3. LITERATURE REVIEW

3.1. Diabetic Retinopathy

*Pathak D. et al., (2012)*\(^6^1\) described about the PKC and VEGF receptors. PKC isomer selective inhibitors and VEGF trap are likely to be new therapeutics, which can delay the onset or stop the progression of diabetic vascular disease. A new promising therapy for diabetic retinopathy is undergoing Phase III trials, in which they proposed to target PKC beta II isomer using ruboxistaurin by oral administration. Besides retina, PKC beta II isomer is found in higher concentration in brain, spleen, etc. So, oral targeting may be a questionable approach since generalized inhibitors may prove toxic in the treatment of diabetic retinopathy and ocular delivery may be a better alternative approach.

*Ahmed M. Abu El-Asrar et al., (2011)*\(^6^2\) described focal/grid photocoagulation is a better treatment than intravitreal triamcinolone acetonide in eyes with diabetic macular edema and should be considered as the first-line therapeutic option. Emerging therapies include islet cell transplantation, fenofibrate, ruboxistaurin, pharmacologic vitreolysis, rennin-angiotensin system blockers, and peroxisome proliferator-activated receptor gamma agonists.

*Anant Pai et al. (2010)*\(^6^3\) discussed the current concepts and the role of these novel therapeutic approaches in the management of diabetic retinopathy. The standard of care for patients with DR include strict metabolic control of hyperglycemia, blood pressure control, normalization of serum lipids, prompt retinal laser photocoagulation and vitrectomy. For patients who respond poorly and who progressively lose vision in spite of the standard of care, intravitreal administration of steroids or/and anti-vascular endothelial growth factor (anti-VEGF) drugs appear to be a promising second-line of therapy.

*T. Khalfaoui et al., (2008)*\(^6^4\) described the substantial overexpression of adhesion molecules ICAM-1, VCAM-1 and of VEGF suggested that these molecules might contribute to the development of fibrovascular membranes in patients with
proliferative diabetic retinopathy, and that they could constitute suitable markers of this pathology.

**Luis Javier Hernandez-Pastor et al., (2008)**\(^{65}\) described that the ranibizumab was not cost-effective when administered on a monthly basis. When administered as needed, ranibizumab was cost-effective compared with photodynamic treatment for AMD.

**Massimo Porta et al., (2004)**\(^{66}\) described the use of thiamine and its analogues in the primary and secondary prevention of early retinopathy and blockers of vascular endothelial growth factor/vascular permeability factor in more advanced stages.

**Małgorzata Mysliwiec et al., (2008)**\(^{67}\) described the role of vascular endothelial growth factor, tumor necrosis factor alpha and interleukin-6 in pathogenesis of diabetic retinopathy and also the relationship between VEGF, TNF-a, IL-6 and the development of the diabetic retinopathy in children with diabetes mellitus type 1.

**John Lowe et al. (2007)**\(^{68}\) described the ranibizumab (Lucentis), a humanized antibody fragment directed against vascular endothelial growth factor (VEGF-A) and also characterize the binding affinity and pharmacological activity of ranibizumab for 3 biologically active forms of VEGF-A: VEGF165, VEGF121, and VEGF110.

**P.R. Harper et al., (2003)**\(^{69}\) have demonstrated the use of a systems modelling approach for the progression of diabetic retinopathy that has been used for cost-effectiveness evaluations of various prevention and patient care options. The adopted framework incorporates retinopathy risk groupings, created using classification and regression tree (CART) analysis, which are then fed into a developed simulation model, at the level of individual diabetic patients.

