WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 11, 1291-1327. Research Article ISSN 2277– 7105

POSSIBLE ROLE OF HEME OXYGENASE (HO)-1 IN ANTI-CATARACT ACTIVITY OF DIABECON (D-400) IN HYPERGLYCEMIA-INDUCED CATARACT IN CULTURED GOAT LENS

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Article Received on
18 June 2022,

Revised on 08 July 2022,
Accepted on 28 July 2022

DOI: 10.20959/wjpr202211-25115

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ABSTRACT

Diabetic retinopathy (DR) causes significant visual loss on a global scale. Treatments for the vision-threatening complications of diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) have greatly improved over the past decade. Heme oxygenase-1 (HO-1), the rate-limiting enzyme that catalyzes the degradation of heme to biliverdin, carbon oxide (CO) and iron, is one of the ARE-regulated phase II detoxifying enzymes and antioxidants, which are regulated by the redox-sensitive transcription factor nuclear factor erythroid 2-related factor (Nrf2). Over expression of HO-1 is neuroprotective in a model of permanent middle cerebral artery occlusion (MCAO) in transgenic mice. In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. Diabecon (D-400) contains Gymnema sylvestre (Meshashringi), Eugenia jambolana (Jambu), Tinospora cordifolia, Pterocarpus marsupium, Ficus glomerata, Momordica charantia (Karela), Ocimum sanctum (Vishnu priya), as its main ingredients. The preservation of beta cell function noted in this study after use of Diabecon (D-400) is also animal studies, indicating the regeneration of rat beta cells. There was a significant reduction in postprandial plasma glucose levels and glycosylated haemoglobin levels in the drug treated group. The present study was designed to evaluate the diabcon (D-400), ayurvedic medicines for their

anti-cataract effect in-vitro hyperglycemia-induced cataract in cultured goat Lens and its mechanism of actions.

KEYWORDS: Diabetic retinopathy, Heme oxygenase-1, Diabecon (D-400).

1. INTRODUCTION

1.1 Introduction for diabetic retinopathy

Diabetic retinopathy is additionally called diabetic disease, may be a medical condition within which damage occurs to the retina because of diabetes and is leading causes of blindness. Diabetic retinopathy may be a chronic progressive, potentially sight-threatening disease of the retinal microvasculature related to the prolonged hyperglycemia and other conditions linked to DM like hypertension.

Diabetic retinopathy (DR) could be a vascular disease of the retina which affects patients with DM. it's the quantity one reason for blindness in people between the ages of 20-64 within the U.S. diabetes is extremely common, so it's not surprising that DR affects 3.4 percent of the population (4.1 million individuals). Of the scores of people with DR, nearly one -fourth have vision -threatening disease (AAO 2008). The likelihood of developing diabetic retinopathy is said to the duration of the disease. Type 2 diabetes has an insidious onset and may go unnoticed for years. As a result, patients may have already got DR at the time of diagnosis. Type 1 diabetics, on the opposite hand, are diagnosed early within the course of their disease, and that they typically don't develop retinopathy until years after the diagnosis is formed. the chance of developing retinopathy increases after puberty. Twenty years after the diagnosis of diabetes, 80% of type 2 diabetics and nearly all type 1 diabetics show some signs of retinopathy (Klein 1984a, Klein 1984b).[1]

1.2 Prevalence of diabetes & diabetic retinopathy

Diabetes is now considered a deadly disease, with the population of patients expected to rise to 380 million by 2025. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. quite 80% of diabetes deaths occur in low- and middle-income countries.[2] Tragically, this may cause approximately 4 million people round the world losing their sight from diabetic retinopathy, the leading reason behind blindness in patients aged 20 to 74 years.[3] Similarly, an incidence of retinopathy, a typical microvascular complication of diabetes, is predicted to rise to alarming levels. Diabetic retinopathy is assessed into nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR),

characterised by the expansion of recent blood vessels (retinal neovascularization). NPDR is further divided into mild, moderate, and severe stages which will or might not involve the event of a macula diabetic macular oedema (DMO).[4] The most important causes of severe impairment are PDR and DMO. Nearly all patients with Type 1 diabetes and >60% of patients with Type 2 diabetes are expected to possess some kind of retinopathy by the primary decade of incidence of diabetes.[5,6]

The risk of developing diabetic retinopathy are often reduced by early detection, timely tight control of glucose, vital sign, and possibly lipids; however, clinically this can be difficult to realize. Laser photocoagulation and vitrectomy are required to treat sight-threatening retinopathy. there's an urgent must understand how diabetes causes damage to the blood vessels within the eye, to drive the event of latest drugs for the treatment of diabetic retinopathy.[3] The Diabetes Control and Complications Trial (DCCT) and uk Prospective Diabetes Study (UKPDS) clinical trials confirmed the strong relationship between chronic hyperglycaemia and also the development and progression of diabetic retinopathy, but the underlying mechanism that ends up in the event of microvascular damage as a results of hyperglycaemia remains unclear.[7,8]

1.3 Pathophysiology of diabetic retinopathy

Variety Of Interconnecting Biochemical Pathways Are Proposed As Potential Links Between Hyperglycaemia And Diabetic Retinopathy. These Include Increased Polyol Pathway Flux, Activation Of Diacylglycerol- (Dag-)Pkc Pathway, Increased Expression Of Proteins Like Vascular Endothelial Growth Factor (Vegf) And Insulin-Like Growth Factor-1 (Igf-1), Haemodynamic Changes, Accelerated Formation Of Advanced Glycation Endproducts (Ages), Oxidative Stress, Activation Of The Renin-Angiotensin-Aldosterone System (Raas), And Subclinical Inflammation And Leukostasis.

1.4 Pathophysiological role of polyol pathway in diabetic retinopathY

The polyol pathway metabolises excess glucose (Figure 1). The enzyme aldose reductase (AR) present within the retina reduces glucose into sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is subsequently converted into fructose by sorbitol dehydrogenase (SDH). Since sorbitol is impermeable to cellular membranes, it accumulates within the cell, and this can be followed by the slow metabolism of sorbitol to fructose.[9,10] NADPH is additionally required for glutathione reductase as a cofactor for regenerating intracellular glutathione in cells, thus reducing the antioxidant

capacity of the cells. The buildup of sorbitol is believed to possess multiple damaging effects in retinal cells including osmotic damage.[11] Additionally, the fructose produced by the polyol pathway will be phosphorylated to fructose-3-phosphate which successively are often degraded to 3-deoxyglucosone, both of which are strong glycating agents and might lead to the assembly of AGEs.[12]

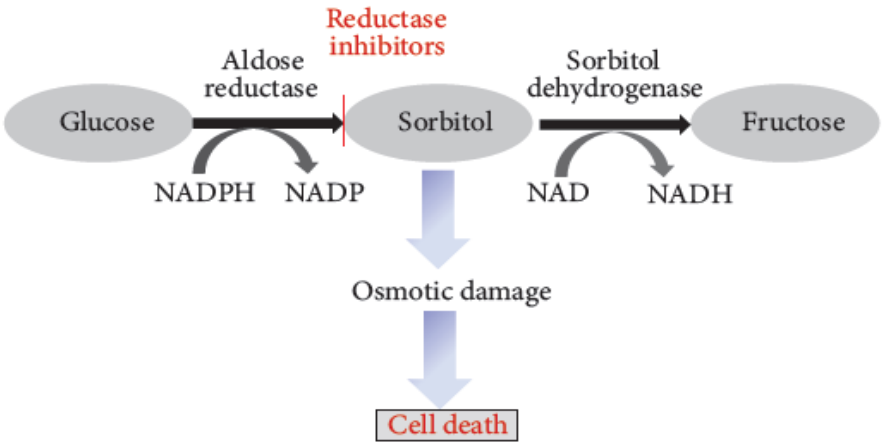

Figure 1: A cascade of Polyol pathway.

