceptor cells and is thickest in the foveolar area. The human retina contains about 4-5 million cones and 77–107 million rods (Wurtz and Kandel, 2000). Only cones are found in the foveola, whereas rods predominate outside the foveola throughout the remaining retina.

Outer Plexiform Layer: In the OPL, photoreceptor cells of the outer nuclear layer form connections with the bipolar and horizontal cells of the inner nuclear layers. This layer
has two components: the axons of the photoreceptor, bipolar and horizontal cells and their synaptic connections.

**Inner Nuclear Layer:** This layer harbors the nuclei of at least five different types of cells: the horizontal, the bipolar, the amacrine, the interplexiform, and the Müller cells. The horizontal cells are located along the outer limit of the inner nuclear layer facing the OPL, whereas the amacrine faces the IPL. The nuclei of the bipolar, inner plexiform and Müller cells take up intermediate positions (Ogden, 1983; Wurtz and Kandel, 2000).

**Inner Plexiform Layer:** It is the second retinal processing layer with networks between bipolar, amacrine, and ganglion cells. It is further sublayered into 6 laminae. This enables the parallel representation and processing of the photoreceptor input through specific interactions between the bipolar, amacrine, and ganglion cells in each of the six lamina of the IPL (Zhu et al., 2000).

**Ganglion Cell Layer:** This layer contains about 1.2 million ganglion cells as well as a number of other cell types, including “displaced” amacrine cells, astrocytes, endothelial cells and pericytes (Curcio et al., 1987).

**Nerve Fiber Layer:** As all the retinal ganglion axon fibers converge on the optic disc, the nerve fiber layer is thicker towards the disc. The axons are accompanied by astrocytes in the nerve fiber layer and are separated into small bundles by the cellular processes of Müller cells and the internal limiting membrane (Hildebrand and Fielder, 2011).

**Inner Limiting Membrane:** The innermost processes of the Müller cell enlarge and flatten on the vitreal side to form the inner limiting membrane. Vitreous collagen fibrils insert into this membrane of the retina, thereby, rendering the retina vulnerable to vitreoretinal traction forces (Hildebrand and Fielder, 2011).

### 2.5. Colour and pigments of the retina

Retinae are not uniformly pigmented. Patches of irregular intensity, area and shape are subject to variability even within individual fundus images. Melanin, the substance of greatest light absorbance coefficient, is present in the retinal pigment epithelium (RPE)
and the choroid. Its absorbance decreases uniformly over the visual range of 400-700nm. Macular pigment, which is distinct from the phototransductive pigments in the rod and cone cell processes, does not interact with light of wavelengths greater than 530 nm (Kilbride et al., 1989).

Oxyhaemoglobin and deoxyhaemoglobin, the greatest light absorbers of the retinal vessels, have absorbance spectra in the range of 500-600nm (Flewelling, 1995). The perceptual difference in colour between venous and arterial blood is attributable to the steeper decline in absorbance of oxyhaemoglobin after 600 nm. The non-pigmented vessel walls (of the larger veins and arteries) have a minor but often distinguishable absorbance from the general fundus (Patel, 1995).

The pigment of the RPE and choroids is visible at the macula. This area is also variably pigmented. Its translucency and its own ‘yellowish’ coloration are perceptible as the darkened area. Fovea, the area of highest visual acuity, lies in the centre of the macula. The fovea is avascular and additionally pigmented. At the fovea, the retina is devoid of vessels, and its metabolic needs are met by diffusion and active transport through the RPE only. The sensory stratum of the rest of the retina has a dual source of perfusion, as both the capillaries within the interior two thirds of the retina (Adler, 1992) and the choirocappilaries are involved. This vasculated volume is approximately 0.4 mm thick throughout most of the retina.

2.6. Diabetic Retinopathy

Diabetic retinopathy (DR) is a complication of diabetes mellitus (DM) that affects the blood vessels of the retina and leads to blindness. The progression of retinopathy is gradual, advancing from mild abnormalities (characterized by increased vascular permeability) to moderate and severe non-proliferative DR (characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous (American Diabetes Association, 2000).

DR is one of the most serious complications of diabetes. Both type-1 (IDDM) and type-2 diabetes (NIDDM) may lead to blindness due to retinopathy, with type-1 being more
prevalent. It was found in a study that blindness due to DR for type 1 was 86%, while the same was 33% for type 2 of the total DM cases (Al-Maskari and El-Sadig, 2007). The prevalence of DR is probably around 30% in type 2 DM, but notably was above this level in five out of six studies reported from the Asian and Pacific Island Nations of the Western Pacific Region (http://www.eatlas.idf.org/Complications/). The same source estimates that 6–9% of patients with proliferative retinopathy or severe non proliferative disease would become blind each year (Amos et al., 1997). Moreover, growing evidence also suggests that after 15 years of diabetes, approximately 2% of patients develop blindness, while about 10% develop severe visual handicap (Amos et al., 1997). According to American Diabetes Association, 2009, more than 12,000 diabetic patients become blind due to retinopathy every year. It possesses enormous public health implications worldwide. The worldwide population of diabetics expected to develop loss of vision is predicted to double by 2030 (Wild et al., 2004). Thus, the early detection of sight-threatening retinopathy and the timely intervention with laser photocoagulation has been shown to be effective in preventing severe visual loss. The annual incidence of retinopathy requiring ophthalmological follow up or treatment has been reported to average 1.5% after one year (Amos et al., 1997).

Several factors have been identified as determinants for the development of DR and its progression; including, type and duration of DM, age, gender, glycemic control, hypertension, body mass index (BMI), smoking, serum lipids and presence of microalbuminuria (Cai et al., 2006, Herrera et al., 2005).

2.7. Pathology and etiology of diabetic retinopathy

Abnormality in functions due to alterations in chemical, molecular biology or retinal physiology in the blood retinal barrier leads to early DR. The chemical changes eventually induce permeability, surface-adhesive, structural and metabolic changes to cells of the retinal capillary wall. This malfunction is the result of the toxicity of high glucose concentrations (hyperglycaemia) as well as the toxic effects of by-products from stressed metabolic pathways. The affected proteins have structural, catalytic or mediator functions; thus there are diverse physical manifestations of early diabetic eye disease.
These small symptoms in turn give rise to more serious manifestations of DR as the retina attempts to compensate. Auto-immune or viral destruction of the pancreatic $\beta$-cells, leading to insulin deficiency, causes Type I diabetes (Forrester and Knott, 1997). Insulin resistance of the cells leads to Type II diabetes. The retinal consequences of both these types (type-1 and 2 diabetes) are similar. Any variability between the consequences arises out of the different time of diabetes diagnosis. Type II diabetics often have sub-clinical disease for many years before receiving health care, whereas the symptoms of type I diabetics are acutely manifested in other organs. The myriad of consequences of hyperglycemia is the increased viscosity of diabetic blood, which is thought to exacerbate the hyperglycemic damage to the endothelial cells and basement membrane. The appearance of fenestrae and the lack of tight junctions influence the permeability of the vessel walls (Ishibashi and Inomata, 1993). This toxic or bio-chemical insult can lead to complete endothelial and pericyte cell loss, so that the capillaries become acellular or composed of the basement membrane alone (Stitt et al., 1995). The pericyte contractile cells, being only a partial structural component of the capillaries, are believed be regulatory in nature. Loss of pericytes cells combined with the loss of smooth muscle mass in the larger retinal vessels may increase the haemodynamic pressure, which causes distension and ballooning of capillaries also known as Microaneurysms or MA. The damaged endothelium attracts and adheres to the mobile and inflammatory cells of the blood, which results in capillary blockage. MAs are the indication of abortive new vessel growth attempting to re-canalize or re-perfuse the blocked vessel (Forrester and Knott, 1997). MAs represent one of the first clear and easily perceptible changes in micro-vascular morphology. The consequences of pericyte loss, endothelial cell damage and MA formation (potentially coupled with impairment of RPE function) are the starting point for the development of DR.