**B.C.P. Polak et al., (2003)**\(^{70}\) demonstrated the vast majority of diabetic patients benefits from intensive glycemic control and intensive ophthalmological care, but these costeffective interventions which are not only complementary, but also substitute each other, require lasting, full compliance by all parties concerned.
Thomas W. Gardner et al., (2002)\textsuperscript{71} described recent observations regarding the cellular anatomy that contributes to the blood–retinal barrier and its breakdown, the alterations of macroglial, neuronal, and microglial cells in diabetes, and how these changes lead to loss of vision, an overview of inflammatory mechanisms and responses in the retina in diabetes is provided. These new observations provide a broader clinical and research perspective on diabetic retinal vascular dysfunction and provide new avenues for improved treatments to prevent loss of vision.

George L. King et al., (1998)\textsuperscript{72} demonstrated that hyperglycemia causes vascular complications of diabetes possible by the activation of protein kinase C (PKC). They studied cultural cells, animal models of diabetes and patients have shown that inhibition of PKC by specific PKC-M inhibitor was able to reverse many of the vascular dysfunctions in the retina, kidney and cardiovascular systems induced by either hyperglycemia or diabetes. In addition high doses of vitamin E were shown to decrease the level of DAG and PKC. Thus animal and clinical studies have shown that high doses of vitamin E treatment could apparently reverse some of the changes in the retinal and renal vessels.

Hans-Peter Hammes et al., (1991)\textsuperscript{73} have demonstrated that the treatment of diabetic rats for 26 weeks with aminoguanidine, an inhibitor of advanced glycosylation product formation, prevented a 2.6-fold accumulation of these products at branching sites of precapillary arterioles where abnormal periodic acid/Schiff reagentpositive deposits also occurred. A dine treatment completely prevented abnormal endothelial cell proliferation and significantly diminished pericyte dropout.

3.2. Biopolymers

Sadeq Hassan Al-Sheraji et al., (2012)\textsuperscript{74} described purification, characterization and antioxidant activity of polysaccharides extracted from the fibrous pulp of Mangifera pajang fruits. The acidic polysaccharides had the highest antioxidant activity and should be considered as a prospective antioxidant.
Wenshui Xia et al., (2010)\textsuperscript{75} have shown that Chitosan and its oligosaccharides, which are known to possess multiple functional properties, have attracted considerable interest due to their biological activities and potential applications in the food, pharmaceutical, agricultural and environmental industries.

Anoop Kumar Singh et al., (2010)\textsuperscript{76} described physical, thermal, sorption and functional properties of a gum obtained from the stem of \textit{mangifera indica} were characterized viz. elemental analysis, Fourier transmittance infra red, particle size analysis, thermo gravimetric analysis, differential scanning colorimetry, scanning electron microscopy and X-ray powder diffraction. Tablets with 5\% w/w binder concentration showed optimum results than standard binder, thus conclusion was drawn that mango gum was found to be useful for the preparation of uncoated tablet dosage form.

Girish K Jani et al., (2009)\textsuperscript{77} described that the gums and mucilages are widely used natural materials for conventional and novel dosage forms. These natural materials have advantages over synthetic ones since they are chemically inert, nontoxic, less expensive, biodegradable and widely available. They can also be modified in different ways to obtain tailor-made materials for drug delivery systems and thus can compete with the available synthetic excipients.

Vipin K. Sharma et al., (2009)\textsuperscript{78} described the dietary fibers have positive effects on human health, both in the prevention and in treatment of chronic diseases. Isabgol husk (\textit{Plantago ovata}) is a natural polymer of plant origin which is mainly composed of polysaccharide chain having (1>3) and (1>4)-\textbeta– xylan system. It is used a bulk forming agent in constipation but due to some other beneficial effects.

V.T.P. Vinod et al., (2008)\textsuperscript{79} describe the morphological, physico-chemical and structural characterization of gum kondagogu (Cochlospermum gossypium). Analysis of acid-hydrolyzed gum by GC–MS, indicated the presence of rhamnose, galacturonic acid, glucuronic acid, b-D-galactopyranose, a-D-glucose, b-D-glucose, galactose, arabinose, mannose and fructose, while the 1D and 2D NMR, revealed the presence of
the following sugar residues and their linkages—(1-2) \( b-D-Gal \), (1-6)-\( b-D-Gal \), (1-4)\( b-D-Glc \) \( A \), 4-O-Me-\( a-D-Glc \) \( A \), (1-2) \( a-L-Rha \), and (1-4) \( a-D-Gal \).