The use of NADPH as a cofactor within the polyol pathway leads to less NADPH availability to be used by glutathione reductase, which is crucial for the generation of reduced glutathione. This decrease within the reduced glutathione available ends up in a diminished protective response against oxidative stress.[13] The aberrant shift of the NADH/NAD+ ratio by SDH has been proposed to trigger NADH oxidase which might cause the increased production of reactive oxygen species (ROS) within the cell.[14]

Initial studies investigating the role of the polyol pathway within the pathogenesis of diabetic retinopathy were performed in diabetic animals fed with galactose.[15–17] These studies showed that aldose reductase inhibitors (ARIs) were ready to reduce the incidence and severity of diabetic retinal lesions occurring within the galactose-fed animals. more moderen studies have demonstrated that increased AR is localised in several retinal cells including pericytes, retinal endothelial cells, Müller cells, retinal pigment epithelial cells, and neurons.[18-21] These studies also demonstrate that increased AR activity is involved within the destruction of retinal cells. Exposure of pericytes or endothelial cells to increased concentrations of glucose or galactose resulted in reduced viability of cells. However, this necrobiosis was reversed upon the addition of ARIs.[22–25]

The polyol pathway has also been implicated in several other pathophysiological changes which occur during diabetic retinopathy, one in all these being the increased thickness of the retinal capillary basement membrane.[26,27] Rat models of diabetes have shown that treatment with ARIs is in a position to forestall the thickening of the basement membrane.[28,29] Another mechanism involved within the development of retinopathy is leukocyte adhesion to endothelial cells or leukostasis[30] as discussed in a while during this paper. A study performed by Hattori et al. demonstrated that addition of an ARI to a diabetic rat model was ready to attenuate the leukocyte adhesion to endothelial cells.[31] a rise in vascular permeability and also the breakdown of the blood retinal barrier, hallmark processes which occur in diabetic retinopathy[32,33] are shown to be prevented by the appliance of ARIs.[34] Genetic polymorphism studies also indicate that AR is involved within the development of diabetic retinopathy.[35]

The administration of ARIs to animal model of diabetes at the onset of diabetes has demonstrated some benefit in preventing diabetic retinopathy.[38] However, ARI clinical trials, like the sorbinil retinopathy trial, have shown little clinical benefit.[38,39] it's thought that the poor performance of sorbinil was because of the insufficient inhibition of the polyol pathway in human tissue.[40] An ARI from a unique structural class of drug, ARI-809, is very selective and potent and seems to stop retinopathylike changes in an animal model of diabetes studies,[40] but has not been tested in humans. Recent evidence suggests that the inhibition of both sorbitol and fructose is required to realize beneficial effects in diabetic retinopathy.[41,42]

1.5 Role of nonenzymatic protein glycation in diabetic retinopathy

Among the several pathogenic mechanisms that will contribute to diabetic retinopathy are the formation and accumulation of AGEs.[43,44] AGEs form at a relentless but slow rate within the normal body starting at the embryonic development and accumulate with time. However, their formation is markedly accelerated in diabetes due to the increased availability of glucose.[45] AGEs are a heterogeneous group of molecules formed from the nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids. The initial product of this reaction is termed a Schiff base, which spontaneously rearranges itself into an Amadori product[46] (Figure 2).

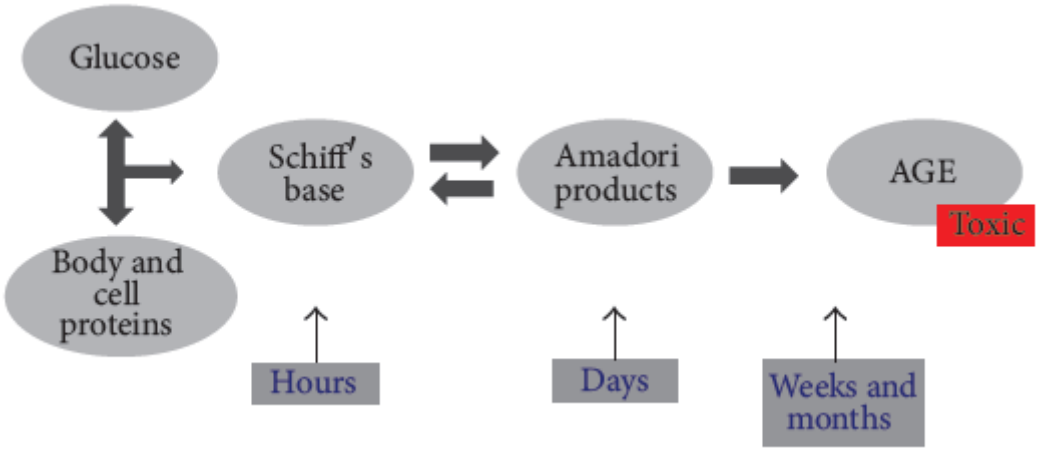

Fig. 2: Formation of advanced glycation end (AGE) products.

A key characteristic of certain reactions or precursor AGEs is their ability for covalent crosslink formation between proteins, which alter their structure and performance, as in cellular matrix, basement membranes, and vessel-wall components. Other major features of AGEs relate to their interaction with a range of cell-surface AGE-binding receptors, including receptor for advanced glycation end products (RAGEs), resulting in cellular activation and prooxidant, pro-inflammatory events.

AGEs affect cells by three main mechanisms: (1) as adducts occurring on modified serum proteins, (2) as endogenous adducts formed as a consequence of glucose metabolism, or (3) as extracellular matrix-immobilised modifications of long-lived structural proteins.[47] AGEs are important pathogenic mediators of virtually all diabetic complications for example, AGEs are found in retinal vessel of diabetic patients, and their levels correlate with thosein the serum moreover like severity of retinopathy. The interaction of AGEs with specific cell surface receptors has been implicated within the development of diabetic retinopathy. These AGE receptors include the craze, galectin-3, CD36, and therefore the macrophage scavenger receptor.[47] Intracellular production old precursors involves thenonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids. Early glycation and oxidation ends up in the formation of Schiff base which spontaneously rearranges itself into an Amadori product. Further glycation of proteins and lipids causes molecular rearrangements that cause the generation of AGEs.

There is evidence that there are three carbohydratederived oxidation products that are increased in diabetes: Nεε-(carboxymethyl) lysine (CML), Nεε-(carboxymethyl) hydroxylysine (CMhL), and pentosidine.[48] The increased concentrations of CML, CMhL

and pentosidine in diabetes provides indirect evidence for a diabetes-related increase in oxidative damage to the protein.[49] CML, and CMhL are formed by oxidative cleavage of Amadori adducts, whereas pentosidine is a fuorescent cross-link formed between lysine and arginine residues in protein.[50] It has been demonstrated that the accumulation of CML in the neural retina and its associated vasculature shows a marked increase during diabetes.[48] During the development of diabetic retinopathy, the enzymatic and nonenzymatic pathogenic mechanisms proceed simultaneously and perhaps synergistically, suggesting that altered mitochondrial function and resultant oxidative stress may exacerbate both tissue damage and AGE formation.[51] The important role of oxidative stress in the pathogenesis of diabetic retinopathy is discussed in the latter part of this section.

