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Inhibition of lens protein glycation and aldose reductase activity using Aqueous extract of Morinda Citrifolia and Ocimum Sanctum in Glucose induced diabetic cataract: A lens organ culture studies

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Abstract---Diabetes mellitus is a chronic disorder characterized by persistent elevated blood glucose level in the body which leads to various secondary complications like cataract. The understanding of mechanisms by which glucose exerts its toxicity is of utmost importance for rational pharmacological interventions to treat diabetic cataract. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The aim of the present study was to explore local antiglycation and anticataract potential of *M. citrifolia* and *O. sanctum* in goat lenses using glucose induced cataract model. A total of 120 goat lenses were divided into four groups of 30 each viz. Normal Control, Experimental diabetic cataract, Experimental diabetic cataract + Extract of *Morinda citrifolia* (0.25%), Experimental diabetic cataract Extract of *Ocimum sanctum* (0.25%). Extent of protein glycation and activity of aldose reductase were measured in lens homogenates. Experimental cataractous lenses showed increased levels of both glycation of protein as well as aldose reductase activity. However, lenses treated with *M. citrifolia* as well as *O. sanctum* showed significant decreased levels of both glycation of protein and aldose reductase activity. Both *M. citrifolia* and *O. sanctum* have showed

antiglycation and anticataract potential in the prevention of diabetic cataract when used tropically.

Keyword---lens protein, glycation, aldose reductase activity, Aqueous extract, diabetic, cataract.

Introduction

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, lipid and protein metabolism characterized by persistent elevated blood glucose level in the body. The prevalence of DM is increasing on a daily basis, according to International Diabetes Federation an estimated 463 million adults aged 20–79 years are currently living with diabetes which represents 9.3% of the world's population in this age group. The total number is predicted to rise to 578 million (10.2%) by 2030 and to 700 million (10.9%) by 2045. Similarly, India ranks second among the countries with the largest population with diabetes, being 77 million in 2019 predicted to rise to 101 million by 2030 and to 134.2 million by 2045 (1). Cataract is the opacification of eye lens, associated with the breakdown of the eye lens micro-architecture, which interferes with transmission of light onto the retina. The etiology of cataract is usually multifactorial. However, certain diseases, like diabetes, are known to accelerate the onset and progression of such disabilities. This is particularly true in the case of cataract development, where diabetes is a well-established accelerative factor (2). The understanding of mechanisms by which glucose exerts its toxicity is of utmost importance for rational pharmacological interventions to treat diabetic cataract. During hyperglycemia the cellular levels of glucose greatly increase in the lens which is independent of insulin for glucose entry. The excess glucose flux can lead to post-translational modification viz. non enzymatic glycation of long-lived lens proteins and also activates polyol pathway causing synthesis and accumulation of excessive sorbitol by aldose reductase utilizing NADPH in the lens fibres and consequent osmotic as well as oxidative stress (3,4).

As the knowledge of heterogeneity of diabetes increases, there is need to look for more effective agents with lesser side effects because of several limitations and side effects of the current synthetic drugs. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications (5,6). Under conditions of diabetes, the need of tight blood glucose control is a key prerequisite to reduce the incidence, progression, and severity of cataract. Yet periods of hyperglycemia in the daily regimen of a diabetic patient cannot be avoided, with all of the aforementioned deleterious consequences of glucose toxicity. Therefore, additional adjunct therapy interfering with the pathological processes at molecular level, e.g., based on antioxidants, aldose reductase inhibitors and anti-glycation agents, is needed to attenuate the noxious effects of glucose (7). Alternative medicine, use of herbs, dietary supplements and neutraceuticals, have become a major part in the clinical treatment of many chronic disorders including diabetes. This is mainly because of their better effectiveness, relatively low cost and fewer side

effects(8). Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.

Morinda citrifolia L. is commonly known as Great Morinda, Indian mulberry, Beach mulberry and noni. The noni fruit has been used in tropical regions as both Food and folk medicine. *Ocimum sanctum* is globally known for more than 2000 years as one of the most versatile medicinal plants, having a wide spectrum of biological activities. It is a popular sacred plant of Indian subcontinent, commonly known as tulsi or holy basil. Both these plants have a high index in a variety of pharmacological activities like antimicrobial, immunomodulatory, antistress, anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, chemoprotective, antihyperlipidemic, cardioprotective, antioxidant, antitussive, radioprotective, memory enhancing, antiarthritic, antifertility, antihypertensive, anticoagulant, anticataract, anthelmintic and antinociceptive activities(9,10). The objective of the present study was to determine the in vitro anticataract potential of aqueous fruit extract of *M. citrifolia* aqueous leaf extract of *O. sanctum* against glucose-induced cataractogenesis using goat lenses.

