Inhibition of diabetic cataract by glucose tolerance factor extracted from yeast

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Abstract

Diabetes leads to many complications; among them is the development of cataract. Hyperglycemia brings to increased polyol concentration in the lens, to glycation of lens proteins, and to elevated level of ROS (Reactive Oxygen Species) causing oxidative stress. The glucose tolerance factor (GTF) was found by several groups to decrease hyperglycemia and oxidative stress both in diabetic animals and humans. The aim of our study was to explore the damages induced by high glucose to the eye lens and to assess the protective effects of GTF both *in vivo* and *in vitro*. The *in vivo* study included control healthy rats, streptozotocin (STZ) diabetic untreated rats, and STZ diabetic rats orally treated with 15 doses of GTF. The diabetic untreated rats developed cataracts, whereas the development of cataract was totally or partially prevented in GTF treated animals. *In vitro* studies were done on bovine lenses incubated for 14 days. Half of the lenses were incubated in normal glucose conditions, and half in high glucose conditions (450 mg%). To one group of the normal or high glucose condition GTF was added. The optical quality of all the lenses was measured daily by an automated scanning laser system. The control lenses, whether with or without GTF addition, did not show any reduction in their quality. High glucose conditions induced optical damage to the lenses. Addition of GTF to high glucose conditions prevented this damage. High glucose conditions affected the activity of aldose reductase and sodium potassium ATPase in lens epithelial cell. Addition of GTF decreased the destructive changes induced by high glucose conditions. The amount of soluble cortical lens proteins was decreased and structural changes were detected in lenses incubated in high glucose medium. These changes could be prevented when GTF was added to high glucose medium. Our findings demonstrate the anticataractogenic potential of GTF.

Keywords: Lens, cataract, glucose tolerance factor, diabetes, aldose reductase, crystallins

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Introduction

Diabetes mellitus is a metabolic disorder with an increasing global prevalence. The disease is associated with disturbed glucose and lipid metabolism. According to World Health Organization report from August 2011, 346 million people in the world were diabetic.[1] The number will increase to 438 million within 20 years.[2] There are devastating complications related to diabetes, among them: atherosclerosis, nephropathy and retinopathy, and a much higher prevalence of cataract.[3] The number of people with cataract is expected to reach 30 million by 2020.[4] Cataract is the major cause of visual loss in older diabetic subjects and occurs at an earlier age than in non-diabetics.[5] Several epidemiological studies presented fivefold frequency of cataract in diabetic subjects compared with non-diabetic population.[3] The pathogenesis of diabetic cataract has not been fully revealed. Most of the studies indicate that hyperglycemia is the major cause for diabetic cataract. Higher glucose levels induce glycation of lens proteins leading to production of ROS (Reactive Oxygen Species) and creation of oxidative stress, decreased antioxidant enzymes activity, and aggregation of lens fibers.[6–8] Several studies presented the major role of polyol in diabetic cataract development.[9–11] All of the above cause a reduction in lens transparency and the development of cataract. Much effort has been invested in development of anticataract medications. Aldose reductase (AR) inhibitors,[9–11] vitamins,[12] and antioxidants[13–15] have been examined throughout the years with only partial effects. Up to date, there is no medication to prevent diabetic cataractogenesis, and surgical removal of the damaged lens is the only available treatment. This procedure poses high economic problem, particularly in the third world.

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The glucose tolerance factor (GTF) was first discovered by Schwarz and Mertz[16] in Brewer's yeast—the richest source for GTF.[17,18] GTF improved glucose tolerance of diabetic animals and humans. Doisy and his group[19] and Offenbacher and Pi-Sunyer[20] showed that treating diabetic patients with GTF remarkably improved their glucose tolerance. Grant and McMullen found a decrease in blood glucose and lipids in diabetic people following treatment with GTF.[21]

We presented in our previous studies that diabetic rats treated with GTF showed a remarkable decline in both glucose and lipids in their blood.[22] We also showed a preventive effect of GTF on nephropathy in diabetic rats following two weeks of oral treatment.[23] *In vitro* studies showed that GTF augmented glucose transport into yeast cells,[24,25] adipocytes,[26] and cardiomyocytes,[27] and elevated glycogen synthesis in hepatocytes following the addition of GTF to the medium.[28] Recent studies done in our laboratory showed that GTF increased the phosphorylation and activation of key proteins along insulin cellular cascade, exhibiting insulin mimetic activity.[29] Our review on GTF summarizes the research done on the subject.[30]

Despite the high importance of GTF, its composition and molecular structure have not been revealed yet, mainly due to the lability of the purified fractions.