There is evidence from animal studies that exposure to high levels of AGEs contributes to renal and vascular complications.[51,52]

1.6 Role of oxidative stress in diabetic retinopathy

Oxidative stress is also defned as an imbalance between the amount of ROS or oxygen radicals and therefore the antioxidant defences in an exceedingly biological system. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and necrobiosis. A hypothetical sequence of events by which oxidative stress could also be linked to tissue damage and also the development of pathophysiology is printed in Figure 4. ROS and reactive nitrogen species (RNS) are the 2 major varieties of oxidants. Under normal physiological conditions, ROS are either detoxified by interaction with various reducing and sequestering agents including thioredoxin, glutathione (GSH), and tocopherol (vitamin E) or by enzymes like superoxide dismutases (SODs), catalase, peroxidase, and thioredoxin reductase.[53-55] Oxidative stress induced by hyperglycaemia is a very important pathway of diabetic microvascular complications, and increasing evidence suggests that the correlation between hyperglycaemia, changes within the redox homeostasis, and oxidative stress is that the key event within the pathogenesis of diabetic retinopathy.[56] it's been hypothesised that both the event and therefore the progression of retinopathy result from increased oxidative species.[57]

The scheme in Figure 3 highlights the varied enzymatic reactions that result in the formation of sources of ROS. These species then target macromolecules causing chemical modification of those biological molecules thus causing damage to the cell and tissue functions, resulting in pathology. Inhibitors and scavengers of ROS can limit the increased accumulation of those

reactive species. Increased oxidative stress may result from over production of precursors to reactive oxygen radicals and/or decreased efficiency of inhibitory and scavenger systems.[53] Animal studies have demonstrated that oxidative stress contributes not only to the event of retinopathy but also to the resistance of retinopathy to reverse after good glycaemic control is reinstituted.[58] Superoxide levels are elevated within the retina of diabetic rats and in retinal cells incubated in high glucose media and oxide content which is increased within the retina of diabetic rats, and membrane lipid peroxidation and oxidative damage to DNA (as a consequence to ROS-induced injury) are elevated within the retina in diabetes.[59,60] In diabetes the activities of antioxidant defence enzymes answerable for scavenging free radicals and maintaining redox homeostasis like SOD, glutathione reductase, antioxidant, and catalase are diminished within the retina and animal studies have provided evidence for and against the utilization of antioxidants to stop experimental diabetic retinopathy.[61,62]

Although the employment of antioxidants has shown some benefit, this has not been supported by clinical trials. Brownlee and colleagues have suggested that oxidative stress may represent a "unifying mechanism" which links all of the damaging biochemical pathways induced by hyperglycaemia in diabetic retinopathy.[63] They propose that mitochondrial-derived ROS causes strand breaks in DNA which successively activates poly-(ADP-ribose)-polymerase (PARP). The activation of PARP inhibits glyceraldehyde phosphate dehydrogenase (GAPDH) activity which causes the buildup glycolytic of metabolites. The metabolites then activate the AGE, PKC$\beta\beta$2, polyol, and hexosamine pathways.[63] Other studies have suggested the important role of NADPH oxidase-derived ROS within the pathogenesis of diabetic retinopathy.[63] Recent evidence has implicated ROS-mediated activation of metalloproteinase-2 (MMP-2) within the development of diabetic retinopathy.[64]

1.7 Prevalence and Pathogenesis of diabetic cataract

Cataract in diabetic patients may be a major explanation for blindness in developed and developing countries. Cataract is taken into account a serious explanation for visual disorder in diabetic patients because the incidence and progression of cataract is elevated in patients with DM.[65,66] The association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies. Thanks to increasing numbers of type 1 and kind 2 diabetics worldwide, the incidence of diabetic cataracts steadily rises. Although cataract surgery, the foremost common surgical ophthalmic procedure worldwide, is a good

cure, the elucidation of pathomechanisms to delay or prevent the event of cataract in diabetic patients remains a challenge. Furthermore, patients with diabetes have higher complication rates from cataract surgery.[67] Both diabetes and cataract pose an infinite health and economic burden, particularly in developing countries, where diabetes treatment is insufficient and cataract surgery often inaccessible.[68]

The pathogenesis of diabetic cataract development remains not fully understood. Recent basic research studies have emphasized the role of the polyol pathway within the initiation of the disease process. The enzyme aldose reductase (AR) catalyzes the reduction of glucose to sorbitol through the polyol pathway, a process linked to the event of diabetic cataract. Extensive research has focused on the central role of the AR pathway because the initiating consider diabetic cataract formation. It's been shown that the intracellular accumulation of sorbitol results in osmotic changes leading to hydropic lens fibers that degenerate and form sugar cataracts.[69,70] within the lens, sorbitol is produced faster than it's converted to fructose by the enzyme sorbitol dehydrogenase. additionally, the polar character of sorbitol prevents its intracellular removal through diffusion. The increased accumulation of sorbitol creates a hyperosmotic effect that leads to an infusion of fluid to countervail the osmotic gradient. Animal studies have shown that the intracellular accumulation of polyols ends up in a collapse and liquefaction of lens fibers, which ultimately leads to the formation of lens opacities.[69,70] These findings have led to the "Osmotic Hypothesis" of sugar cataract formation, emphasizing that the intracellular increase of fluid in response to AR-mediated accumulation of polyols ends up in lens swelling related to complex biochemical changes ultimately resulting in cataract formation.[69,70] Furthermore, studies have shown that osmotic stress within the lens caused by sorbitol accumulation induces apoptosis in lens epithelial cells (LEC) resulting in the event of cataract.[71-73] The role of osmotic stress is especially important for the rapid cataract formation in young patients with type 1 DM thanks to the extensive swelling of cortical lens fibers.[74,75] A study performed by Oishi et al. investigated whether AR is linked to the event of adult diabetic cataracts.[76] Levels of AR in red blood cells of patients under 60 years old with a brief duration of diabetes were positively correlated with the prevalence of posterior subcapsular cataracts. A indirect correlation has been shown in diabetic patients between the quantity of AR in erythrocytes and therefore the density of lens epithelial cells, which are known to be decreased in diabetics compared to nondiabetics suggesting a possible role of AR during this pathomechanism.[77]

The polyol pathway has been described because the primary mediator of diabetes-induced oxidative stress within the lens.[78] Osmotic stress caused by the buildup of sorbitol induces stress within the endoplasmic reticulum (ER), the principal site of protein synthesis, ultimately resulting in the generation of free radicals. ER stress may additionally result from fluctuations of glucose levels initiating an unfolded protein response (UPR) that generates reactive oxygen species (ROS) and causes oxidative stress damage to lens fibers.[79]

There are numerous recent publications that describe oxidative stress damage to lens fibers by radical scavengers in diabetics. However, there's no evidence that these free radicals initiate the method of cataract formation but rather accelerate and aggravate its development. peroxide (H2O2) is elevated within the humour of diabetics and induces the generation of hydroxyl radicals (OH–) after entering the lens through processes described as Fenton reactions.[80] The atom gas (NO•), another factor elevated within the diabetic lens and within the humour.[81,82] may result in an increased peroxynitrite formation, which successively induces cell damage thanks to its oxidizing properties.

Furthermore, increased glucose levels within the humor may induce glycation of lens proteins, a process leading to the generation of superoxide radicals (O2) and within the formation of advanced glycation endproducts (AGE).[83] By interaction old with cell surface receptors like receptor for advanced glycation endproducts within the epithelium of the lens further O2− and H2O2 are generated.[84] Additionally to increased levels of free radicals, diabetic lenses show an impaired antioxidant capacity, increasing their susceptibility to oxidative stress. The loss of antioxidants is exacerbated by glycation and inactivation of lens antioxidant enzymes like superoxide dismutases.[85] Copper-zink SOD 1 (SOD1) is that the most dominant enzyme isoenzyme within the lens,[86] which is vital for the degradation of superoxide radicals (O2 −) into peroxide (H2O2) and oxygen.[87] The importance of SOD1 within the protection against cataract development within the presence of diabetes has been shown in various in vitro and in vivo animal studies.[88,89] Therefore, it suggested that the initiating mechanism in diabetic cataract formation is that the generation of polyols from glucose by AR, which ends up in increased osmotic stress within the lens fibers resulting in their swelling and rupture.[65] Aldose reductase enzyme and particularly its inhibition by aldose reductase inhibitors (ARIs), has been gaining attention over the last years from the pharmaceutical community, because it appears to be a promising pharmacotherapeutic target.[90] Several authors have studied and reported on variety of structurally diverse present

and artificial AR inhibitors that have proven to be effective for the prevention of diabetic complications in experimental animals, moreover as in clinical trials.[91,92]