\textit{Nazar A. El Nasri et al.,} (2007)\textsuperscript{80} have described emulsion and foaming properties of fenugreek protein concentrate showed that they were greatly affected by pH levels and salt (NaCl) concentration. The minimum values of both emulsion and foam properties were attained at pH 4.5 which was the isoelectric point of the protein; maximum values were obtained at pH 2 and pH 12.

\textit{Ebubekir Altuntas et al.,} (2005)\textsuperscript{81} demonstrated some physical properties of fenugreek seeds were evaluated as a function of moisture content. The average length, width, thickness, geometric mean diameter and unit mass of the seed ranged from 4.01 to 4.19mm, 2.35 to 2.61mm, 1.49 to 1.74mm, 2.40 to 2.66mm and 0.0157 to 0.0164g as the moisture content increased from 8.9% to 20.1% d.b. respectively.

\textit{Gowthamarajan et al.,} (2003)\textsuperscript{82} investigated \textit{Borassus flabellifer} mucilage as gelling agent for nimesulide gel formulation. The physical characteristics of mucilage such as solubility, swelling index, loss on drying, pH and viscosity were studied. Different batches of drug loaded gels were prepared and evaluated for \textit{in vitro} diffusion profiles. The gel prepared with 3% mucilage showed ideal drug release characteristics and was found to be suitable gelling agent.

\textit{Gowthamarajan et al.,} (2002)\textsuperscript{83} evaluated fenugreek mucilage as gelling agent in pharmaceutical gels containing diclofenac diethylammonium as model drug and glycerin and PEG-400 as plasticizers. The \textit{in vitro} diffusion profile was studied. The gel prepared with 3.25% of mucilage and 10% glycerin as plasticizer showed better drug release when compared with the marketed formulations.

\textit{Kulkarni et al.,} (2002)\textsuperscript{84} studied the binding properties of \textit{Plantago ovata} and \textit{Trigonella foenum graecum} mucilages in uncoated tablets containing paracetamol as model drug. They found that the tablets prepared with 8 and 9% concentration of the mucilages were ideal in their physical properties and \textit{in vitro} drug release.
Susi Burgalassi et al., (2001)\textsuperscript{85} has carried out a series of test for the prospective ocular permeation enhancers; benzalkonium chloride (BAC), cetlypyridinium chloride (CPC), ethylenediaminetetraacetic acid (EDTA), polyoxyethylene (20) stearyl ether (PSE) and polyethoxylated castor oil (PCO) were tested for cytotoxicity on cultures of rabbit (RCE) and human (HCE) corneal epithelial cells.

Bhardwaj et.al. (2000)\textsuperscript{86} presented a review of natural gums and modified natural gums as carriers in sustained release medications, including agar beads, sodium alginate, carrageenans, cellulose ethers, chitosan, dried molasses, gellan gum, guar gum, acacia, karayagum, glucomannan gel, locust bean gum, modified straches, pectins, sclerogluccan and xanthan gum.

3.3. Anti-angiogenic modulators

Preetha Anand et al., (2010)\textsuperscript{87} demonstrated that curcumin-loaded PLGA nanoparticles formulation has enhanced cellular uptake, and increased bioactivity \textit{in vitro} and superior bioavailability \textit{in vivo} over curcumin.

Yogeeswari P et al.,(2005)\textsuperscript{88} described that betulinic acid is a naturally occurring pentacyclic triterpenoid and has been shown to exhibit a variety of biological activities including inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic, anti-angiogenic and antioxidant properties.