Methods and Materials

Institutional Ethics Committee approval was taken for the present study.

Study design

The study was done by "Lens Organ Culture Technique" in 120 fresh isolated goat lenses. These 120 lenses were grouped into 4 groups (Table 1).

Table 1
Study design

Groups	Name of the group	Composition
Group 1	Normal Control	Lens + KRB buffer + Glucose 5.5mM
Group 2	Experimental diabetic cataract	Lens + KRB buffer + Glucose 55mM
Group 3	Experimental diabetic cataract + Extract of *M. citrifolia*	Lens + KRB buffer + Glucose 55mM + Extract of *M. citrifolia*
Group 4	Experimental diabetic cataract + Extract of *O. sanctum*	Lens + KRB buffer + Glucose 55mM + Extract of *O. sanctum*

Preparation of plant water extracts

Dry powders of *M. citrifolia* fruits and *O. sanctum* leaves were taken and 25% w/v water extracts were prepared. The concentration of solution of each extract used for the study was 0.25%.

Preparation of lens culture

Goat eyeballs were obtained from the slaughter house and were transported to the laboratory in an ice box. Once reached laboratory lenses were removed from the eyeballs by intracapsular lens extraction method. The lenses were incubated in Krebs-Ringer Bicarbonate Buffer (KRB buffer) pH 7.8 with Cefixime 500 mg for 72 hrs (11).

Preparation of lens homogenate

At the end of 72 h of incubation, lenses from each group were removed and homogenised in 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000xg for 30 min at -4 ºC in a refrigerated centrifuge. The supernatant was subjected to the estimation of biochemical parameters

Estimation of biochemical parameters
Measurement of extent of Protein Glycation

Protein glycation was determined in terms of AEGs using an assay kit according to the manufacturer's recommendations. In summary, adding 50 microliters (µL) of lens supernatant to AGEs conjugated coated well and incubated for 10 minutes. After that, 50 µL of the diluted anti-AGE antibody was added and incubated for 1 hour. Wells were washed with 250 µL with wash buffer. 100 µL of diluted secondary antibody-HRP Conjugate was added per well and incubated for another 1 hour. Washing was repeated with wash buffer. In the next step, 100 µL of substrate solution was added and incubate for 10-20 minutes. Finally, the enzyme reaction was stopped by adding 100 µL of stop solution to each well. Absorbance was read immediately on a microplate reader using 450 nm. The total amounts of AGEs were determined as µg/mg of protein. Results were calculated using the standard curves plotted in assay.

Determination of Aldose Reductase (AR) Activity

Aldose reductase activity was measured according to the method of *Hayman* and *Kinoshita.* (12). The assay mixture in 1 ml contained 0.7 ml phosphate buffer (0.07 M), 0.1 ml of NADPH (0.125mM), 0.1 ml of lens supernatant, 0.1 ml of D L-glyceraldehyde (5×10^{-4}M). Appropriate reference blanks were employed for corrections containing except the substrate, D L-glyceraldehyde. The enzymatic reaction was started by the addition of substrate and the absorbance was recorded in UV- Spectrophotometer at 340 nm for at least 3 min at 30 sec intervals. AR activity was expressed as Δ OD/min/mg protein.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 6). All results were expressed in mean ± SD. Student's "t" test was used to compare results of biochemical parameters between the Group 1 and Group 2 (Normal control and Experimental diabetic cataract). For comparing the results of biochemical parameters between Group 2, Group 3, and Group 4, "One-way Analysis of variance" (ANOVA) was performed. A p value < 0.05 was considered significant.

Results

Effect of *M. citrifolia* and *O. sanctum* on lens protein glycation (AGEs)

Effect of *M. citrifolia* and *O. sanctum* on lens protein glycation was revealed in Fig. 1, shown that, the extent of protein glycation in lens incubated with 55 mM glucose (group 2) (4.58 ± 0.50 µg/mg protein) is significantly increased as compared with lens incubated with 5.5 mM glucose (group 1) (7.77 ± 1.22 µg/mg protein). The increase was statistically significant ($p<0.001$). Effect of fruit extract of *M. citrifolia* on lens protein glycation was found to be significant were extent of protein glycation deceased (Group 3) in the lenses incubated with fruit extract of Effect of *M. citrifolia* ($p<0.001$). Similar effects were also observed in lenses incubated with leaf extract of O. sanctum (Group 4) ($p<0.001$).