Although, some synthetic aldose reductase inhibitors (ARIs) are developed as drug candidates, however, virtually all haven't been successful in clinical trials because of adverse pharmacokinetic properties, inadequate efficacy, and toxic side effects. The utilization of medicinal plants, plant extracts or plant-derived pure chemicals to treat human ailments is a crucial alternative therapeutic approach. The medicinal values of plants lie their component phytochemicals like alkaloids, tannins, flavonoids and other phenolic compounds, which produce an exact physiological action on the figure. a scientific look for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research.[90]

1.8 Dysregulation of nrf2 signaling in diabetes

Oxidative stress could be a central feature of diabetes and plays a causal role within the pathogenesis of diabetic complications. The nuclear factor-like 2 (Nrf2) transcription factor mediates the induction of antioxidant and cytoprotective genes and may be a major regulator of the endogenous antioxidant and detoxification systems.[93] Heme-oxygenase-1 (HO-1) can provide an endogenous protective anti-oxidant system associated with the rise in biliverdin and bilirubin, which possess anti-oxidant and anti complement activity, and may reduce lipid peroxidation.[94] additionally,it has been suggested that CO may additionally have anti-inflammatory properties, including inhibition of pro-inflammatory cytokine expression. HO-1 is that the rate limiting enzyme in heme degradation and leads to the discharge of biliverdin, CO, ferrous iron and biliverdin product, being subsequently reduced to bilirubin.[94]

HO-1 may be a rate-limiting enzyme that catalyzes the oxidative degradation of heme into biliverdin, carbon monoxide gas and iron. Importantly, the transcription factor Nrf2 by binding at the promoter site of antioxidant response elements (ARE), regulate the expression of a collection of genes code for electrophile-conjugating enzymes and antioxidant enzymes like HO-1, NAD(P)H:quinone oxidoreductase 1, glutathione S transferases, glutamate–cysteine ligase, and glutathione peroxidase.[95] Multiple aspects of the Nrf2 signaling pathway are aberrant in diabetes and simultaneously targeting the dysregulated aspects of the Nrf2 signaling pathway must be considered.[93] Compelling evidence exists to suggest that oxidative stress plays a causal role within the pathogenesis of diabetic complications like nephropathy, neuropathy, and cardiac hypertrophy, suggesting oxidative stress could be a

central feature of the disease. Improving the endogenous cellular antioxidant and detoxification system is under investigation as a therapeutic approach to reducing oxidative stress and attenuating complications in diabetes. Nrf2 may be a Cap 'n' Collar (CNC) basic-region leucine zipper transcription factor that functions as a serious regulator of the endogenous antioxidant and detoxification system and provides cells the power to adapt to oxidative stress and electrophiles by mediating the induction of the cytoprotective genes. it's expressed altogether tissues of the physical structure, and is important in maintaining cellular redox status.[96]

Research in humans has also shown that Nrf2 function is decreased in subjects with T2DM.[97,98] Siewart and colleagues[97] compared prooxidant status and mRNA expression of Nrf2 and HO-1 in 40 patients with T2DM and 30 age-matched controls. Using the thiobarbituric acid-reactive substances (TBARS) method, the authors found that blood from patients with T2DM exhibit a roughly 100% increase in oxidative stress compared to healthy controls. Furthermore, Nrf2 and HO-1 organic phenomenon was significantly lower in leukocytes from patients with T2DM in comparison to healthy controls.

1.9 Historical aspect of herbs as therapeutic agents

In India, Ayurveda medicine has used many herbs like turmeric possibly as early as 1900 BC.[99] Many other herbs and minerals employed in Ayurveda were later described by ancient Indian herbalists like Charaka and Sushruta during the first millennium BC. The Sushruta Samhita attributed to Sushruta within the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources and 57 preparations supported animal sources.[100] In these herbs were discussed for his or her healing properties and nutmeg, senna, sandalwood, rhubarb, myrrh, cinnamon and rosewater were also discussed for his or her biological properties. The Malaga authored Corpus of Simples, the foremost complete Arab herbal which introduced 200 new healing herbs, including tamarind, aconite and medicine.[101]

1.10 Nuclear factor-like 2 (nrf2) transcription factor as anti-oxidant

Oxidative stress may be a central feature of diabetes and plays a causal role within the pathogenesis of diabetic complications. Compelling evidence exists to suggest that oxidative stress could be a central feature of diabetes and diabetic complications like nephropathy, neuropathy, diabetic cataract and retinopathy. The nuclear factor-like 2 (Nrf2) may be a Cap 'n' Collar (CNC) basic-region leucine zipper transcription factor that functions as a significant regulator of the endogenous antioxidant and detoxification system and provides

cells the flexibility to adapt to oxidative stress and electrophiles by mediating the induction of the cytoprotective genes. it's expressed altogether tissues of the chassis, and is important in maintaining cellular redox status. Antioxidant/electrophile response element (ARE/EpRE)-regulated clinical test detoxifying enzymes and antioxidants is one among the foremost antioxidant pathways involved in counteracting increased oxidative stress and maintaining the redox status in many tissues.[102-133] The genes of GSH-Px, HO-1, -GCS, and NQO1, belonging to the ARE, are the downstream target genes of Nrf2. Cells have highly developed endogenous antioxidant defense systems to counteract the oxidative stress generated in many diseases.[104,105] Heme oxygenase-1 (HO-1), the rate-limiting enzyme that catalyzes the degradation of heme to biliverdin, carbon oxide (CO) and iron, is one in all the ARE-regulated phase II clinical trial detoxifying enzymes and antioxidants, which are regulated by the redox-sensitive transcription factor nuclear factor erythroid 2- related factor (Nrf2)[106] Overexpression of HO-1 is neuroprotective during a model of permanent middle artery occlusion (MCAO) in transgenic mice.[107] Furthermore, pharmacological induction of HO-1 has been shown to safeguard the retina from angle-closure glaucoma -induced ischemia-reperfusion injury.[108] Genetic overexpression of Nrf2 prevents the onset of T2DM in mice and little molecule activation of Nrf2 reduces oxidative stress, and a myriad of diabetic complications, including cardiovascular complications, nephropathy, and neuropathy.[109,110] Improving the endogenous cellular antioxidant and detoxification system is under investigation as a therapeutic approach to reducing oxidative stress and attenuating complications in diabetes.[111] Multiple aspects of the Nrf2 signaling pathway are aberrant in diabetes and targeting the dysregulated aspects of the Nrf2 signalling pathway should be considered nearly as good therapeutic target.[112] The potential of the Nrf2 pathway as a panacea for the features of the diabetic milieu has given rise to research aimed toward developing pharmacological therapies that focus on the Nrf2 pathway.

1.11 Benefical role of nrf2/ho-1 pathway

Grape-seed proanthocyanidin extract (GSPE), an extract obtained from red grape seeds, which are rich in plant flavonoids, proanthocyanidin oligomers and polymerized oligomers, has been widely marketed in worldwide. GSPE exhibits a good range of biologic properties for resisting oxidative stress. Grape Seed Proanthocyanidin (GSPE) extract has shown to provide protection against diabetic complications yet as its potent antioxidant and anti inflammatory activity.[113] GSPE also activated nuclear erythroid2-related factor2 (Nrf2), which may be a key antioxidative transcription factor, with the concomitant elevation of

downstream hemeoxygenase-1 (HO-1). Growing evidences clearly suggest that GSPE produces beneficial effects through counteracting oxidative injury by regulating the Nrf2 pathway, in various experimental disease conditions, including zearalenone-induced oxidative damage of liver in Kunming mice[114] and DM-induced dysfunction and morphological damage of the bladder in rat.[115] Inaddition, Activation of the Nrf2/HO-1 Antioxidant Pathway Contributes to the Protective Effects of boxthorn Polysaccharides, is that the liquid fraction of the Duke of Argyll's tea tree berries (Wolfberry), a standard Chinese medicine with proposed anti-aging effects, extracted by a process involving the removal of the lipid soluble components, like zeaxanthin and other carotenoids with alcohol, within the Rodent Retina after Ischemia-Reperfusion-Induced Damage.[116]