2.8. Terms Related To Diabetic Retinopathy:

i. **Microaneurysms**: These are the first clinical abnormality to be noticed in the eye. They may appear in isolation or in clusters as tiny, dark red spots or looking like tiny haemorrhages within the light sensitive retina. Their sizes ranges from 10-100
microns i.e. less than 1/12th the diameter of an average optics disc and are circular in shape (The Berries: Diabetic Retinopathy, http://www.theberries.ns.ca/ARchives/2006Winter/diabetic_retinopathy.html), at this stage, the disease is not eye threatening.

ii. **Haemorrhages**: Occurs in the deeper layers of the retina and are often called ‘blot’ haemorrhages because of their round shape.

iii. **Hard exudates**: These are one of the main characteristics of diabetic retinopathy and can vary in size from tiny specks to large patches with clear edges. As well as blood, fluid that is rich in fat and protein is contained in the eye and this is what leaks out to form the exudates. These can impair vision by preventing light from reaching the retina.

iv. **Soft exudates**: These are often called ‘cotton wool spots’ and are more often seen in advanced retinopathy.

v. **Neovascularisation**: This can be describe as abnormal growth of blood vessels in areas of the eye including the retina and is associated with vision loss. This occurs in response to ischemia, or diminished blood flow to ocular tissues. If these abnormal blood vessels grow around the pupil, glaucoma can result from the increasing pressure within the eye. These new blood vessels have weaker walls and may break and bleed, or cause scar tissue to grow that can pull the retina away from the back of the eye. When the retina is pulled away it is called a retinal detachment and if left untreated, a retinal detachment can cause severe vision loss, including blindness. Leaking blood can cloud the vitreous (the clear, jelly-like substance that fills the eye) and block the light passing through the pupil to the retina, causing blurred and distorted images. In more advanced proliferate retinopathy; diabetic fibrous or scar tissue can form on the retina (Vallabha et al., 2004).

### 2.9. Pathogenesis of Diabetic Retinopathy

The changes in micro and macro-vascular function and structure can lead to reduction or total loss of visual acuity (Edward, 2008). The progression, consequences and
manifestations of the break down of the blood-retina barrier are different in each individual. A healthy diabetic retina may develop none, some or all of the lesions typical of early retinopathy. Lesions such as MA, seemingly so integral to the development of DR may be present, absent and or undetectable in early DR. Yet despite these irregularities the terminology used to describe the status and stages of DR is linear; starting with a normal or healthy retina, and ending up with a phase of significant scaring and retinal tissue loss. Examples of such scales are published by Klein et al. (1989a), Feman et al. (1995), ETDRS Research Group (1991b) and Davis et al. (1998). In between these two extremes there are usually two general categories describing the progression of DR. These two categories are Non-Proliferative and Proliferative DR, or NPDR and PDR respectively.

2.10. Diabetes-associated Retinal Dysfunctions Before the Onset of Clinical Diabetic Retinopathy

Abnormalities in both vascular and neuronal retinal functions in diabetes can be detected before the clinical diagnosis of DR, based on the appearance of its morphological characteristics. One of the earliest retinal changes in diabetes is a decrease in retinal blood flow (Bursell et al., 1999), which has been attributed to the prolongation of blood transit time through retinal arterioles and capillaries. Although this hemodynamic change provides early evidence of retinal vascular dysfunction, the long term significance of diabetes-induced changes in blood flow is not yet available. Diabetes has been shown to increase retinal vascular permeability (RVP) and the adherence of leukocyte to the retinal endothelium, suggesting the local activation of inflammatory processes. Diabetes without DR has also been associated with prolonged retinal implicit time delays detected using a multifocal electroretinogram (Fortune et al., 1999), suggesting early and regional abnormalities in neuroretinal responses or conduction. Although these abnormalities in retinal function associated with diabetes may provide biomarkers for DR, it is not known whether the mechanisms that induce these early functional changes are the same as the mechanisms that drive the morphological changes associated with advanced stages of DR.
2.11. Classification of Diabetic Retinopathy

2.11.1. Non-proliferative Diabetic Retinopathy (NPDR)

The degree and severity of microaneurysms and intraretinal hemorrhages compared with well-established clinical photograph standards is a common marker used to define the level of non-proliferative (NP) DR, graded as mild, moderate and severe.

2.11.1.1. Mild NPDR

The first clinical signs of DR include the appearance of small and local lesions of microaneurysms, small retinal hemorrhages and calibre distortions called Intra-Retinal Microvascular Abnormalities or IRMA which are typical signs of mild NPDR. The occurrence of retinal hemorrhages indicates disruption of endothelium and basal lamina, enabling blood components to diffuse into the neuroretina. Histological studies have revealed decreased numbers of retinal pericytes and the appearance of acellular capillaries. These changes indicate a breakdown of small-vessel and capillary endothelium integrity.

Fig 2. 3 Microaneurysms, Small and punctuate red dots
Fig 2. 4 Hemorrhages (HA), dispersed red blotches with irregular perimiters.

Fig 2. 5 IRMA, Irregular calibres and tortousity of the smaller retinal vessels

2.11.1.2.       Moderate NPDR

Moderate NPDR is characterized by extensive intra-retinal hemorrhaging and a poorly perfused retina within which the venous vascular walls may distend. The distension alters the diameter and occasionally the path of the vessel. In other words, the vessels become
tourtous-of-path or beaded- in-appearance. At this point, further deterioration of the blood retina-barrier would take DR from a moderate non-proliferative state to severe state.

Fig 2.6 Venous beading, irregular distension of large and or smaller retinal vein calibres, excerpt of ETDRS Std.

2.11.1.3. **Severe NPDR**

Pericytes play a critical role in the maintenance of endothelial tight junctions and microvascular blood flow. Pericyte loss, along with the appearance of acellular capillaries, indicates microvascular damage that could eventually lead to areas of nonperfusion. Capillary blockage and capillary dropout may lead to larger areas of ischemic retina. Focal areas of non-perfusion or infarcts lead to accumulation of axoplasmic fluid, leading to soft exudation. These are diffuse white or greyish smears, also called cotton wool spots. Additional retinal abnormalities including hard exudates; where failure to reabsorb, or the accumulation of the leaked lipoproteins present small, well defined and bright patches and vitreoretinal abnormalities can also emerge during the progression of NPDR. Although these early retinal abnormalities are not typically
associated with vision loss, it is believed that these pathological changes contribute to the sequel of advanced DR.

Fig 2. 7 Soft exudates (Cotton wool spots), areas of axoplasmic accumulation

Fig 2. 8 Hard exudates, areas of lipoprotein mal-absorption, yellowish well defined contours.

2.11.2. Proliferative Diabetic Retinopathy (PDR)

PDR is characterized by the appearance of new pathological blood vessels that grow from existing retinal vasculature and vitreous/pre-retinal hemorrhage. Retinal ischaemia results in the production of vasoproliferative substances and to the development of neovascularization due to widespread capillary non perfusion. This neovascularization
can involve the retina, optic disc or the iris. The new blood vessels are fragile and tend to
grow in an anterior direction into the interface zone between the vitreous and the retina.
This zone expands with age and the new vessels rupture resulting in vitreous or pre-
retinal haemorrhage. After Neovascularisation Disk (NVD) or Neo-Vascularisation
Elsewhere (NVE) scar tissues arise in an attempt to heal and clear the haemorrhage.
These scars can contract and detach the retina with consequent damage to the retina.

Retinal neovascularization in DR is believed to be primarily driven by a hypoxic
response caused by microvascular damage, reduced retina perfusion, and local ischemia
that developed during NPDR. The newly formed blood vessels in PDR do not
appreciably improve retinal perfusion and can exacerbate vision loss by causing vitreous
hemorrhage and development of fibrous tissue that can lead to traction retinal
detachments.

Fig 2. 9 Neo Vascularisation Elsewhere, (NVE) Growth of capillary like new vessels on fundus but
not OD
Fig 2. 10 NeoVascularisationDisk, (NVD), Development of microvessels into the vitreo-retinal interface at OD

Fig 2. 11 Pre-retinal Haemorrhage, extravasated blood between retina and vitreous ETDRS Std.

**Diabetic macular oedema**

Among the lesions associated with diabetic retinopathy, macular oedema (MO) is most notable for its early and asymptomatic development (Klein et al., 1989b; Klein et al., 1995) and its successful treatment (ETDRS Research Group, 1985; ETDRS Research
Group, 1991c). It corresponds to a swelling in the macula, one of the areas of the retina. When some of the small blood vessels in the retina become blocked, the surrounding ones dilate to compensate for this. Increased vascular permeability results in the accumulation of plasma-like fluid in the dilated vessels, which in turn causes the macula to swell and cease to function.

Retinal swelling may also occur in the absence of significant extravasated fluid. In this case the cells of the matrix of structural cells engorge or swell. Unlike the other lesions there is less of a progression or development of MO; the severity being defined primarily by location, and only to some extent by the volume of the swelling or fluid. It is the involvement of the macula in oedema which is the critical factor. Macular oedema is hardly perceptible via non-stereoscopic images yet, hard exudates may be an indicator of MO, especially if circularly distributed. However, the relationship between the presence of MO and hard exudation is unknown.