Mukherjee R et al., (2004)\textsuperscript{89} described that betulinic acid significantly caused cytotoxicity to endothelial cell line ECV304 (IC\textsubscript{50} 1.26 ±0.44 lg/mL) in a 5-day MTT assay. Novel and more potent derivatives of betulinic acid (2, 4, 6–8) have been synthesized with IC\textsubscript{50} less than 0.4 lg/mL. The endothelial cell specificity against human tumor cell lines DU145, L132, A549, and PA-1 were determined. Further betulinic acid inhibited TLS formation of ECV304 cells on Matrigel TM by 5.5% while its derivatives caused an inhibition of 13.1–49.2%.
Hideshima T et al., (2006) described that lenalidomide is an analogue of the established drug thalidomide, but it enjoys a better safety profile than thalidomide. Also, unlike many other agents used in the treatment of these diseases, it is available as an oral capsule.

3.4. Nanoparticle and Ocular gel

Ilva D. Rupenthal et al. (2011) compared a number of anionic polysaccharides (gellan gum, xanthan gum, carrageenan and alginate) to an uncharged (HPMC) and a positively charged (chitosan) polymer system with emphasis on the gelling behaviour, rheological and textural properties, gel microstructure, contact angle and in vitro release characteristics. All systems exhibited physically entangled polymer networks that were able to disentangle upon shear stress and significantly prolonged the in vitro release of a model hydrophilic drug compared to a solution.

Narayan Bhattarai et al., (2010) described chitosan-based hydrogels for controlled, localized drug delivery. They investigated the newest developments in chitosan hydrogel preparation and define the design parameters in the development of physically and chemically cross-linked hydrogels.

Gilhotra Ritu Mehra et al., (2010) described the enhanced miotic potential of pilocarpine by tamarind gum based in-situ gelling ocular dosage form. The formulations were tested for drug content uniformity, bioadhesive strength, gelation, in vitro release study and in vivo miotic activity which provide an excellent potential alternative ophthalmic sustained-release formulation of pilocarpine for clinical use.

Sirish Vodithala et al., (2010) formulated ion activated ocular gels of ketorolac tromethamine were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro drug release, ocular irritancy studies (as per Draize test) and ex vivo corneal permeation studies using isolated goats cornea. The developed formulations showed sustained release of drug for upto 6 hrs were found to be non-irritating with no ocular damage.
Himanshu Gupta et al., (2010) developed and evaluated a new colloidal system, that is, poly (dl-lactide-co-glycolide) (PLGA) nanoparticles for sparfloxacin ophthalmic delivery, to improve precorneal residence time and ocular penetration. Nanoparticles were prepared by nanoprecipitation technique and characterized for various properties such as particle size, zeta potential, in vitro drug release, statistical model fitting and stability.

J. Araujo et al., (2010) developed a novel nanostructured lipid carrier (NLC) for the intravitreal targeting delivery of triamcinolone acetonide (TA) by direct ocular instillation. A five-level central composite rotatable design was used to study the influence of four different variables on the physicochemical characteristics of NLCs. The analysis of variance (ANOVA) statistical test was used to assess the optimization of NLC production parameters.


Maria de la Fuente et al., (2008) assessed the effectiveness and investigate the mechanism of action of a new type of nanoparticle made of two bioadhesive polysaccharides, hyaluronic acid (HA) and chitosan (CS), intended for the delivery of genes to the cornea and conjunctiva. The results give evidence of the potential of HA-CS nanoparticles for the targeting and further transfer of genes to the ocular surface.

Tarl W. Prow et al., (2008) evaluated the safe delivery of genes via chitosan, PCEP (poly{[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium iodide] ethyl phosphate}), and magnetic nanoparticles (MNP) in the eye. They concluded that the MNP nanoparticle evaluated in vivo was the least toxic nanoparticle.
Chinnaraj Premanand et al., (2006)\textsuperscript{100} investigated the effect of high glucose on the proliferation of human retinal endothelial cells (HRECs) and to elucidate the possible mechanisms of antiangiogenic activity of curcumin. They suggested that curcumin induced the apoptosis in HRECs by the regulation of intracellular ROS generation, VEGF expression and release, and VEGF-mediated PKC-βII translocation.