Diabecon (D-400) contains Gymnema sylvestre (Meshashringi), Eugenia jambolana (Jambu), Tinospora cordifolia, kino, Ficus glomerata, gourd (Karela), Ocimum sanctum (Vishnu priya), as its main ingredients[117] The preservation of cell function noted during this study after use of Diabecon (D-400) is additionally animal studies, indicating the regeneration of rat beta cells.[118] A double-blind, placebo-controlled trial of Diabecon (D-400) using forty consecutive NIDDM patients with secondary failure to oral hypoglycemic agents (OHA). There was a big reduction in postprandial plasma glucose levels and glycosylated haemoglobin levels within the drug treated group.[119]

Both clinical and animal studies clearly evidencing the efficacy as anti-hyperglycemic and anti-hyperlipidemic activity and safety of Diabecon (D-400) within the treatment of diabetes.[120-121] Further, administeration of Diabecon, 2 tablets thrice daily for 12 weeks, additionally to traditional antidiabetic treatment to patients with ketosis-resistant diabetes mellitus (NIDDM) and insulin-Dependent DM (IDDM) (n=30), shown to significant reductions in haemorrhages (suggest that helps in resolution of retinal and vitreal haemorrhages), exudation was reduced significantly indicating the anti-inflammatory effect and also inhibited the proliferative changes in retina and controlled progressive retinal damage. sharp-sightedness of all the patients was recorded before the administering the drug and at the tip of drug therapy. it absolutely was observed that 60% of patients showed improvement of vision by a minimum of one line on Snellen's chart. On funduscopic examination, the development in neovascularisation and determination of exudates was significant.[122]

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Drugs, Reagents and Solvents used

Diabecon (D-400) was obtained from Himalaya pharma, Makali, Bangalore. K-lyte, common salt, bicarbonate, sodium orthophosphate, and salt were purchased from Central Drug House (CDH), India; glucose, zinc protophorphyrin-IX (ZnPP) were purchased from Fischer scientific (India), ethanoic acid, and ethylenediaminetetraacetic acid (EDTA) were purchased from Qualigens, India; thiobarbituric acid, Tris-HCl and ammonium sulfate were purchased from sigma chemical company. All other chemicals used were of analytical grade. Triple water was employed in the experiment. ZnPP were dissolved in 0.5 mol/L NaOH, then titrated to pH 7.4 with HCl and reconstituted with normal saline. the answer was prepared darkly just before use and guarded from light.

2.2 Methods

2.2.1 Isolation and Culture of goat Lens

Fresh goat eyeballs were obtained from slaughterhouse immediately after slaughter and transported to the laboratory at 0-4°C. The Lenses were removed by lateral incision of the attention and incubated in artificial aqueous humour (NaCl 140 mM, KCl 5 mM, MgCl 2 2 mM, NaHCO3 0.5 mM, NaH (PO4) 2 0.5 mM, CaCl2 0.4 mM and Glucose 5.5 mM) at temperature and pH 7.8 for 72 h. Penicillin 32mg attempt to streptomycin 250 mg there have been added to the culture media to forestall bacterial contamination.[123,124]

2.2.2 Generation of cataract

Glucose in an exceedingly concentration of 55 mM was accustomed induce cataract. At high concentrations, glucose within the lens was metabolized through sorbitol pathway and accumulation of polyols (sugar alcohols), causing over hydration and oxidative stress. This led to cataractogenesis.

2.2.3 Photographic evaluation

Lenses incubated in glucose 5.5 µM remained transparent, whereas, the lens incubated in 55 µM glucose developed dense opacities. The opacity increased towards centre with complete opacification at the tip of 72 h. The Anti-cataract activity are often evaluated by assessments of whether treatment of medicine can retarded the event of opacity.[125]

Experimental protocol (Table 1)

S. No.	Group	Treatment
1	GP-I: Normal control	Normal lens [Control (Glucose 5.5 mM)]
2	GP-II: Disease Control	Glucose 55 mM exposed
3	GP-V: 200 D-400 treated	Glucose 55 mM + D-400 200 µg/ml
4	GP-V: 400 D-400 treated	Glucose 55 mM + D-400 µg/ml
5	GP-V: 400 Vit.C treated	Glucose 55 mM + Vit. C 400 µg/ml
6	GP-V: 400 D-400 + ZnPP treated	Glucose 55 mM + D-400 µg/ml+ ZnPP (10 µg/ml)
7	GP-V: 400 Vit.C + ZnPP treated	Glucose 55 mM + Vit. C 400 µg/ml + ZnPP (10 µg/ml)

2.3 Biochemical estimations

2.3.1 Measurement of Lipid-peroxidation

Lipid-peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by using the thiobarbituric acid test.[126] In brief, 0.2ml of homogenate was added to 0.8% thiobarbituric acid,8.1% sodium dodecyl sulfate (SDS) and acetic acid (20%) in distilled water. After heating for 60min in a water bath at 95° C, the mixture was then cooled and extracted with a mixture of n-butanol/pyridine (15:1,v:v). The absorbance of the reaction product present in the upper organic layer separated by centrifugation was measured spectrophotometrically at 532nm. The results were expressed in µM of MDA/mg of protein.

2.3.2 Estimation of protein content

The protein content of the samples was determined by the method of Lowry et al., using bovine serum albumin as the standard.[127]

2.3.3 Estimation of antioxidant enzyme levels

Superoxide dismutase (SOD)

SOD was assessed by utilizing the technique of Kakkar *et al.*,[128] A single unit of enzyme was expressed as %inhibition of nitroblutetrazolium (NBT) reduction/min. The results were expressed in SOD units/mg of protein.

Reduced Glutathione (GSH)

GSH was estimated by the method of Ellman.[129] In brief, 10% TCA was added to the homogenate and the mixture was centrifuged 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5'-dithiobisnitro benzoic acid in 100ml of 0.1% sodium nitrate)

and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was measured spectrophotometrically at 410nm. The results were expressed in mg of GSH/mg of protein.

2.3.4 Estimation of Nitrite/Nitrate

Nitrite level in tissue homogenate was estimated using Greiss reagent method.[130] In brief, 200 µl of protein-free supernatant, 30 µl of 10% NaOH was added followed by 300 µl of tris-HCl buffer and mixed well. To this, 530 µl of Griess reagent (0.3% N-1[Napthyl-ethylene diamine-dihydrochloride] in distilled water +3% Sulphanilamide in 1M HCl) was added and incubated in the dark for 10-15 minutes, and the absorbance was read at 540nm (Spectrophotometer, Beckman DU 640B, Switzerland). The results were expressed in µM of nitrite/mg of protein.

Bilirubin determination in tissue homogenate

Briefly, Aliquots of 500 µl of occular tissue homogenate were added to 250 mg of BaCl2 and vortex-mixed thoroughly, as described by Foresti et al. with some modifications. Then, 0.75 ml of benzene was added to the mixture, and tubes were vigorously vortex-mixed again. Benzene phase containing extracted bilirubin was separated from the aqueous phase by centrifugation at 13,000 g for 30 min. A standard bilirubin curve was obtained using commercial bilirubin. Bilirubin was measured spectrophotometrically, as the absorbance difference between 450 and 600 nm and expressed as mg/Dl.[131,132]

2.4 Statistical analysis

The results were expressed as mean ± standard error mean (S.EM) and analyzed using analysis of variance (ANOVA) followed by Tukey's multiple comparison test. p value < 0.05 was considered as statistically significant. Statistical analysis was performed using Graph Pad Instant Software, (Version 5.01).

3. RESULT

3.1 Effect of various doses of grape seed Diabecon (D-400) and Proanthocyanidin extract on tissue homogenate TBARS, nitrite/nitrate levels

There was a significant increase in TBARS level (measured as MDA) and nitrite/nitrate levels were observed in disease control group, as compared to the normal control, and levels were slightly recovered on GSPE per se. Supplementation of diabecon (D-400) (200 µg/ml and 400 µg/ml) produced a dose-dependently decreased TBARS levels, as compared to vehicle control group. Interestingly, Both Diabecon (D-400) has produced greater decrease in

TBARS level, even as compared to that of standard drug, Vitamin C treated group ($P \leq 0.05$) However, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly reverse the observed beneficial effects of both diabecon (D-400) (Table. 1).