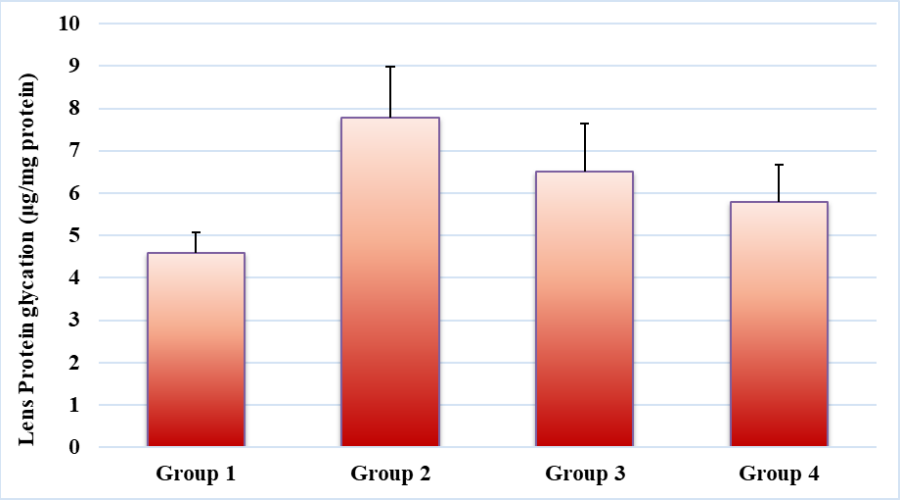

Fig. 1. Effect of *M. citrifolia* and *O. sanctum* on lens protein glycation (AGEs) in (Group 1) Normal control, (Group 2) Experimental diabetic cataract, (Group 3) Experimental diabetic cataract + Extract of *M. citrifolia* and (Group 4) Experimental diabetic cataract + Extract of *O. sanctum*. Data are mean ± SD. (n = 30)

Effect of *M. citrifolia* and *O. sanctum* on Aldose reductase activity

Aldose reductase activity in various study groups was shown in Fig. 2. Aldose reductase activity in normal control (Group 1) was found to be (0.004 ± 0.002 nmol/min/mg protein) whereas aldose reductase activity in experimental diabetic cataract (Group 2) was found to be (0.008 ± 0.004 nmol/min/mg protein). This increase in aldose reductase activity in group 2 was almost double and statistically significantly ($p<0.001$) when compared with group 1. Aldose reductase activity in lenses incubated with *M. citrifolia* (Group 3) and *O. sanctum* (Group 4) were decreased when compared with experimental diabetic cataract (Group 2). This decreased activity was much significant in group 3 than group 4.

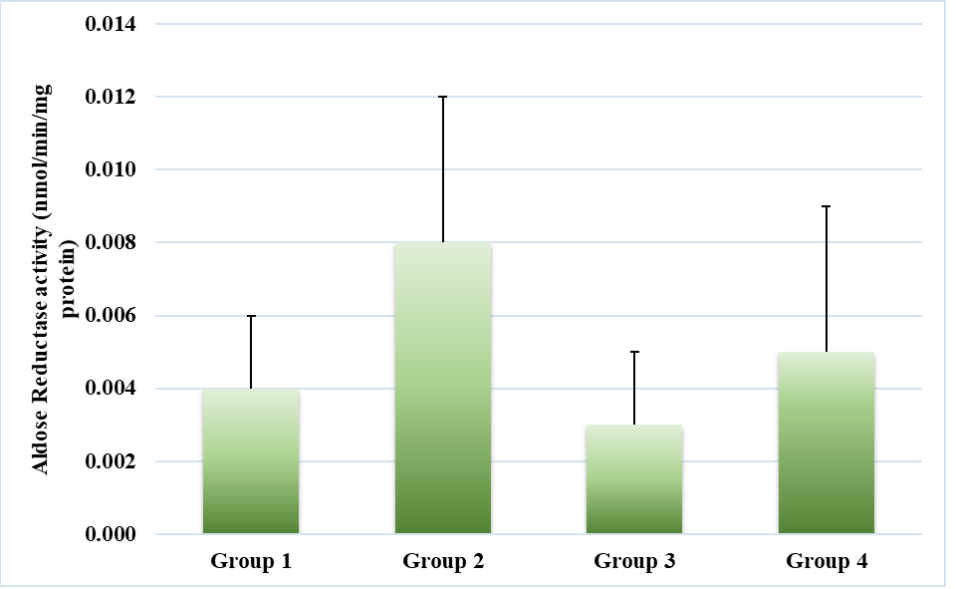

Fig. 2. Effect of *M. citrifolia* and *O. sanctum* on activity of aldose reductase of (Group 1) Normal control, (Group 2) Experimental diabetic cataract, (Group 3) Experimental diabetic cataract + Extract of *M. citrifolia* and (Group 4) Experimental diabetic cataract + Extract of *O. sanctum*. Data are mean ± SD. (n = 30).