We developed a method for extraction and isolation of GTF from brewer yeast, concluding with an active and stable material, as presented in our articles.[18,22,29]

A good *in vitro* model for studying the effects of high glucose on cataractogenesis is lens organ culture. We developed an *in vitro* system that enables keeping lenses intact for long-term experiments.[31] We established a method for monitoring lens optical quality during culture period.[32] This method can be used to detect early damage in the eye lens: focal changes appear when the lens is still clear.

The purpose of the present study was to examine the influence of high glucose conditions on cataract formation in diabetic animals *in vivo* and in lenses incubated in high glucose medium, *in vitro*. We also aimed at examining the effect of the antidiabetic agent, GTF, on the eye lens both *in vivo* and *in vitro*. In the current paper, we show the effects of high glucose levels on the eye lens in diabetic rats and in organ culture, and the protective effects of GTF in both conditions.

Materials and methods

Preparation of GTF

GTF was extracted and partially purified from commercial yeast extract according to a method developed in our laboratory, as detailed previously.[18,22]

The methanolic extract was filtered through two membranes (Cut off 3500 and 1000 dalton), and loaded and eluted from anionic (Diethylaminoethyl cellulose), and cationic (Dowex 50W) columns. Biological active fractions were further purified by HPLC C18 and gel filtration columns.

In vivo experiments

Five-week-old male Sprague-Dawley rats weighing 120–130 g were purchased from Harlan Israel. STZ, 60 mg/kg BW, dissolved in 50 mM citrate buffer, pH 4.5 was injected subcutaneously. Diabetic rats with non-fasting glycemia > 200 mg/dl were chosen for the experiment. Animal studies were approved by the committee for animal use and care of the University of Haifa.

Diabetic rats were divided into two groups of 10 animals: One group was treated with 15 daily doses (0.7 g/rat) of GTF (via a stomach tube); another group of diabetic animals was treated only with water (the vehicle). Additional 10 healthy rats served as a control group. Blood glucose from the tail was monitored once a week, and at indicating dates, the animals were administered with GTF. Rats eyes were examined once a week by a slit lamp microscope. Cataract appearance and progression of lens opacity were monitored as described by Suryanarayana *et al*.[33,34] Briefly, we graded lens opacity as follows: clear—clear lenses with no vacuoles, partial opacity, and mature cataract. At the end of the experiment photographs of the representative animals' eyes were taken.

Lens organ culture. Bovine lenses were carefully removed from the eyes. Each lens was placed in a specially designed culture chamber, consisting of two compartments. The lens was placed in a clear space filled with medium. The lens was incubated at 35 °C in 24 mL culture medium M199 with Earle's salt, 3% fetal calf serum, and antibiotics (penicillin and streptomycin). The medium was changed every 24 h.

Measurements of optical quality. Lens optical monitoring was done by an automated laser scanning system that records focal length according to Sivak *et al*.[32] A low power helium-neon laser mounted on a computer driven X-Y table with two video cameras and a video digitizer was used. The laser was programmed to scan across the lens in the axial direction in small steps. A custom software program determined the focal length. The optical center was determined by finding the position of minimum refraction for both X and Y directions. Focal length variability represents the variation in focal lengths of 22 beams and is calculated as the standard error of the mean of the average of the 22 focal lengths.

High glucose medium and GTF treatment

The lenses were divided into four groups: (1) control lenses, (2) lenses incubated in high glucose (450 mg%), (3) control lenses incubated with GTF (0.1 mg/mL), (4) lenses incubated in high glucose medium plus GTF (0.1 mg/mL).

Preparation of rats' lenses for protein and enzymatic analysis

The lenses were removed carefully under dissecting microscope and homogenized for 1 min in cold, in 2 mL phosphate buffer pH 7.4 containing 1.15% KCl. Samples of the homogenate were taken for protein and enzymatic determination.