3.2 Effect of various doses of Diabecon (D-400) on tissue homogenate SOD and Glutathione levels

SOD and glutathione levels in various groups are shown in table 1. Their levels were slightly, but not significantly increased in disease control group, as compared to the normal control. However, supplementation of both diabecon (D-400) (200 µg/ml and 400 µg/ml) SOD and glutathione levels were more significantly and dose dependently increased, as compared to vehicle control as well as standard drug Vitamin C treatment group ($P \leq 0.05$). However, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly reverse the observed beneficial effects of diabecon (D-400) (400 µg/ml) (Table. 2).

Table 2: Effect of various doses of Diabecon (D-400) on tissue homogenate TBARS, nitrite/nitrate, SOD, and glutathione levels. Values are expressed as mean± SEM; n=6; a denotes for P<0.05 vsnormal control, b denotes for P < 0.05 vs DC; c denotes for P<0.05 vs GSPE 200µg/ml treated; d denotes for P<0.05 vs GSPE 400µg/ml treated; Abbreviations: DC- diseases control.

Treatment	MDA (µmoles/mg protein)	Nitrite/nitrate (µmoles/mg protein)	SOD (U/mg protein)	Glutathione (µM of GSH/mg of protein)
NC	12.39 ±1.32	0.41 ±0.03	22.67 ±2.05	5.14 ±0.32
DC	19.98 ±2.15[a]	0.65 ±0.08[a]	28.12 ±1.65[a]	2.1 ±0.12[a]
ZnPP *per se*	13.02 ±1.85	0.67 ±0.09	23.35 ±2.75	2.08 ±0.15
D-400 (200µg/ml treated)	15.83 ±1.56 [b]	0.59 ±0.04	32.41 ±2.33[b]	3.93 ±0.17 [b]
D-400 (400µg/ml treated)	13.86 ±1.73 [b,c]	0.46 ±0.05 [b,c]	36.82 ±2.72 [b,c]	5.18 ±0.49 [b,c]
Vit.C (400µg/ml treated)	14.28 ±0.66[b]	0.51 ±0.03 [b]	26.44 ±1.83[b]	2.97 ±0.12[b]
ZnPP+D-400 (400µg/ml	18.35 ±1.69 [d]	0.69 ±0.07 [d]	29.82 ±2.72 [d]	3.08 ±0.49 [d]

treated)				
ZnPP+Vit.C (400µg/ml treated)	14.42 ±0.61	0.56 ±0.03	23.43 ±1.81	3.07 ±0.14

3.3 Photographic evaluation of effect of Diabecon (D-400) on glucose-induced opacity of lenses

Lenses incubated in glucose 5.5 µM remained transparent, whereas, the lens incubated in 55 µM glucose developed dense opacities. The opacity increased towards centre with complete opacification at the top of 72 h. supplementation of diabecon (D-400) (200 µg/ml and 400 µg/ml) retarded the event of opacity in dose-dependent fashion [Fig. 3]. Also, Vit C has shown to provide beneficial effect against glucose-induced lens opacity, but the effectiveness observed even at high dose is a smaller amount as compared to both diabecon (D-400) at a dose of 400 µg/ml. However, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly reverse the observed beneficial effects of diabecon (D-400) [Fig. 3].

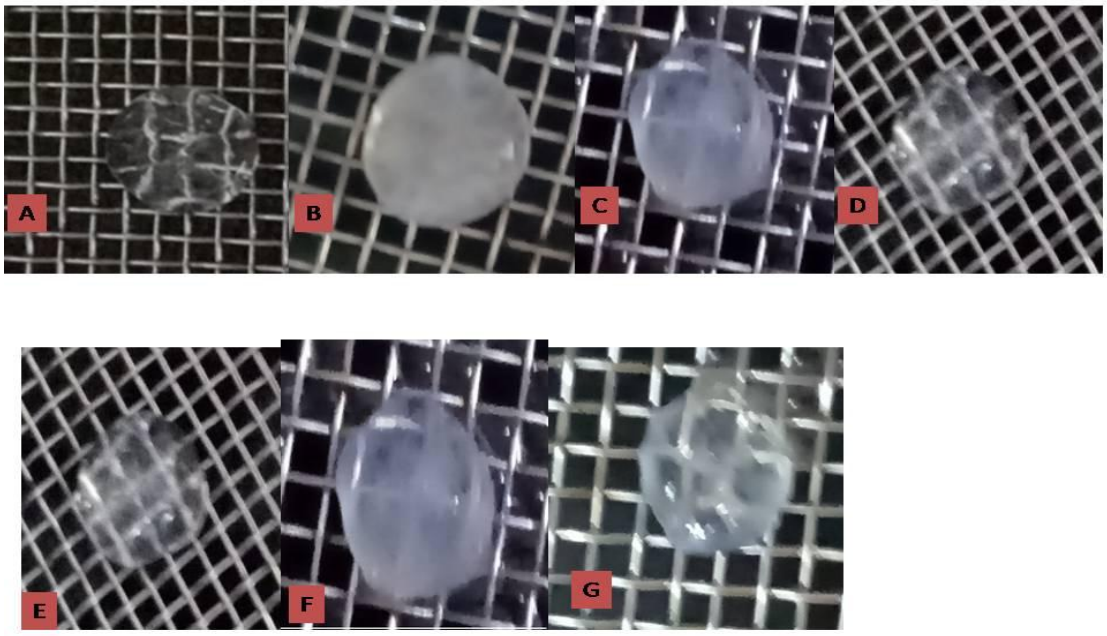

Figure 3: (A) Group I: Normal lens after 72 hours of incubation in glucose 5.5 mM (Lens transparency maintained, squares clearly visible). (B) Group III: Complete cataractogenesis after 72 hours of incubation in glucose 55 mM (Absolute loss of transparency, no squares visible through lens). (C) Group IV: After 72 hours of incubation in glucose 55 mM + Diabecon (D-400) 200 µg/ml, lens appears slightly hazy (less no. of squares slightly visible). (D) Group V: After 72 hours of incubation in glucose 55 mM +

Diabecon (D-400) 400 µg/ml, lens appears slightly hazy (More no. of squares slightly visible). (E). Group VIII: After 72 hours of incubation in glucose 55 mM + Vit C 200 µg/ml, lens appears slightly hazy (Moderate no. of squares visible). (F). Group IX: After 72 hours of incubation in glucose 55 mM + ZnPP (10 µg/ml + Diabecon (D-400) 200 µg/ml, lens appears hazy ((G). Group XI: After 72 hours of incubation in glucose 55 mM + ZnPP (10 µg/ml + Vit C 200 µg/ml, lens appears slightly hazy (Moderate no. of squares visible).

3.4 Effect of various doses of Diabecon (D-400) on tissue homogenate bilirubin levels

Bilirubin, a product of the HO-1 system cleavage, is slightly, but not significantly increased in the hyperglycemia-induced in cataract lens tissue, when compared to normal control group. However, supplementation of diabecon (D-400) (200 µg/ml and 400 µg/ml) have significantly and dose dependently increased bilirubin, as compared to vehicle control as well as standard drug Vitamin C treatment group ($P \leq 0.05$) (Table.). Therefore, we can infer that during hyperglycemia-induced cataract formation, there was an increase in the HO-1 expression, probably as a response to oxidative stress. However, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly reverse the observed beneficial effects of diabecon (D-400) (400 µg/ml). However, the role of specific HO-1 enzymatic products in the ameliorative effects observed was not completely elucidated. Our results indicate that the HO-1/biliverdin/CO pathway plays a protective role against hyperglycemia-induced cataract and that these preventive effects probably result from decreased free radical production [Fig. 4].