Otilia M. Koo et al., (2005)\textsuperscript{101} described the role of nanotechnology in targeted drug delivery. The principles of passive and active targeting of nanosized carriers to inflamed and cancerous tissues with increased vascular leakiness, overexpression of specific epitopes, and cellular uptake of these nanoscale systems were discussed.

Kimberly L. Douglas et al., (2005)\textsuperscript{102} introduced a new procedure to prepare alginate–chitosan nanoparticles and examined several experimental parameters in relation to their formation and characteristics. Using DLS and TEM analysis, nanoparticle formation was shown to be predominantly affected by the ratio of alginate to chitosan, the molecular weight of the biopolymers and the solution pH.

J. Vandervoort et al., (2004)\textsuperscript{103} were prepared gelatin nanoparticles encapsulating pilocarpine HCl or hydrocortisone as model drugs by desolvation method. The influence of a number of preparation parameters on the particle properties was investigated. For the pilocarpine HCl-loaded spheres, an influence of the pH during particle preparation on the size was observed. Slightly negative zeta potential values were measured for all samples.

Lifeng Qi et al., (2004)\textsuperscript{104} evaluated the \textit{in vitro} antibacterial activity of chitosan nanoparticles and copper-loaded nanoparticles against various microorganisms. Chitosan nanoparticles were prepared based on the ionic gelation of chitosan with tripolyphosphate anions. Copper ions were adsorbed onto the chitosan nanoparticles mainly by ion-exchange resins and surface chelation to form copper-loaded nanoparticles. The physicochemical properties of the nanoparticles were determined by size and zeta potential analysis, atomic force microscopy (AFM), FTIR analysis, and XRD pattern.
**Sinjan De et al., (2003)** described the preparation of chitosan–alginate nanospheres and their properties compared to the poly-L-lysine–alginate system. The mass ratio range of sodium alginate: CaCl: cationic polymer (poly-L-lysine [PLL] or chitosan) to prepare nanospheres was 100:17:10. This mass ratio ensured that the calcium alginate was maintained in the pre-gel phase and sufficient cationic polymer was present to form nanospheres. At low cationic polymer concentrations, nanospheres were not formed, whereas microspheres were formed at higher concentrations.

**Hongwei Li et al., (2002)** discussed a number of recent developments in the use of polymers for the fabrication of nanostructures via lithographic and self-assembling strategies. The natural length scales of polymer chains and their morphologies in the bulk, which lie in the nanometer domain, make polymers ideal building blocks for nanotechnology.

**Katarina Edsman et al., (1996)** studied the ocular residence time of carbomer gels have been monitored in humans and the gels were also rheologically characterised. The contact time of Carbopol 974P and Carbopol 1342NF was concentration dependent and was approximately 2-2.5 h for a 2% gel. There was a good correlation of the human contact time and the elastic properties of the gels.

### 3.5. *In-Vitro In-Vivo* Ocular irritation and Antineovascularization Studies

**Hong-yan Ge et al., (2011)** evaluated the antiangiogenic activities of lipid-mediated subconjunctival injection of the modified RGDRGD (arginine-glycin-aspartic- arginine- glycin- aspartic- endostatin gene in a rabbit model of neovascularization in vivo. Subconjunctival injection of both native endostatin and modified *RGDRGD*-endostatin genes resulted in a significant suppression of CNV *in-vivo*, with modified *RGDRGD*-endostatin being more effective than native endostatin.

**Jung Sub Kim et al., (2010)** investigated the anti-angiogenic effect of topical curcumin on corneal neovascularization in a rabbit model. The concentration of vascular endothelial growth factor (VEGF) mRNA in the corneal tissue was measured
by reverse transcriptase-polymerase chain reaction (RT-PCR), and the activation of NF-kappaB was examined by immunofluorescent staining. They concluded that the topical application of curcumin was useful in reducing experimental corneal neovascularization and could be used to inhibit angiogenesis in the cornea.