Preparation of lens samples for enzymatic analysis

Dissection of lens epithelium by a cut along the equators was done under a stereomicroscope. The epithelium was immersed in 200 µL 50 mM phosphate buffer pH 7 at

0–4 °C. The tissue was sonicated twice in a MSE Ultrasonic Disintegrator at 50 W for 10 s, following by centrifugation at 14,000g for 10 min. AR activity was determined in the supernatant. ATPase activity was measured in the homogenate without centrifugation.

AR assay

AR activity was examined according to Hayman and Kinoshita,[35] by monitoring the production of NADP at 340 nm, using a Varian DMS 200 spectrophotometer with a thermo regulated cell at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer, pH 6.0, with 5 mM 2 mercaptoethanol, 0.1 mM βNADPH, 0.4 M Li_2SO_4, and 10 mM DL-glyceraldehyde. One unit of enzyme activity is defined as the amount oxidizing 1 µmol of NADPH per minute at 25 °C.

Sodium potassium ATPase (Na/K ATPase) assay

ATPase activity of the homogenates was measured according to Hightower and McCready[36] and Sheeham and Hrapchak.[37]

Protein determination

Protein concentration in the homogenate and the soluble supernatant was measured by the procedure of Lowry *et al*.[38]

Analysis of cortical lens proteins

Lens samples were taken on the 12th day of culture. The cortical samples were kept in 50 mM phosphate buffer, pH 7.4 in cold (-80 °C). To examine cortical proteins, Tris buffer pH 6.8 containing 3% EDTA (Ethylenediaminetetraacetic acid) was added. The samples were centrifuged in cooled centrifuge at 5000 rpm for 10 min. The supernatant was collected and used for analysis of lens soluble proteins.

The samples were dissolved in dissociation buffer pH 6.8 according to Leammli[39] and were loaded on polyacrylamide gel. The gel was run in 60 mA for 90 min. The proteins were transferred to nitrocellulose membranes and incubated overnight at 4 °C with specific antibodies for lens soluble proteins. The immunoblots were developed with ECL (chemiluminescent reagent for Western blotting) detection reagents by using Image analyzer. Quantitative analysis of lens soluble protein bands was performed by densitometry of the films using Image J. program (NIH). The results are expressed as arbitrary units.

Statistical analysis

The results are presented as means ± SEM. Statistical analysis was done by SPSS program. Statistically significant differences were analyzed via one-way ANOVA-Duncan test. $P < 0.05$ or 0.01 was considered as statistically significant.

Results

In vivo study

While glucose values measured for healthy control rats were low (102 ± 5 mg/dL) along all eight weeks of the experiment, the values detected in diabetic untreated rats at the end of the experiment reached 450 ± 25 mg/dL. Blood glucose values measured in diabetic rats treated with GTF were lower and reached the value of 252 ± 17 mg/dL, indicating a positive influence of GTF on reducing hyperglycemia in the treated diabetic animals.

Figure 1 presents the eyes of healthy animals where all the lenses looked clear (a), untreated diabetic animals where a full opacity of the lens is detected (b), and diabetic animals treated with 15 oral daily doses of GTF, where an inhibition of cataract appearance is clearly detected (c, d).