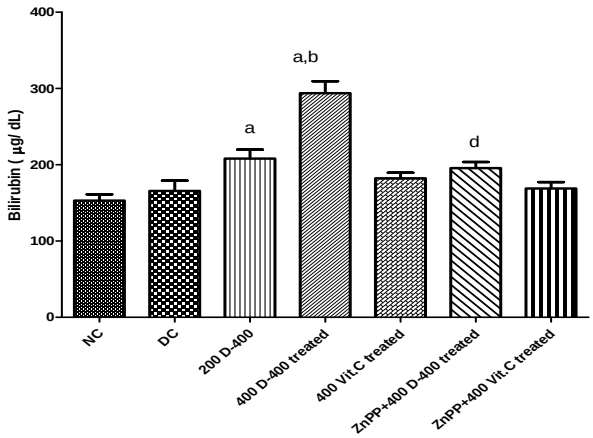

Fig 4. Effect of various doses of Diabecon (D-400), on tissue homogenate bilirubin levels. Values are expressed as mean± S.E.M; n=6; a denotes for P<0.05 Vs. normal control, b denotes for P < 0.05 vs DC; c denotes for P<0.05 vs Diabecon 200µg/ml treated, d denotes for P<0.05 vs Diabecon 400µg/ml treated; Abbreviations: DC- diseases control.

4. DISCUSSION

Diabetes is now thought to be a plague, with the population of patients expected to rise to 380 million by 2025. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. quite 80% of diabetes deaths occur in low- and middle-income countries.[1,2] Tragically, this may result in approximately 4 million people round the world losing their sight from diabetic retinopathy, the leading explanation for blindness in patients aged 20 to 74 years.[3] With diabetes now recognised as a world epidemic, the incidence of retinopathy, a typical microvascular complication of diabetes, is predicted to rise to alarming levels.

The pathogenesis of diabetic cataract development continues to be not fully understood. Recent basic research studies have emphasized the role of the polyol pathway within the initiation of the disease process. The enzyme aldose reductase (AR) catalyzes the reduction of glucose to sorbitol through the polyol pathway, a process linked to the event of diabetic cataract. Extensive research has focused on the central role of the AR pathway because the initiating consider diabetic cataract formation. It's been shown that the intracellular accumulation of sorbitol results in osmotic changes leading to hydropic lens fibers that degenerate and form sugar cataracts.[69,70] Within the lens, sorbitol is produced faster than it's converted to fructose by the enzyme sorbitol dehydrogenase. Additionally, the polar character of sorbitol prevents its intracellular removal through diffusion. The increased accumulation of sorbitol creates a hyperosmotic effect that leads to an infusion of fluid to countervail the osmotic gradient. Animal studies have shown that the intracellular accumulation of polyols results in a collapse and liquefaction of lens fibers, which ultimately ends up in the formation of lens opacities.[69,70] These findings have led to the "Osmotic Hypothesis" of sugar cataract formation, emphasizing that the intracellular increase of fluid in response to AR-mediated accumulation of polyols ends up in lens swelling related to complex biochemical changes ultimately resulting in cataract formation.[69,70] Furthermore, studies have shown that osmotic stress within the lens caused by sorbitol accumulation induces apoptosis in lens epithelial cells (LEC) resulting in the event of cataract.[71-73] The role of osmotic stress is especially important for the rapid cataract formation in young patients with type 1 diabetes thanks to the extensive swelling of cortical lens fibers.[74,75] Cataract is that the opacification of eye lens, related to the breakdown of the attention lens micro-architecture, which interferes with transmission of sunshine onto the retina. Cataract remains the leading reason behind blindness and disability worldwide, particularly in

developing countries.[1] While effective surgical procedures are available for treatment, the matter of post-operative complications, cost of surgery and high number of individuals requiring surgery pose a considerable economic burden. except aging, various risk factors of cataract include: nutritional inadequacy, metabolic and inherited defects, UV radiation and smoking. Diabetes has been considered to be one amongst the main risk factors of cataract and age-attenuated prevalence of cataract in India is 3 times that of the u. s.. Cataract is that the most prevalent disorder resulting in visual disorder, the prospect of pharmacological intervention to inhibit or to delay the onset of cataract continues to be at the experimental stage. The enzyme aldose reductase catalyzes the reduction of glucose to sorbitol through the polyol pathway, which may be a process that's linked to cataract development in diabetes. Because sorbitol doesn't readily diffuse across cell membranes and since it demonstrates slow conversion to fructose, sorbitol accumulation under hyperglycemic conditions induces osmotic stress within the cell, resulting in lens fiber cell swelling and eventually to membrane rupture. Because aldose reductase is primarily localized to the lens epithelium, a rise in osmotic stress via sorbitol accumulation occurs in these cells first.[171] Of importance to the current study is that the polyol pathway and particularly the role of aldose reductase enzyme within the pathway. Aldose reductase is that the first and rate-limiting enzyme within the polyol pathway and reduces glucose to sorbitol utilizing NADPH as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase. Normally, the polyol pathway represents a minor route of glucose utilization, accounting for <3% of glucose consumption. However, within the diabetic state, the activity of this pathway is substantially increased and will represent up to 30% of total glucose consumption. Sorbitol doesn't readily diffuse across cell membranes, and also the intracellular accumulation of sorbitol has been implicated within the microvascular complications of diabetes like peripheral neuropathy, nephropathy, retinopathy, and cataracts.[172] Among these aldose reductase inhibitors, only epalrestat is on the market within the Japanese marketplace for the treatment of diabetic neuropathy. Thus, there's an urgent need for brand new aldose reductase inhibitorS. Interestingly, several herbal extracts and nutritional supplements (such as naturceuticals) have shown anti-cataract activities.[173] Activation of polyol pathway because of increased aldose reductase activity is one in every of the several mechanisms that are implicated within the development of assorted secondary complications of diabetes. Though numerous synthetic aldose reductase inhibitors are tested, these haven't been very successful clinically. Therefore, variety of common plant/ natural products utilized in Indian culinary are evaluated for his or her aldose reductase inhibitory

potential. Current try at discovering effective anticataract agent is concentrate on evaluating Aldose reductase inhibition (ARI) capacities of medicinal plants and plant extracts. Most of those plant/spice sources are expected to be largely free from adverse effects as they're being employed during a kind of dietary preparations and as traditional medicines also.[174]

Overall, free radicals have been implicated in the pathogenesis of various diseases and particularly diabetes and its secondary complication especially cataract. Free radicals that have been implicated in cataract formation include superoxide anion (O2 −), nitric oxide (NO), hydrogen peroxide (H2O2), and hydroxyl radicals (OH·). Superoxide anionin itself is not highly toxic but it may react with other molecules, such as NO, yielding more reactive compounds. An excess of NO, produced by inducible nitric oxide synthases (iNOS) upon stimulation, is thought to cause cell injury by nitrosative stress and this may occur in certain diseases. In the eye, NO contributes to glaucoma, diabetic retinopathy, and also cataract.[175,176] OH·, another highly reactive free radical has been shown to contribute to lens crystalline modification.[177] Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. In addition to hyperglycemia, diabetes mellitus is usually accompanied by increased generation of free radicals or impaired antioxidant defenses. Several hypothesis have been put forth to explain the genesis of free radicals in diabetes.