![Diabetic macular oedema](image)

**Fig 2. 12 Diabetic macular oedema**

### 2.12. Pathways And Molecular Mechanisms Involved In Diabetic Retinopathy

There is an abundant evidence that diabetic retinopathy is associated with a range of systemic vascular complications, however, the underlying pathophysiological mechanisms remain obscure, which reflects, an incomplete understanding of the pathogenesis of the disease (Krentz et al., 2007; Frank, 2004).
Several mechanisms explaining pathophysiology of DR have been hypothesized. Earlier, it was believed that increased risk of systemic vascular complications in type 2 diabetic patients with retinopathy was due to their concomitant cardiovascular risk factors such as hypertension and dyslipidemia (Klein et al., 2002a,b). However, this is unlikely the case as many studies have demonstrated that cardiovascular risk factors alone cannot fully justify the observed associations (Cheung and Wong, 2008; Juutilainen et al., 2007), suggesting the existence of other biological mechanisms. It has been suggested that retinopathy is a manifestation of generalized vascular dysfunction caused by endothelial dysfunction or genetically determined alterations in the basement membrane metabolism associated with hyperglycemia. These vascular effects result in increased arterial permeability and leakage, which may develop retinopathy and nephropathy (Cheung and Wong, 2008).

There is a large body of literature available which indicates that retinopathy is associated with high blood glucose. In the presence of hyperglycemia, the endothelial cells lining the microvasculature, including the retinal microcirculation, experience oxidative stress and become activated. This in turn promotes adhesive interactions between circulating inflammatory cells along with their activation or priming. 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 act in concert with the flow disturbances and other physical factors. Thus, microvascular disease may play a critical intermediate pathogenic role, by providing the inflammatory drive in patients with DR. Support for this hypothesis is derived from numerous clinical and experimental studies showing that inflammation is a key factor in the development of diabetic retinopathy (Hansson, 2005; Toda and Nakanishi-Toda, 2007).

Thus, a number of physiological and pathological factors act together in the progression of DR and are related to each other. None of the factors can challenge alone to be the mechanism behind causing the disease. Here we present different pathways and
molecular mechanisms which contribute in some form or the other, in the development of DR.

2.12.1. AGEs

High glucose both in type 1 and type 2 diabetes leads to formation of reactive derivatives via non-enzymatic protein glycation, i.e. nonenzymatic condensation reaction between reducing glucoses and amine residues of proteins, lipids, or nucleic acids. These derivatives undergo a series of complex reaction to give an irreversible cross-linked complex group of compound termed advanced glycation end products (AGEs) (Fig. 2.13), which are implicated in endothelial cell injury and blood–retinal barrier (BRB) dysfunction (Stitt, et al., 2000). Endothelial cell injury occurs as AGEs increase vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), and intercellular adhesion molecule-1 (ICAM-1) expression in microvascular endothelial cells through intracellular ROS generation (Ola, et al., 2012). Although, other factors like increased polyol pathway activity, PKC activation, oxidative stress, all of which lead to induce VEGF, can be improved by strict glycemic control, but the AGEs are resistant to degradation and continue accumulating in ocular tissues even in patients with good glycemic control (Toyoda et al., 2011). Retinal pericytes, which play an important role in the maintenance of microvascular homeostasis, accumulate AGEs during diabetes. Increase in AGEs formation correlate with degree of DR and their accumulation has been found in retinal blood vessels, serum and vitreous of diabetic patients and animals (Goh & Cooper, 2008; Stitt, 2001).

Among the numerous AGEs formed, carboxymethyllysine (CML) is thought to be the major product causing ocular complications in diabetes (Ghanem et al., 2011; Boehm et al., 2004; Hirata et al., 2004). CML accumulation can be identified even in early-stage DR, while in the advanced stages, accumulation of CML accompanies progression of DR (Hammes et al., 1999).

AGEs interact with the receptor of AGEs (RAGE) and form AGE–RAGE complex. The RAGE is a member of the immunoglobulin superfamily of cell surface molecules. AGE–
RAGE interaction triggers the activation of critical cell signaling pathways, such as p21ras, mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) (Fig. 2.13), which lead to the activation of proinflammatory responses and the cellular damage resulting in the inflammation, neurodegeneration, microvascular dysfunction and other complications of DR (Ehlermann et al., 2006; Yamagishi, 2009; Zong, et al., 2011). However, there’s still a need to discover the cellular and molecular processes that initiate the AGEs-induced progression of DR so that specific target of AGE-RAGE axis can be diagnosed.


2.12.2. PKC

In the case of diabetic retinopathy, tight glucose and blood pressure control are difficult to achieve and maintain in practice, yet still only provide partial protection against sight threatening complications in the lifetime of a person with diabetes (Zhang, et al., 2001).
Hyperglycemia leads to increased de novo synthesis of diacylglycerol (DAG), which is synthesized *de novo* in cells with a low aldose reductase activity such as endothelial cells, from the glycolytic intermediates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Way, et al., 2001). DAG is the endogenous activator of various isoforms of protein kinase C (PKC), a family of serine/threonine kinases that mediates a variety of functional and structural abnormalities in vascular tissues like retina in diabetic retinopathy (Geraldes & King, 2010). PKC causes phosphorylation of numerous substrate proteins thereby triggering a cascade of pathophysiological responses. In diabetic retinopathy, it leads to changes in endothelial permeability, blood flow and formation of angiogenic growth factors which in turn contribute to retinal leakage, ischaemia and neovascularisation. Some of the changes due to PKCs activation include increase in blood flow, basement membrane thickening, extracellular matrix expansion, vascular permeability, apoptosis, angiogenesis, leukocyte adhesion, cytokine activation and loss of capillary pericytes, all early features of diabetic retinopathy (Das Evcimen & King, 2007; Donnelly et al., 2004).

### 2.12.3. Polyol pathway

Polyol pathway is a metabolic pathway where a part of excess glucose gets metabolized. Secondary to hyperglycemia, the intracellular glucose level is elevated and subsequently reduced to sorbitol by the enzyme aldose reductase (AR) and then to fructose. The role of augmented polyol pathway in diabetic retinopathy has been debated for long. The increased level of glucose under diabetic condition activates polyol pathway, making excess sorbitol using NADPH. It reduces NADPH to NAD (Fig.2.14). NADPH is required not only by glutathione reductase for the reduction of oxidized glutathione (GSSG) to glutathione (GSH), but also by AR for conversion of glucose to sorbitol. It results in less glutathione and increased oxidative stress, the major factors in retinal damage (Ola, et al., 2012; Barba et al., 2010; Lorenzi, 2007). Polyol pathway activation thus initiates several mechanisms of cellular damage by activation and interaction of AR and other pathogenetic factors such as formation of AGE, activation of oxidative–
nitrosative stress, PKC pathway, and PARP that may initiate inflammation and growth factor imbalances (Obrosova & Kador, 2011).

Fig 2. Polyol Pathway

2.12.4. Hexosamine pathway

Under normal conditions, about 3% glucose inside the cell enters the hexosamine pathway. As the vascular effects of the hexosamine pathway, in which fructose 6-phosphate is converted into glucosamine 6-phosphate by the enzyme glutamine:fructose-6-phosphate amidotransferase, UDP-N-acetylhexosamine (UDP-HexNAc), is synthesized. UDP-HexNAc is an essential substrate for the synthesis of glycosyl side chains for proteins and lipids (Fig.2.15). A little change in the amount of glucose flux through this pathway may have diverse effect on protein function. Hexosamine content increases in retinal tissues of diabetic humans and rats (Giacco & Brownlee, 2010). An increased flux through this pathway results in modulation of transcription factors and pathological changes in gene expression. It can also impair insulin signaling in retina.
(Nakamura et al., 2001). Hexosamine pathway, therefore is considered as a potential pathway implicated in DR.

![Hexosamine pathway diagram](image)


2.12.5. Oxidative stress

Oxidative stress holds an important role in diabetic complications, and reactive oxygen species (ROS) are considered a causal link between hyperglycemia and abnormal metabolic changes important in the development of diabetic complications. Retina and capillary cells experience increased oxidative damage in the diabetic mellitus, and the antioxidant defense mechanism is impaired (Kowluru and Kanwar, 2009).