Discussion

Three molecular mechanisms may be involved in the development of diabetic cataract: nonenzymatic glycation of eye lens proteins, oxidative stress, and activated polyol pathway in glucose disposition. All of these changes accelerate generation of reactive oxygen species (ROS) and increases in oxidative chemical modification of proteins in the lens of diabetic patients (13). Worldwide, researcher and scientists are working on prevention of diabetic complications. Use of naturally occurring compounds in the treatment of variety of chronic disorders and illnesses is growing, and many extracts and isolated compounds are becoming better alternatives to synthetic drugs, diabetes and its complications can be prevented and/or decreased using these natural molecules (14). *M. citrifolia* and *O. sanctum* both plants share a high index in a variety of pharmacological activities due to presence of various glycosides, alkaloids, flavonoids and many more bioactive compounds. We have selected these medicinal plants based on its antidiabetic activity and evaluated its antioxidant and AR inhibitory activity.

Hyperglycemia in diabetes causes uncontrolled entry of glucose in lens which triggers activation of the polyol pathway in the lens (15), this leads to synthesis of excessive amount of polyol products especially the sorbitol. The enzyme AR reduces glucose into sorbitol more rapidly in contrast sorbitol is more difficult to metabolize and its oxidation take place at very slower rate; as a results sorbitol accumulates in the lens epithelial cells. Furthermore, sorbitol being a polar and highly hydrophilic molecule not able to penetrate through lipid bilayer and thus

accumulates intracellularly (16,17). Intracellular accumulation of sorbitol creates hyperosmotic pressure that results into increase in water influx, fibre swelling and rupturing of lens cells results into leakage and disorganization of cellular proteins consequently develops into cataract (4). Since AR induced/mediated changes being major insults in the development of diabetic cataract, the inhibition of aldose reductase is, therefore, one of the potential pharmacological approach that has been proposed to treat or ameliorate secondary complications of diabetes including cataract. Our results have shown, significant increased AR activity in lens incubated with high glucose concentration, whereas significant decreased AR activity in lens incubated with *M. citrifolia* and *O. sanctum*. This show in-vitro inhibitory potential of these medicinal plants against lens AR. Both aqueous extracts demonstrated potential inhibitor of AR activity. However, aqueous fruit extract of *M. citrifolia* demonstrated a consistently better effect as compared to the aqueous leaf extract of *O. sanctum.* The effects found in the present study support the findings in past related to *M. citrifolia* (18).

Since the lens proteins are long-lived, they are highly susceptible to post-translational modification such as glycation which is believed to enhance protein unfolding, changing not only the physiochemical properties of lens proteins but also its function of the lens. Non enzymatic glycation is the process in which glucose reacts with e-amino group of lysine resulting in the Schiff base (SB) formation. This SB undergoes an Amadori rearrangement via Maillard reaction to form Amadori product. Later, the Amadori product undergoes dehydration and rearrangement to form cross-links of proteins. Resulting in protein aggregation or advanced glycation end products (AGEs) (19). In order to provide an efficient therapeutic strategy for diabetes and its associated complications, natural antiglycation compounds which have limited side effects have to be isolated from plants. In the present work, similar emphasis is given to evaluate the efficacy of two medicinal plants as antiglycation agent.

The experimental results of present study clearly indicate that incubation of lens in 55mM of glucose causes significant increase in lens protein glycation and addition of aqueous extract of *M. citrifolia* and *O. sanctum* in respective cultured lenses containing 55mM glucose prevented the levels of lens protein glycation significantly. Increased lens protein glycation in the experimental cataract model developed in the present study mimicked these effects seen in humans (19). Both aqueous extracts able to demonstrated the protection against formation of cataract by inhibiting non-enzymatic glycation in the experimental model.

Conclusion

Aqueous extract of *M. citrifolia* and *O. sanctum* were evaluated for their anticataract properties in goat lenses using lens organ culture technique. Aqueous extract of *M. citrifolia* showed strong inhibitor of aldose reductase, a key enzyme in polyol pathway involved in diabetic complication like cataract and it also showed inhibitor of lens protein glycation. *O. sanctum* also showed significant anti-cataract property by inhibiting activity of aldose reductase as well as lens protein glycation. Our findings are strongly suggestive of the possible usefulness of *M. citrifolia* and *O. sanctum* in the prevention of diabetic cataract when used

tropically, since ocular diseases are known to be treated more effectively by administering the treatment compounds topically.

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