Oxidative stress is a central feature of diabetes and plays a causal role in the pathogenesis of diabetic complications. Compelling evidence exists to suggest that oxidative stress is a central feature of diabetes and diabetic complications such as nephropathy, neuropathy, diabetic cataract and retinopathy. The nuclear factor-like 2 (Nrf2) is a Cap ‘n’ Collar (CNC) basic-region leucine zipper transcription factor that functions as a major regulator of the endogenous antioxidant and detoxification system and provides cells the ability to adapt to oxidative stress and electrophiles by mediating the induction of the cytoprotective genes. It is expressed in all tissues of the human body, and is essential in maintaining cellular redox status. Antioxidant/electrophile response element (ARE/ EpRE)-regulated phase II detoxifying enzymes and antioxidants is one of the major antioxidant pathways involved in counteracting increased oxidative stress and maintaining the redox status in many tissues.[141-143] The genes of GSH-Px, HO-1, -GCS, and NQO1, belonging to the ARE, are the downstream target genes of Nrf2. Cells have highly developed endogenous antioxidant

defense systems to counteract the oxidative stress generated in many diseases.[142,143] Heme oxygenase-1 (HO-1), the rate-limiting enzyme that catalyzes the degradation of heme to biliverdin, carbon oxide (CO) and iron, is one of the ARE-regulated phase II detoxifying enzymes and antioxidants, which are regulated by the redox-sensitive transcription factor nuclear factor erythroid 2- related factor (Nrf2)[144] Overexpression of HO-1 is neuroprotective in a model of permanent middle cerebral artery occlusion (MCAO) in transgenic mice.[145] Furthermore, pharmacological induction of HO-1 has been shown to protect the retina from acute glaucoma -induced ischemia-reperfusion injury.[146] Multiple aspects of the Nrf2 signaling pathway are aberrant in diabetes and simultaneously targeting the dysregulated aspects of the Nrf2 signaling pathway ought to be considered.[93] HO-1 is the rate limiting enzyme in heme degradation and results in the release of biliverdin, CO, ferrous iron and biliverdin product, being subsequently reduced to bilirubin.[94]

Grape Seed Proanthocyanidin (GSPE) extract has shown to produce protection against diabetic complications as well as its potent antioxidant and anti-inflammatory activity.[150] GSPE also activated nuclear erythroid2-related factor2 (Nrf2), which is a key antioxidative transcription factor, with the concomitant elevation of downstream hemeoxygenase-1 (HO-1). Growing evidences clearly suggest that GSPE produces beneficial effects through counteracting oxidative injury by regulating the Nrf2 pathway, in various experimental disease conditions, including zearalenone-induced oxidative damage of liver in Kunming mice[151] and DM-induced dysfunction and morphological damage of the bladder in rat.[152] Inaddition, Activation of the Nrf2/HO-1 Antioxidant Pathway Contributes to the Protective Effects of Lycium Barbarum Polysaccharides, is the liquid fraction of the Lycium barbarum berries (Wolfberry), a traditional Chinese medicine with proposed anti-aging effects, extracted by a process involving the removal of the lipid soluble components, such as zeaxanthin and other carotenoids with alcohol, in the Rodent Retina after Ischemia-Reperfusion-Induced Damage.[153]

In the present study, treatment with Diabecon D-400 has been associated with significant increase in the levels of bilirubin, an end product of HO-1 pathway. Simil On the other hand, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly abolished the observed elevated levels of bilirubin associated with diabecon (D-400). This may indicate that activation of Nrf2/HO-1 pathway may responsible for observed beneficial effects of diabecon (D-400) in this study. However, more data using

enzyme-linked immunosorbant assay (ELISA) and Western blotting is needed to implicate the more clearly, whether increased expression and/or increased activity of HO-1.

Diabecon (D-400) contains Gymnema sylvestre (Meshashringi), Eugenia jambolana (Jambu), Tinospora cordifolia, Pterocarpus marsupium, Ficus glomerata, Momordica charantia (Karela), Ocimum sanctum (Vishnu priya), as its main ingredients[154] The preservation of beta cell function noted in this study after use of Diabecon (D-400) is also animal studies, indicating the regeneration of rat beta cells.[155] A double-blind, placebo-controlled trial of Diabecon (D-400) using forty consecutive NIDDM patients with secondary failure to oral hypoglycemic agents (OHA). There was a significant reduction in postprandial plasma glucose levels and glycosylated haemoglobin levels in the drug treated group.[156]

Both clinical and animal studies clearly evidencing the efficacy as anti-hyperglycemic and anti-hyperlipidemic activity and safety of Diabecon (D-400) in the treatment of diabetes.[157-158] Further, administeration of Diabecon, 2 tablets thrice daily for 12 weeks, in addition to conventional antidiabetic treatment to patients with non-insulin-dependent diabetes mellitus (NIDDM) and insulin-Dependent Diabetes Mellitus (IDDM) (n=30), shown to significantly reduce in haemorrhages, exudation and also inhibited the proliferative changes in retina and controlled progressive retinal damage. It was observed that 60% of patients showed improvement of vision by at least one line on Snellen's chart.[154]

CAT and GSH catalyze the transformation of H2O2 within the cell to harmless by products, thereby curtailing the amount of cellular destruction inflicted by products of lipid peroxidation. a reduction within the activities of those enzymes in tissues has been related to the buildup of highly reactive free radicals, resulting in deleterious effects like loss of integrity and performance of cell membranes.[178-180] Reduction within the levels of reduced glutathione is observed during cataract of any etiology. GSH plays a number one role in preserving lens clarity. It also acts as antioxidant and stabilize proteins in reduced form.[181] this study showed that GSPE remarkably decrease the elevated levels of nitrite/nitrate. this could result to GSPE induced decrease in iNOS and NO activity Taken as an entire, these data provide evidence that reactive oxygen species play a vital role within the pathogenesis of diabetic cataract. Thus, scavenging free radicals, reducing the depletion of endogenous anti-oxidant enzymes, and counteracting GSH deficiency may be the target for therapeutic intervention in diabetic cataract. These studies have implicated the generation of oxygen-derived free radicals and lipid peroxidation jointly of the foremost important mechanisms

involved within the pathogenesis of diabetic cataract. Antioxidants are known to inhibit lipid peroxidation and scavenge free radicals. Therefore, the grape seed proanthocyanidin might exert its activity in one or more aforementioned assays. This provides favourable results showing anti-cataract potential of GSPE secondary to reduction of oxidative-nitrosive stress. Phytoconstituents from herbal drugs may ultimately inhibit expenditure of GSH through oxidation leaving the-SH groups intact. On the opposite hand, they will directly stimulate reduced glutathione synthesis which can flow from to a modulating effect on reduced glutathione related enzymes within the lens. The restoration of reduced glutathione levels by diabecon (D-400) could also be responsible anti-cataract potential.

5. SUMMARY AND CONCLUSION

The present study was designed to evaluate the diabcon (D-400), ayurvedic medicines for their anti-cataract effect in-vitro hyperglycemia-induced cataract in cultured goat Lens and its mechanism of actions.

On the basis of the results obtained, the following salient findings have emerged;

A) An in-vitro hyperglycemia(55 mM glucose) has shown to induce cataract in cultured goat Lens, which is associated a significant increase in TBARS level (measured as MDA) and nitrite/nitrate levels were observed in disease control group, as compared to the normal control, while, SOD and glutathione levels were slightly, but not significantly increased in disease control group.

B) Supplementation of diabcon (D-400) (200 µg/ml and 400 µg/ml) produced a dose-dependent decrease in the levels of TBARS and nitrates, as compared to vehicle control group.

C) Bilirubin, a product of the HO-1 system cleavage, is slightly, but not significantly increased in the hyperglycemia-induced in cataract lens tissue, when compared to normal control group. However, Supplementation of diabcon (D-400) (200 µg/ml and 400 µg/ml) has more significantly and dose dependently increased biliverdin, as compared to vehicle control as well as standard drug Vitamin C treatment group ($P \leq 0.05$). Therefore, we can infer that during hyperglycemia-induced cataract formation, there was an increase in the HO-1 expression, probably as a response to oxidative stress. However, co-administration of zinc protophorphyrin-IX (ZnPP; 10µg/ml), a selective HO-1 inhibitor, has significantly reverse the observed beneficial effects of diabecon (D-400) at a dose of (400 µg/ml).

On the basis of above, it may be concluded that diabecon showed promising in vitro activity against glucose cataract in an isolated goat lens model. Our results also indicate that the activation of HO-1/biliverdin/CO pathway and descresed oxido-nitrosative stress, which may be responsible for the observed beneficial effects against hyperglycemia-induced cataract

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