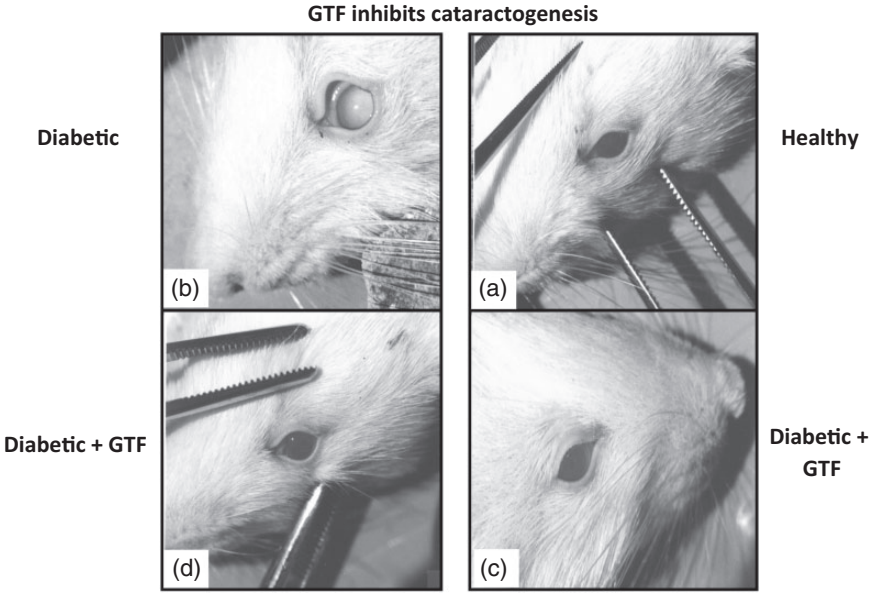

Figure 1 Representative rats eyes at the end of eight weeks study, of healthy, diabetic, and diabetic treated with 15 oral doses of GTF (0.7 g/rat). Note the decrease in turbidity of lenses of animals treated with GTF compared to diabetic untreated lens, where a severe cataract can be seen

Slit lamp detection indicated that none of the healthy rats developed cataract during the experimental period. Cataract could be detected from the fourth week and on from the induction of diabetes. It reached a full opacity for most of the diabetic animals (eight of 10) at the end of the eight weeks experiment, while two animals showed partial opacity in their lenses (Table 1). In diabetic animals treated with GTF a decreased number of cataracts developed (only four rats developed partial lens opacity and six animals did not develop any cataract) during the period of the experiment.

The protein content of the lenses decreased in diabetic lenses but returned to control values when the diabetic animals were treated with GTF (Figure 2).

We examined the activity of AR at the end of the eight-week study (Figure 3). While AR activity significantly increased in lenses removed from diabetic rats, the activity of this enzyme was reduced in lenses taken from animals treated by 15 repeated doses of GTF.

Table 1 Number of lenses in different stages of cataractogenesis at the end of the study

Cataract stage	Healthy control (n=10)	Diabetic untreated (n=10)	Diabetic treated with GTF (n=10)
Clear	10	0	6
Partial opacity	0	2	4
Mature cataract	0	8	0

Number of lenses at the end of eight weeks study, of healthy, diabetic, and diabetic treated with 15 oral doses of GTF (0.7 g/rat), at the different stages of cataract formation. Rats' eyes were examined once a week using a slit lamp biomicroscope. Cataract initiation and progression of lens opacity was monitored according to three grades: clear, partial opacity, mature cataract.

In vitro study

Three hundred and sixty-eight bovine lenses were used in this study. The optical quality of the lenses was detected daily, for two weeks. There was almost no change in focal length variability in the control lenses all experimental days (Figure 4). Lenses incubated in high glucose medium showed optical damage on day 4, recovery of the damage from day 5 to day 10, and increased damage from day 11 and on, as shown by an increase in focal length variability. Addition of 0.1 mg/mL GTF to the high glucose medium (containing 450 mg% glucose) reduced the optical damage almost to control values.

Figure 5 shows focal length profiles on day 12 of the culture of three different treatments: Figure 5(a) presents focal length profile of control bovine lens. This lens has good optical characteristics: almost all the + signs are at a similar distance to that of the focal length. Figure 5(b) demonstrates focal length profile of a lens incubated in high glucose medium. This lens shows increased variability of focal length indicating an optical damage. Figure 5(c) demonstrates the focal length profile of lens treated with high glucose in the presence of GTF. The ability of the lens to focus light when treated with both high glucose and GTF is much higher than that of the lens treated with glucose alone, indicating a defending effect of GTF on lens optical quality.

Na/K ATPase activity of bovine lenses during the first 10 days of culture is demonstrated in Figure 6. The activity of this enzyme decreased in lenses incubated in high glucose conditions. Addition of GTF to the high glucose medium reduced the glucose damage and brought Na/K ATPase activity close to control values.