Hyperglycemia generates ROS by activation of AGE, AR, hexosamine, and PKC pathways. Other sources of oxidative stress are the activation of NADPH oxidase which may increase superoxide, induction of xanthine oxidase, impaired activities of antioxidant
defense enzymes- superoxide dismutase and catalase, and decreased level of glutathione (Al-Shabrawey et al., 2008). In diabetic retina, high glucose stimulates flux through the glycolytic pathway, and increases tissue lactate-pyruvate ratios, cytosolic NADH and tricarboxylic acid cycle flux which may load the mitochondria with electrons, thereby producing excess of ROS (Fig. 2.16) (Madsen-Bouterse & Kowluru, 2008). Recent clinical studies have substantiated the concept of ‘metabolic memory’ in the pathogenesis of DR, which defines some basic causes of chronic damage in diabetic vessels that are not easily reversed, even on subsequent good blood glucose control (Kowluru & Chan 2010; Zhong & Kowluru, 2011). It is established that hyperglycemia might not be the major source of mitochondrial ROS in diabetes for sustained production of oxidative stress. This is the reason that even intensive therapy to control blood glucose has some long-term effects on the risk of DR. Several studies have shown that despite controlled blood glucose, altered lipoprotein metabolism, excess level of excitatory amino acids and altered growth factors may act as potential sources of ROS generation (Kanwar & Kowluru, 2009; Zheng & Kern, 2009). Along with the production of ROS, oxidative stress also activates other metabolic pathways that are detrimental to the development of DR. It is still unclear whether oxidative stress has a primary role in the pathogenesis of diabetic complication occurring at an early stage in diabetes or whether it is a consequence of the tissue damage (Fernandes, Hosoya, & Pereira, 2011; Izuta et al., 2010). The development of novel therapeutic strategies that specifically target major pathways and the sources of ROS is desired for DR patients.
Fig 2.16 Hyperglycemia-induced biochemical alterations caused by oxidative stress leading to diabetic retinopathy. (Modified from Balasubramanyam et al., 2002).

2.12.6. Renin Angiotensin System (RAS)

In patients with DR, tight control of blood pressure delays the progression of the disease, and growing evidence suggests that RAS plays an important role in the regulation of blood pressure. Renin–angiotensin system (RAS), active both systemically and locally in the eye, is a potential angiogenic mechanism of DR. It is an enzymatic cascade which begins with the conversion of the prorenin, to active renin (Satofuka et al., 2009). Renin converts angiotensinogen, the precursor of angiotensin peptides, to angiotensin I (Ang I) which is further cleaved by angiotensin-converting enzyme (ACE) to angiotensin II (Ang II). Ang II is the main effector peptide of the RAS, acting primarily on two receptors, the angiotensin-1 (AT-1) and angiotensin-2 (AT-2). Renin, ACE and AT-2 receptors are distributed throughout the retinal and choroidal vessels. Plasma and intravitreal prorenin, renin, and Ang II concentrations are increased in DR patients and correlate with its severity (Noma et al., 2009; Wright and Dodson, 2010). Angiotensin has been shown to be an angiogenic growth factor in animal experiments (Sarlos et al., 2003) promoting differentiation, apoptosis, and the deposition of extracellular matrix (Otani, et al., 2001).
Angiotensin stimulates growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor, VEGF, and connective tissue growth factor, thereby inducing cell growth, proliferation and the deposition of extracellular matrix proteins (Ruperez et al., 2003). Ang II induces RAGE expression in hypertensive eye and potentiates deleterious effect of AGEs, indicating a link between AGE-RAGE, and thus, RAS is involved in the pathogenesis of DR. this has led to a major interest in RAS inhibitors to prevent retinopathy.

2.12.7. Inflammation

Inflammation is a nonspecific response to injury that includes a variety of functional and molecular mediators. Inflammation although beneficial on an acute basis, can however have undesirable effects if persisting chronically. The increased expression of many inflammatory proteins is regulated at the level of gene transcription through the activation of proinflammatory transcription factors, including NF-κB. These proinflammatory transcription factors are activated and play a critical role in amplifying and perpetuating the inflammatory process. Proinflammatory proteins like COX-2, interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α) etc can contribute to cell damage and death in tissues including brain and retina (Vincent and Mohr, 2007).

2.12.7.1. IL-1β

IL-1β, a 17kDa glycoprotein, produced by monocytes, macrophages, lymphocytes or fibroblasts, stimulates various cells to release a cascade of inflammatory signals (Meacock et al., 2000). In diabetic conditions, the levels of this cytokine are increased in the endothelial cells of the retina (Abu et al., 1992; Yuuki et al., 2001; Carmo et al., 1999; Kowluru and Odenbach, 2004). IL-1β can induce the expression of many genes whose promoters are regulated through complex interactions with NF-kB (Kowluru and Odenbach, 2004). NF-kB is a heterodimer which is localised in the subretinal membranes and in the microvessels (Hammes et al., 1999). It consists of two subunits, p50 and p65, and is usually stored in its inactive form in the cytosol, which gets activated on stimulation of IL-1β. Activation of NF-κB typically involves the phosphorylation of
cytoplasmic IκB by the IκB kinase (IKK) complex, resulting in IκB degradation via the proteosomal system. The degradation of IκB releases the NF-κB heterodimers to translocate to the nucleus where they bind to nuclear DNA, leading to activation and transcription of specific subsets of genes involved in apoptosis (Timothy, 2007). It transcriptionally activates various cellular genes associated with immune response, inflammation, and oxidative stress (Teng, et al, 2002). In high glucose condition, this activation of NF-kB in the retina and capillary cells leads to accelerated loss of pericytes as observed in diabetic retinopathy. It causes diabetic-induced retinal inflammation by up-regulation of ICAM-1, MCP-1, and VEGF (Ola et al., 2012). Thus, the NF-kB activation is an early event in the development of diabetic retinopathy, and it exerts proapoptotic effects on retinal endothelial cells that are aggravated in high glucose conditions.

IL-1β is also known to be one of the most potent stimuli for inducible form of nitric oxide synthase (iNOS). High glucose enhances the IL-1β induced NO production by the transcriptional induction of iNOS protein and by the augmentation of iNOS activity (Ueda et al., 2003). Evidences suggest that oxidative stress and changes in nitric oxide formation play significant roles in the onset of diabetic retinopathy. In hyperglycemic conditions iNOS and NO levels are upregulated in the retina and its capillary cells (Du et al., 2004; Kowluru et al., 2003). iNOS oxidizes arginine to citrulline in the presence of biopterin, NADPH, and oxygen. These increased oxidative stress and subsequent activation of NF-kB contribute to the development of diabetic retinopathy. NF-kB enhances nitric oxide production, which is a mediator of islet beta-cell damage. Nitric oxide may react with superoxide anion radical to form reactive peroxyl nitrite radicals (Maritim et al., 2003). It can thus be suggested that increase in IL-1β in the diabetic retinal cells contributes to the increased levels of NO and apoptosis in the retina and its capillary cells. The increased inflammatory IL-1β and high glucose are together involved in the course and progression of diabetic retinopathy.
2.12.7.2. TNF-α

Tumor necrosis factor (TNF)-α is a pleiotropic/angiogenic cytokine, whose levels are upregulated in diabetic retina and vitreous and is implicated for early inflammatory changes in the retinopathy (El-Remessy, et al., 2006). Astrocytes and Muller cells of retina are potential source of TNF-α in diabetes (Joussen et al., 2002; Demircan et al., 2006). It is involved in the up-regulation of receptors for adhesion molecules on leukocytes (Joussen et al., 2004; Schram et al., 2005; Carin et al., 2008). It has been shown that TNF-α immunoreactivity is significantly higher in retinal (Limb et al., 1996) and plasma specimens from both type 1 and type 2 diabetic patients with PDR and this elevation is correlated with an increased activity of the glycosylating enzyme core 2 GlcNAc-T in polymorphonuclear cells (Ben-Mahmud et al., 2006). Increased TNF-α expression may further drive NF-κB-dependent gene transcription (Song et al., 1997). Alternatively, TNF could enhance apoptosis through indirect mechanisms, for example, by reducing the expression of other pro-apoptotic factors (Alikhani et al., 2005) or reduce leukostasis that can promote endothelial injury through various pathways (Koizumi et al., 2003). Whatever the mechanism be, but TNF-α plays a major role in the loss of microvascular endothelial cell and pericyte apoptosis as a pro-apoptotic cytokine (Behl et al., 2008).
2.12.8. Growth Factors