**Vinoth Prabhu Veeramani et al., (2010)**

described various current techniques used in angiogenesis assay. Angiogenesis activity can be evaluated by using in-vivo, in-vitro and organ culture assay systems. *In-vitro assays* were performed by using different types of endothelial cells isolated from either capillaries or large vessels. Mostly used cell lines are Bovine aortic endothelial cells (BAECs), Chicken endothelial cells (CECs) and Human umbilical vein endothelial cells (HUVECs) as well as by Langendorff isolated heart model. In *in-vivo models*, the matrigel plug assay is the easiest model when compared to hind limb ischemia and all other models. When compared to all other angiogenesis quantification methods capillary density estimation source was given a better accuracy and precession.

**A. Sairam Kishore et al., (2009)**

attempted to know whether the validated *in vitro* alternative models established for chemicals, drugs, pesticides are suitable for nanomaterials, since these materials differ largely and may interfere with commonly used test systems. *In vitro* and *in vivo* studies on ocular and dermal irritation were carried out with two different sizes of multi-walled carbon nanotubes (MWCNT). The results of acute eye irritation toxicity studies with two different sizes of MWCNT in rabbits demonstrated reversible conjunctival redness and discharge and exhibited minimal concern while acute dermal irritation studies indicated that MWCNT of two sizes were non-irritant to the skin of rabbits.

**Anne M. Goodwin et al., (2007)**

described various *in vitro* assays that can be used to assess the activity of agents that affect angiogenesis. Means of quantifying endothelial cell matrix degradation, migration, proliferation, apoptosis and morphogenesis were discussed, as are embryoid body, aortic ring and metatarsal assays of vessel outgrowth. Agents that stimulate angiogenesis can improve blood flow in patients with ischemic diseases, whereas anti-angiogenic agents are used to treat disorders ranging from macular degeneration to cancer.
**Robert P A Manzano et al., (2006)** evaluated the effect of topically administered bevacizumab (Avastin) on experimental corneal neovascularisation in rats. In the bevacizumab-treated eyes, neovascularisation covered, on average, 38.2% (15.5%) (mean (SD)) of the corneal surface compared with 63.5% (5.0%) in the control group (p < 0.02, Mann–Whitney U test). They concluded that the topically administered bevacizumab (Avastin) at a concentration of 4 mg/ml limits corneal neovascularisation following chemical injury in the rat model.

**Akiyoshi Uemura et al., (2006)** described an overview of our current understanding of the process of retinal angiogenesis and describe a number of methodologies applicable to experimental manipulation of the retinal vascular system. The vascular system of the mouse retina provides a useful model for analyzing the molecular and cellular mechanisms regulating angiogenesis because (1) hierarchical vascular networks are newly formed only after birth, (2) the cellular components involved in angiogenesis are well characterized, and (3) all the processes are accessible for monitoring and manipulation.

**Theodore leng et al., (2004)** described the use of chick chorioallantoic membrane (CAM) as a model system for the study of the precision and safety of vitreoretinal microsurgical instruments and techniques. The CAM’s ease of use, low cost, and anatomic structure make it a convenient model for surgical retinal and retinal vascular modeling.

**W. J. W. Pape et al., (1999)** have demonstrated that red blood cell test (RBC haemolysis test) is part of the COLIPA Validation Project on Alternatives to Draize Eye Irritation. It showed good intra- and inter laboratory reproducibility (reliability) and represents one of the promising in vitro alternatives of this project with a good prediction models (relevance) for the assessment of acute ocular irritancy caused by certain classes of chemicals (mainly surfactants) and formulations.

**H. Spielmann et al., (1993)** has discussed about the validation study which is an alternative to the Draize eye irritation test in Germany, which included cytotoxicity testing and HET-CAM test with 136 industrial chemicals.