2.12.8.1. Vascular Endothelial Growth Factor (VEGF)

In DR, there are typical functional alterations in the retinal pathology which include thickening of the basement membrane, hyperpermeability and formation of microaneurysms. These changes are followed by microvascular occlusions leading to a progressive retinal ischemia/hypoxia that induces the release of VEGF, a potent mitogen for endothelial cells, also known as vascular permeability factor. VEGF is related to platelet-derived growth factor in structure and is the strongest angiogenic cytokine known and a potent enhancer of vascular permeability. It triggers proliferation, migration and tube formation leading to growth of new blood vessels which are fragile and may break. Several experiments in animal models of hypoxia-induced ocular neovascularization have shown that VEGF is upregulated several fold before the formation of new blood vessels and that blocking its action inhibits retinal neovascularization. VEGF is thus, the major regulator of physiological and pathological angiogenesis (Ferrara, 2009) and plays a key role in the development of DR (Wirostko et al., 2008; Penn et al., 2008). The expression of VEGF in diabetic retina can be regulated by different pathways, such as Rho/Rho Kinase, ERK1/2, and PKC (Ye et al., 2010; Yokota et al., 2007; Clarke and Dodson, 2007). The exact mechanism whether how PKC exerts its modulation on VEGF expression, is not clear. However, it is suggested that a molecular cascade involving PKCβ, the mRNA-binding protein (RBP) HuR and VEGF exist in the retinal pericytes (Amadio et al., 2010). HuR is an ubiquitously expressed member of the Embryonic Lethal Abnormal Vision (ELAV) family, highly conserved RBPs which act post-transcriptionally as positive regulators of gene expression (Bolognani and Perrone-Bizzozero, 2008). In normal conditions VEGF mRNA is extremely labile, however, its half-life increases by 2–3-fold during hypoxia, such as following retinal capillaries occlusion, due to the stabilizing effect of HuR (Amadio et al., 2010). Moreover, it is reported that acute ischemia induces a rapid binding of HuR to the VEGF mRNA leading to increased levels of the corresponding VEGF protein (Tang et al., 2002). HuR seems to protect VEGF mRNA from ribonucleases by masking specific instability signals within
the VEGF mRNA sequence itself, thus, reduces its decay rate on the one hand and enhances its translation on the other (Amadio et al., 2010).

2.12.8.2. Insulin-like growth factor-I (IGF-I)

The pituitary factor has been identified as growth hormone and the mitogenic mediator of its action is Insulin-like growth factor-I (IGF-I) (Smith et al., 1997). An acute increase in serum levels of IGF-I lead to the onset of PDR in animal models (Grant et al., 1993). Subsequently, increased IGF-I levels were measured in the vitreous of patients with PDR (Lee et al., 1994) indicating that IGF-I may play a role in retinal neovascularization. It has been proposed that leakage across the blood retina barrier and high serum levels of IGF might be the major source for vitreous IGF levels. IGF-I can induce almost all steps of the angiogenesis process including endothelial cell proliferation, migration and basement membrane degradation (Nicosia et al., 1994). It exerts its effect on endothelial cells via coupling with the IGF-I receptor (IGF-IR), and is regulated by a family of insulin-like growth factor binding proteins (IGFBPs) (Burren et al., 1996) which can inhibit or potentiate IGF-I activity depending upon their affinity for IGF-I, the biological system in question and posttranslational modifications (Tollefsen et al., 1991). IGFBPs 1, 2 and 3 have been reported to be significantly increased in vitreous from patients with PDR but not in non-ischaemic eye disease (Burgos et al., 2000).

2.12.8.3. Basic Fibroblast Growth Factor (bFGF)

bFGF and hypoxia act synergistically to not only induce mitogenesis in endothelial cells, but also to upregulate VEGF in smooth muscle cells and endothelial cells, resulting in retinal angiogenesis (Stavri et al., 1995). However, the fact that bFGF-deficient animal models develop the same degree of retinal neovascularization as wild-type animals argues against a major angiogenic role for bFGF in diabetic retinopathy (Ozaki H et al., 1998). Although bFGF may not directly induce retinal neovascularization, it can regulate VEGF expression in retinal vascular cells (Seghezzi et al., 1998).
2.13. Changing paradigms in the treatment of Diabetic Retinopathy

Five large randomized controlled trials provided the scientific basis for care in the diabetic patient to preserve vision. The Diabetes Control and Complications Trial, the United Kingdom Prospective Diabetes Study, the Diabetic Retinopathy Study, the Early Treatment Diabetic Retinopathy Study and the Diabetic Retinopathy Vitrectomy Study demonstrated that strict metabolic control early in the course of diabetes, tight blood pressure control, panretinal photocoagulation, focal/grid laser photocoagulation, and early vitrectomy in patients with type 1 diabetes who had recent vitreous hemorrhage were effective at slowing the progression of retinopathy and reducing visual loss.

2.13.1. The diabetes control and complications trial (DCCT)

The DCCT randomized 1441 type 1 diabetic patients to receive intensive glycemic or conventional therapy. Over 6.5 years of follow-up, long-term observational DCCT data showed that despite gradual equalization of HbA1c values after study termination, the rate of DR progression remained significantly lower in the intensively treated group than in the conventional group (White et al., 2008), emphasizing the importance of instituting tight glycemic control early in the course of diabetes.

In the DCCT, the long-term benefits of intensive insulin treatment greatly outweighed the risks of early worsening of DR. Therefore, ophthalmoscopic monitoring before initiation of intensive treatment and at 3-month intervals for 6–12 months thereafter seems to be appropriate when intensive treatment is initiated in patients with long-standing poor glycemic control, particularly if retinopathy is at or past the moderate non-proliferative stage (Abu El-Asrar and Al-Mezaine, 2011).

2.13.2. The United Kingdom prospective diabetes study (UKPDS)

The UKPDS randomized 3867 patients with newly diagnosed type 2 diabetes to receive intensive or conventional therapy. After 12 years of follow-up, the progression of DR was reduced by 21% and the need for laser photocoagulation by 29% in the intensive versus the conventional treatment group (UKPDS Group 33, 1998). The UKPDS also investigated the influence of tight blood pressure control. A total of 1148 hypertensive
patients with type 2 diabetes were randomized to less tight (<180/105 mmHg) and tight blood pressure control (<150/85 mmHg). With a median follow-up of 8.4 years, patients assigned to the tight control group had a 34% reduction in progression of retinopathy and a 47% reduced risk of deterioration in visual acuity of three lines compared with the less tight control group (UKPDS Group 38, 1998).

2.13.3. The diabetic retinopathy study (DRS)

The DRS investigated whether scatter (panretinal) photocoagulation, compared with indefinite deferral, could reduce the risk of vision loss from PDR. After 2 years, photocoagulation was shown to significantly reduce severe visual loss from PDR. The benefit persisted through the entire duration of follow-up and was greatest among patients whose eyes had high-risk characteristics (Anonymous, 1978).

2.13.4. The early treatment diabetic retinopathy study (ETDRS)

The ETDRS demonstrated that focal/grid laser photocoagulation reduced the risk of vision loss from clinically significant macular edema by 50% or more (ETDRS, 1985). ETDRS analyses also indicated that type 2 diabetic patients should consider scatter photocoagulation at the time of the development of severe NPDR or early PDR (Ferris, 1996).

Diabetic Retinopathy Clinical Research Network also stated that focal/grid photocoagulation remains the standard management technique for diabetic macular edema (Aiello et al., 2010).

2.13.5. The diabetic retinopathy vitrectomy study (DRVS)

The DRVS randomized 616 eyes with vitreous hemorrhage reducing visual acuity to 5/200 or less. After two years of follow-up, 25% of the early vitrectomy group had visual acuity of 10/20 or better compared with 15% of the deferral group. Type 1 diabetic patients, who were on average younger and had more severe PDR, early vitrectomy was beneficial. However, no such advantage was found in the type 2 diabetic group (DRVS, 1985).
The DRS and the ETDRS showed that laser photocoagulation for DR is effective at slowing the progression of retinopathy and reducing visual loss, but the treatment usually does not restore lost vision. Because these treatments are aimed at preventing vision loss and retinopathy can be asymptomatic, it is important to identify and treat patients early in the disease. To achieve this goal, patients with diabetes should be routinely evaluated to detect treatable disease. Guidelines for the frequency of diabetic eye examinations have been largely based on the severity of retinopathy (Fong et al., 2003).

2.14. Emerging therapies

Due to the limitations of the current treatments, new therapeutic approaches are being developed.

2.14.1. Intravitreal triamcinolone acetonide

Intravitreal triamcinolone acetonide (IVTA) generates favorable results in the treatment of diffuse DME. However, its major limitation is its relatively short duration of action of not more than 3 months (Beer et al., 2003), which results in the recurrence of DME, thus necessitating repeated applications of IVTA which carries risk and is inconvenient for patients (Kang et al., 2006, Gillies et al., 2006; Rudnisky et al., 2009; Yilmaz et al., 2009). However, the possibility of combining IVTA with focal/grid photocoagulation may produce greater benefit for DME than either IVTA or focal/grid photocoagulation alone (Diabetic Retinopathy Clinical Research Network, 2008), which has been proved in clinical trials. Several small randomized clinical trials demonstrated that the combination of laser photocoagulation (panretinal and macular) with IVTA resulted in improved visual acuity and decreased central macular thickness and total macular volume when compared with laser photocoagulation alone for the treatment of PDR and macular edema (Lam et al., 2007; Maia et al., 2009). On the other hand, Mirshahi et al., 2010 demonstrated no beneficial effect of combined IVTA plus panretinal photocoagulation and macular photocoagulation in eyes with coexisting high-risk PDR and clinically significant macular edema as compared with panretinal photocoagulation and macular photocoagulation as standard treatment in those patients.
Also, two studies compared the morphological and visual acuity outcomes associated with a single IVTA versus bevacizumab for the treatment of DME. These studies concluded that one single intravitreal injection of triamcinolone showed better results in reducing DME and in the improvement of visual acuity than that of bevacizumab in the short-term management of DME. However, intravitreal bevacizumab had the advantage of intraocular pressure stability compared with the triamcinolone injection (Paccola et al., 2008; Shimura et al., 2008).

2.14.2. Anti-vascular endothelial growth factor (VEGF) treatment

Currently, there are four anti-VEGF agents which have been used in the management of diabetic retinopathy, including pegaptanib (Macugen; Pfizer, Inc., New York, USA), ranibizumab (Lucentis; Genentech, Inc., South San Francisco, California, USA), bevacizumab (Avastin; Genentech, Inc.), and VEGF Trap-Eye (Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA).

2.14.3. Pegaptanib

A phase II clinical trial of intravitreal pegaptanib in patients with DME demonstrated better visual acuity outcomes, reduced central retinal thickness, and reduced need for additional photocoagulation therapy (Cunningham et al., 2005). Patients with retinal neovascularization at the baseline showed regression of neovascularization after intravitreal pegaptanib administration (Adamis et al., 2006). Querques et al. (2009) demonstrated that repeated intravitreal pegaptanib produced significant improvement in best-corrected visual acuity and reduction in mean central macular thickness in patients with diabetic macular edema. González et al. (2009) showed that intravitreal pegaptanib produced short-term marked and rapid regression of diabetic retinal neovascularization. All these data suggest that VEGF blockade is a safe and efficacious adjuvant treatment to panretinal photoagulation in PDR.

2.14.4. Ranibizumab

Ranibizumab is a recombinant humanized monoclonal antibody fragment with specificity for all isoforms of human VEGF-A. Pilot studies of intravitreal ranibizumab
demonstrated reduced foveal thickness and maintained or improved visual acuity in patients with DME (Chun et al., 2006). Nguyen et al. (2009) demonstrated that during a span of 6 months, repeated intravitreal injections of ranibizumab produced a significantly better visual outcome than focal/grid laser treatment in patients with DME. Nguyen et al. (2010) showed that intraocular injection of ranibizumab provided benefit for diabetic macular edema for at least 2 years, and when combined with focal or grid laser treatments, the amount of residual edema was reduced, as were the frequency of injections needed to control edema.

2.14.5. Vascular endothelial growth factor Trap-Eye

VEGF Trap is a 115 kDa recombinant fusion protein consisting of the VEGF binding domains of human VEGF receptors 1 and 2 fused to the Fc domain of human IgG1. A pilot study showed that a single intravitreal injection of VEGF Trap-Eye was well tolerated and was effective in patients with DME (Do et al., 2009).

2.14.6. Bevacizumab

Bevacizumab is a full length recombinant humanized antibody active against all isoforms of VEGF-A. It is FDA-approved as an adjunctive systemic treatment for metastatic colorectal cancer. Several studies reported the use of the off-label intravitreal bevacizumab (IVB) to treat DME, PDR complications, and iris neovascularization.

To date, all studies regarding IVB (1.25 mg) for DME therapy, have demonstrated transient beneficial effects with a requirement for repeated injections (Arevalo et al., 2009a; Roh et al., 2008; Fang et al., 2008). Lam et al. (2009) demonstrated that IVB was more effective in eyes without previous DME treatment, which included focal or grid laser photocoagulation. Two studies demonstrated that IVB at doses of 1.25 and 2.5 mg seems to have similar treatment efficacy in patients with DME (Lam et al., 2009; Arevalo et al., 2009a). Bonini-Filho et al. (2009) showed that IVB for DME with severe capillary loss was associated with beneficial effects on vision, central macular thickness, and total macular volume. Soheilian et al. (2009) reported that IVB in patients with DME yielded a better visual outcome compared with macular photocoagulation.
Several studies demonstrated that IVB injection resulted in marked regression of retinal and iris neovascularization, and rapid resolution of vitreous hemorrhage in patients with PDR (Arevalo et al., 2009b; Jiang et al., 2008; Wakabayashi et al., 2008).

The use of preoperative IVB injection few days before planned pars plana vitrectomy for the treatment of complications of PDR was also found to be efficacious and safe as an adjuvant treatment to facilitate surgery, prevent rebleeding, and accelerate postoperative vitreous clear-up (Ahmadieh et al., 2009; di Lacro et al., 2010). However, tractional retinal detachment may occur or progress shortly following administration of IVB in these patients (Arevalo et al., 2008). Several studies determined the clinical effectiveness of IVB combined with cataract surgery for the management of the postoperative increase of retinal thickness in patients with DME. The short-term results suggest that IVB has the potential not only to prevent the increase in retinal thickness, but also reduce the retinal thickness of eyes with DME after cataract surgery (Takamura et al., 2009; Lanzagorta-Aresti et al., 2009).

2.14.7. Vitrectomy for persistent diffuse diabetic macular edema

Vitrectomy with removal of the premacular posterior hyaloid for persistent diffuse macular edema is widely accepted. Several studies evaluating the efficacy of vitrectomy have yielded conflicting results. Stolba et al. (2005) showed in a prospective randomized trial that vitrectomy with internal limiting membrane peeling was superior to observation in eyes with persistent diffuse DME that previously failed to respond to conventional laser treatment and positively influenced distance and reading visual acuity. Other studies suggested that vitrectomy with and without internal limiting membrane peeling may provide anatomic and visual benefit in eyes with diffuse non-tractional unresponsive DME refractory to laser photocoagulation (Kumagai et al., 2009; Yamamoto et al., 2007). On the other hand, studies have also shown that the benefits of vitrectomy for DME in terms of visual acuity and macular thickness were limited to patients who exhibited signs of macular traction (Shah et al., 2006; Figueroa et al., 2008). The factors associated with favorable outcomes after vitrectomy for DME were also evaluated (Flaxel et al., 2010). Greater visual acuity improvement occurred in eyes with worse baseline acuity and in
eyes in which an epiretinal membrane was removed. Greater reduction in central subfield thickness occurred with worse baseline visual acuity, greater preoperative retinal thickness and removal of internal limited membrane.

Few studies reported that there was no difference in the absorption rate of macular edema or the functional outcome after vitrectomy (Kumagai et al., 2009; Shiba et al., 2009).


Knowing the role of the vitreous body in DR, investigators have used pharmacologic vitreolysis in the management of diabetic retinopathy. A phase III clinical trial has shown that 55 IU of highly purified ovine hyaluronidase (vitrease) helps to clear vitreous hemorrhage 1 month after intravitreal application (Kuppermann et al., 2005a,b). No serious safety issues were reported (Kuppermann et al., 2005a,b). In particular, the incidence of retinal detachment was not statistically different between treated eyes and control groups.

Quiram et al. (2007) demonstrated that intravitreal injection of microplasmin with induction of the combination of posterior vitreous detachment (PVD) and vitreous liquefaction increased intravitreal oxygen tension. On the other hand, hyaluronidase-induced vitreous liquefaction without PVD induction failed to increase intravitreal oxygen tension. Moreover, when microplasmin treated animals were exposed to 100% oxygen, there was an accelerated increase in oxygen levels in the midvitreous cavity compared to control or hyaluronidase treated eyes. These findings suggest that the beneficial effects of surgical vitrectomy in increasing oxygen tension in the vitreous cavity (Stefansson, 2009) may be reproduced with enzymatic induction of PVD and vitreous liquefaction without the time, risks, and expense of surgery.

It is more difficult to separate the vitreous cortex from the internal limiting membrane in diabetic eyes than in nondiabetic eyes. This is likely due to the effects of diabetes on the macromolecules of vitreous and the structural consequences (Zhi-Liang et al., 2009). In an experimental rat model of diabetes, the combination of hyaluronidase causing vitreous
liquefaction and plasmin acting as a PVD inducer was more effective than plasmin alone in inducing complete PVD (Zhi-Liang et al., 2009).

Pilot clinical studies in diabetic eyes found that autologous plasmin was a safe and effective adjunct to vitrectomy for DME and PDR. Intravitreal injection of autologous plasmin enzyme before surgery was useful in inducing a pharmacologic PVD, and thus mechanical PVD was not necessary in the eyes with DME secondary to posterior vitreous cortex contraction (Sakuma et al., 2006; Azzolini et al., 2004; Asami et al., 2004). Plasmin-assisted vitrectomy allowed a more complete and less traumatic posterior vitreous cortex removal with a smooth retinal surface. The internal limiting membrane removed during the plasmin-assisted vitrectomy from eyes with diabetic macular edema demonstrated cleaner and flatter surfaces, whereas the internal limiting membrane removed without the use of autologous plasmin enzyme had remnants of the vitreous cortex more frequently (Asami et al., 2004). Autologous plasmin enzyme was also beneficial in the surgical management of PDR. The proliferative membranes became softened and were easily peeled without retinal tears (Hirata et al., 2007).

Recently, Diaz-Llopis et al. (2009) demonstrated that intravitreal injection of autologous plasmin enzyme without the performance of vitrectomy induced complete PVD and effectively reduced macular thickening due to refractory diffuse diabetic macular edema and improved visual acuity. Therefore, atraumatic pharmacologic separation of the posterior vitreous cortex with clean cleavage between the internal limiting membrane and the posterior hyaloids without performing a vitrectomy can reduce the risk of intraoperative iatrogenic damage such as retinal tears, and damage to the nerve fibers, and the postoperative sequelae.

2.14.9. Fibrates

Fibrates are widely prescribed lipid-lowering drugs in the treatment of dyslipidemia. Their main clinical effects, mediated by peroxisome proliferative activated receptor alpha activation, are a moderate reduction in total cholesterol and low-density lipoprotein cholesterol levels, a marked reduction in triglycerides and an increase in high-density
lipoprotein cholesterol. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study demonstrated that long-term lipid-lowering therapy with fenofibrate reduced the progression of diabetic retinopathy and the need for laser treatment in patients with type 2 diabetes, although the mechanism of this effect does not seem to be related to plasma concentration of lipids (Keech et al., 2007). Recently, ACCORD Study Group (2010) demonstrated that fenofibrate for intensive dyslipidemia therapy reduced the rate of progression of diabetic retinopathy in persons with type 2 diabetes (ACCORD Study Group, 2010).

2.14.10. Renin-angiotensin system (RAS) blockers

Several studies suggested that RAS blockers might reduce the burden of diabetic retinopathy. The findings of the Eurodiab Controlled trial of Lisinopril in Insulin-dependent Diabetes (EUCLID) suggested that blockade of the renin-angiotensin system with the angiotensin-converting enzyme inhibitor lisinopril could reduce both incidence and progression of retinopathy in type 1 diabetes (Chaturvedi et al., 1998). Recently, the Diabetic Retinopathy Candesartan Trials (DIRECT) demonstrated that the angiotensin-receptor antagonist candesartan reduced the incidence of retinopathy in patients with type 1 diabetes (Chaturvedi et al., 2008), and might induce improvement of retinopathy in type 2 diabetic patients with mild-tomoderate retinopathy (Sjølie et al., 2008).

2.14.11. Peroxisome proliferator-activated receptor gamma (PPARc) agonists

The PPARc agonist rosiglitazone inhibited both the retinal leukostasis and retinal leakage observed in the experimental diabetic rats. In addition, the decreased expression of the endogenous PPARc in mice leads to the aggravation of retinal leukostasis and retinal leakage in diabetic mice (Muranaka et al., 2006). Rosiglitazone maleate (Avandia; GlaxoSmithKline, North Carolina, USA) is an orally administered medication used to improve glycemic control in patients with diabetes mellitus. This medication activates the PPARc and leads to insulin sensitization in adipose and other tissues, with potential antiangiogenic activity. Recently, Shen et al. (2008) demonstrated that rosiglitazone may delay the onset of PDR in patients with severe nonproliferative diabetic retinopathy at the
baseline. Several studies showed that the use of glitazone class of drugs was associated with DME (Fong and Contreras, 2009). However, another retrospective study concluded that rosiglitazone is not linked to DME (Tatti et al., 2008).

2.14.12. Ruboxistaurin

Hyperglycemia activates protein kinase C (PKC) by inducing de novo synthesis of diacylglycerol, a physiologic activator of PKC. Substantial data suggest that the β isoform may play an important role in the development of diabetic microvascular complications. Increased PKC β isoform activity induces retinal vascular permeability and neovascularization in animal models. Ruboxistaurin (RBX) (LY333531; Lilly Research Laboratories, Indianapolis, Indiana, USA) is a PKC β-selective inhibitor with adequate bioavailability to permit oral administration once daily. In the Protein Kinase C β inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS2), oral administration of RBX (32 mg per day) reduced sustained moderate visual loss, need for laser treatment for macular edema, and macular edema progression, while increasing occurrence of visual improvement in patients with nonproliferative retinopathy (PKC-DRS2 Group, 2006). In the Protein Kinase Cβ inhibitor Diabetic Macular Edema Study (PKC-DMES), RBX treatment also showed a beneficial effect on DME progression relative to placebo (PKC-DMES Study Group, 2007). More recently, Davis et al. (2009) demonstrated that RBX treatment appears to ameliorate DME-associated visual decline.

2.14.13. Islet cell transplantation

Recent studies demonstrated that improved islet transplant outcomes could be observed with enhanced islet isolation, glucocorticoid- free immunosuppression, and provision of an adequate islet mass of more than 10,000 islet equivalents per kg of body weight. These improvements have resulted in benefits to type 1 diabetic subjects, including long-term c-peptide secretion, improved glycemic control, and reduced hypoglycemic episodes. Recently, it was demonstrated that islet transplantation yields improved HbA1c and less progression of retinopathy compared with intensive medical therapy during 3 years of follow-up (Warnock et al., 2008; Thompson et al., 2008).
2.15. Drugs Evaluated

In the present investigation, we have made an attempt to evaluate the anti diabetic retinopathy potential of the following herbal drugs and constituent in streptozotocin induced rat model of diabetic retinopathy in type 1 and type 2 diabetes.

- *Momordica charantia*
- *Boerhaavia diffusa*
- *Eugenia jambolana*
- *Tinospora cordifolia*
- Polyherbal combination of the above drugs
2.15.1.  *Momordica charantia*

**Common name:** Bitter gourd; bitter melon, karela

**Order:** Cucurbitales

**Family:** Cucurbitaceae

**Genus:** Momordica

**Species:** M. charantia

**Habitat:** Tropical regions of Asia, Africa and the Carribean

The tendril-bearing vine of *M. charantia* grows to 5 meters. It bears simple, alternate leaves 4–12 cm across, with 3–7 deeply separated lobes. Plant bears yellow flowers. Flowering occurs during June to July and fruiting during September to November.

**Chemical Constituents:**

Biologically active compounds present in *M. charantia* chiefly are momordicin I & II and cucurbitacin B.
Bioactive glycosides: momordin, charantin, charantosides, goyaglycosides, momordicosides.

Other terpenoid compounds: momordicin-28, momordicinin, momordicilin, momordenol and momordol.

Charantin

Pharmacological studies of *Momordica charantia*

1. **Antidiabetic** (Sridhar et al., 2008; Kar et al., 2003; Rathi et al., 2002)

2. **Diabetic complications** (nephropathy, neuropathy, cataract and insulin resistance) in experimental animals (Grover et al., 2002, 2001; Rathi et al., 2002; Vikrant et al., 2001)

3. **Anti-inflammatory activity** (Choi et al., 2002)

4. **Anti-oxidant potential** (Dhar et al., 1999)

5. **Other activities:** Anthelmintic (Beloin et al., 2005); Antiviral (Jiratchariyakul et al., 2001, Nerurkar et al., 2006); Antimalarial (Waako et al., 2005); Cardioprotective effect by down-regulating the NF-kB inflammatory pathway (Gadang et al., 2011); Antibacterial; (Yesilada et al., 1999); Anticancer (Kobori et al., 2008; Kohno et al., 2004; Ray, 2010); Anti-ulcer (Gurbuz et al., 2000); Immunomodulatory (Leung et al., 1987), Hypotensive and anti-prothrombin (Wang and Ng, 2001) and Hypcholesterolemic (Ahmed et al., 2001).
2.15.2. **Boerhaavia diffusa**

*Common name:* Punarnava, tar vine, Hog Weed, Pig Weed, Horse Purslane

*Kingdom:* Plantae

*Order:* Caryophyalles

*Family:* Nyctaginaceae

*Genus:* Boerhavia

*Species:* B. diffusa

*Habitat:* Widely dispersed, occurring throughout India, the Pacific and southern United States.

**Chemical Constituents of Boerhaavia diffusa**

B. diffusa contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. potassium nitrate (Chopra et al., 1923), hypoxanthine 9- L-arabinofuranoside (Ahmad and Hossain, 1968), ursolic acid (Mishra and Tiwari, 1971), Punarnavine (Surange and Pendse, 1972), a glycoprotein (Verma and Awasthi, 1979), punarnavoside (Jain and Khanna, 1989), boeravinone A-F (Lami et al., 1990,1992), liirodendrin (Aftab et al., 1996), caffeoyltartaric acid (Ferreres et al., 2005), boeravinone G and H (Borrelli et al., 2005), quercetin and kaempferol (Pereira et al., 2009). The herb contains 15 amino acids such
as argentine, alanine, aspartic acid, methioine, leucine, praline, ornithine, serine, threonine, asparagine, glycine, valine, tryptophane, (Anonymous, 1999). the main rotenoids (known as boeravinones).

It also contains β-Sitosterol, α-2-sitosterol, palmitic acid, ester of β-sitosterol, tetracosanoic, hexacosonoic, stearic, arachidic acid, urosilic acid, Hentriacontane, β-Ecdysone, triacontanol etc.

**Pharmacological Studies of *B. diffusa***

1. **Antidiabetic**- Nalamolu et al. (2004) and Satheesh and Pari (2004) reported the significant antidiabetic activity from the chloroform extract of *B. diffusa*.

2. **Antioxidant**- Mili (2007) reported the antioxidant activity and genoprotective actions.

3. **Anti-inflammatory**- Hiruma-lima et al. (2000) reported significant immunomodulatory activities and anti-inflammatory effects of the aqueous extracts of *B. diffusa* leaves.

4. According to Mehrotra et al. (2002) the ethanolic extract of B. diffusa was capable to inhibit T-cell mitogen phytohemagglutinin and concanavalin A-stimulated proliferation of human peripheral blood mononuclear cells.

5. Chandan, et al. (1991) investigated that an alcoholic extract of *B. diffusa* exhibited hepatoprotective activity against experimentally induced carbon tetrachloride hepatotoxicity in rats and mice. The extract also produced an increase in normal bile flow in rats suggesting a strong choleric activity.

6. According to Singh et al. (1992) B. diffusa is clinically proved safe drug in the patients of nephritic syndrome.

7. Manu and Kuttan (2007) reported about the enhanced effect of Punarnavine on the cell-mediated immune (CMI) response against metastatic progression of B16F-10 melanoma cells in mice.
Uses: According to Ayurveda Boerhavia is bitter, anaemia, cooling, heart diseases, astringent to bowels, useful in biliousness, blood impurities, leucorrhoea, asthma, inflammations, alternatives etc. The leaves are useful in dyspepsia, tumours, abdominal pains, and spleen enlargement. According to Unani system of medicine, the leaves are appetizers, alexiteric, useful in opthalmia and in joint pains. Seeds are tonic expectorant, carminative, useful in lumbago, scabies. The seeds are considered as promising blood purifier. Roots are used to treat gonorrhea, all internal inflammation and edema. Roots stimulate the emptying of the gallbladder, as a diuretic, for all types of liver disorders (including jaundice and hepatitis), gallbladder pain and stones, urinary tract disorders, renal disorders, kidney stones, cystitis, and nephritis.
2.15.3.  *Eugenia jambolana*

*Common names:* Jamun, Jambu, jambola, Java plum, jambolan, black plum, Jambolan plum, Malabar plum

*Kingdom:* Plantae

*Order:* Myrtales

*Family:* Myrtaceae

*Genus:* Syzigium

*Species:* *E. jambolana/S. cumini*

*Habitat:* an evergreen tropical tree in the flowering plant, native to Bangladesh, India, Nepal, Pakistan, Sri Lanka and Indonesia.

**Chemical Constituents**

Methylxanthoxylin, corilagin, ellagitanins, ellagic acid and gallic acid, jambosine, volatile oil, jambolin, quercetin, ferulic acid, veratrole, guajacol and caffeic acid.
Uses:

*E. jambolana* induces apoptosis in specific tumor cells.

It acts as antioxidant by protecting the cell from oxidative stress.

It is strong acid acting against reactive oxygen scavenging systems.

It reduces the blood sugar level in body.

Volatile oil obtained from *E. jambolana* significantly inhibits the growth of bacteria

**Pharmacological studies of *E. jambolana***

1. **Antidiabetic:** The fruit and seed of *Syzygium cumini* are used as antidiabetic agent by decreasing kidney’s catalase activity. This causes a significant reduction in serum glucose by regeneration of pancreatic islet cells. It stimulates insulin secretion and eliminates risk of hyperglycemia and cardiovascular complication (Kohli and Singh 1993; Sahana et al., 2010).

2. **Diabetic nephropathy** (Grover et al., 2002)

3. **Diabetic neuropathy** (Grover et al., 2002)

4. **Diabetic cataract** (Rathi et al., 2002)

5. **Anti-inflammatory** (Muruganandan et al., 2011)

6. **Other activities:** Antiulcer (Chaturvedi et al., 2009), Hypolipidemic effect: (Sharma et al., 2008; Ravi et al., 2005; Viswanath et al., 2008). Cardioprotective effects: (Mastan et al., 2009). Anti-diarrheal effects: (Mukherjee et al., 1998). Antifertility activity: (Rajasekaran et al., 1988). Anti-allergic effects: (Brito et al., 2007). Antipyretic effects: (Chaudhuri et al., 1990). Neuropsychopharmacological effects: (Chakraborty et al., 1986; Kumar et al., 2007). Antineoplastic effects: (Barh and Viswanathan, 2008; Li, et al., 2009; Sharma et al., 2010). Chemopreventive effects: (Parmar et al., 2010; Goyal et al., 2010); Radioprotective effects: (Jagetia et al., 2008, Manjeshwar et al., 2011).
2.15.4. *Tinospora cordifolia*

*Common name:* Guduchi, giloe, gurcha

*Kingdom:* Plantae

*Division:* Magnoliophyta

*Class:* Magnoliopsida

*Order:* Ranunculales

*Family:* Menispermaceae

*Genus:* Tinospora

*Species:* *T. cordifolia*

*Habitat:* indigenous to the tropical areas of India, Myanmar and Sri Lanka
Chemical constituents

Glucoside, alkaloids, bitter principles, crystalline components. The bitter principles have been identified as columbin, chasmanthin and palmarin. The alkaloid tinosporin was also identified.

Other active constituents are diterpene compounds, polyphenols, and polysaccharides, including arabinogalactan (biopolymer consisting of arabinose and galactose monosaccharides) polysaccharide.

Pharmacological studies of *T. cordifolia*

2. Anti-inflammatory effects (Pendse et al., 1977; Gulati and Pandey, 1982; Sherlekar et al., 2001)
3. Anti-oxidant effects (Prince and Menon, 1999; Mathew and Kuttan, 2001)
4. Immuno-modulatory Action (Bishayi et al., 2002)
6. Hepatic Effect (Bishayi et al., 2002)
7. Anti-infective Effects (Jeyachandran R et al., 2003)
9. Antistress effect (Patil et al., 1997)
10. Radio-protective effects (Goel et al., 2004)

2.15.5. Polyherbal combination

*(Momordica charantia + Boerhavia diffusa + Eugenia jambolana + Tinospora cordifolia)*