The activity of AR in lenses incubated for 10 days in organ culture is demonstrated in Figure 7. The activity of control lenses is stable along the 10 days of culture. Incubation of the lenses in the presence of 450 mg% glucose increased significantly AR activity from day 5 to day 8 of

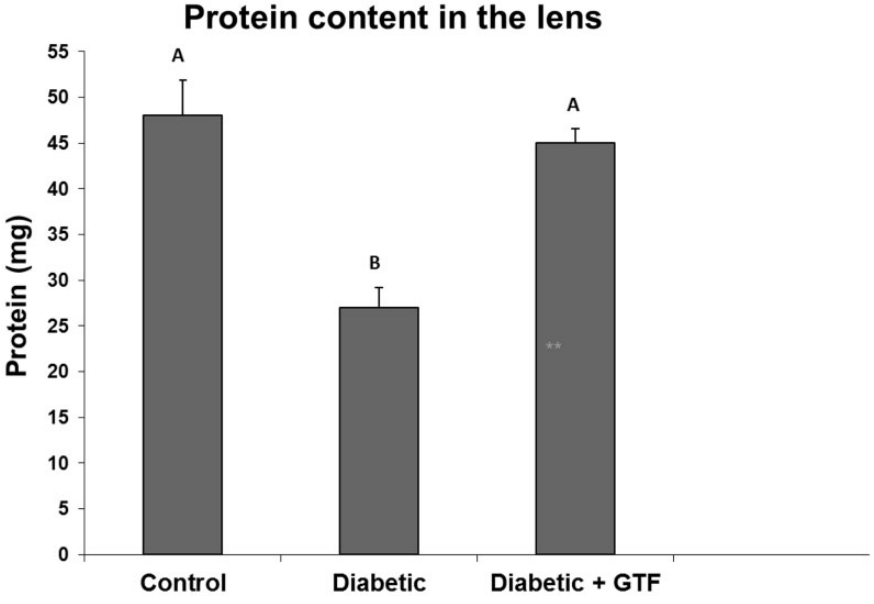

Figure 2 Protein content in the lens. Total protein content in rat lenses under different treatments: control lenses, lenses removed from diabetic rats, lenses removed from diabetic rats treated with 15 daily doses of GTF (0.7 g/rat)

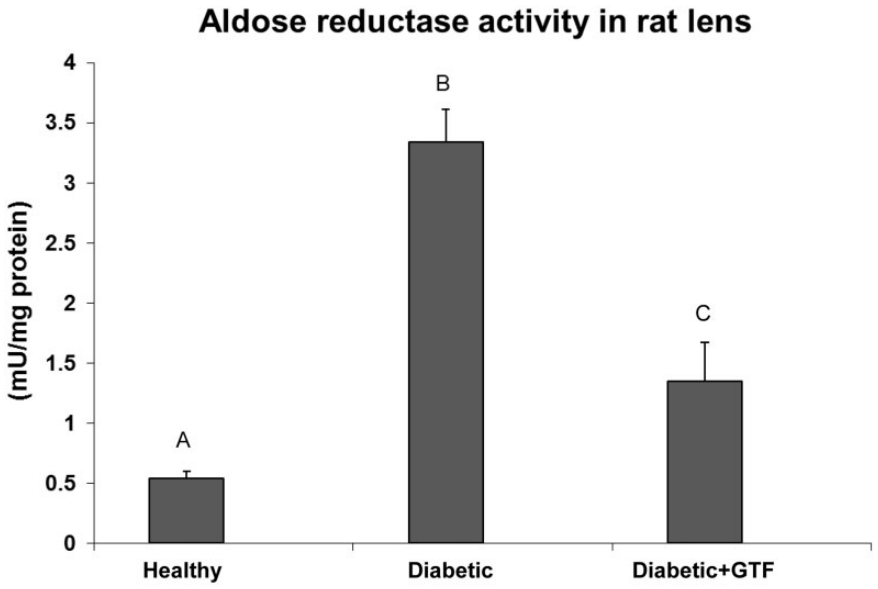

Figure 3 Effect of GTF treatment on aldose reductase activity in rat lenses. Aldose reductase specific activity (mU/mg protein) in lenses derived from healthy, diabetic, and diabetic rats treated with 15 repeated oral doses of GTF (0.7 g/rat)

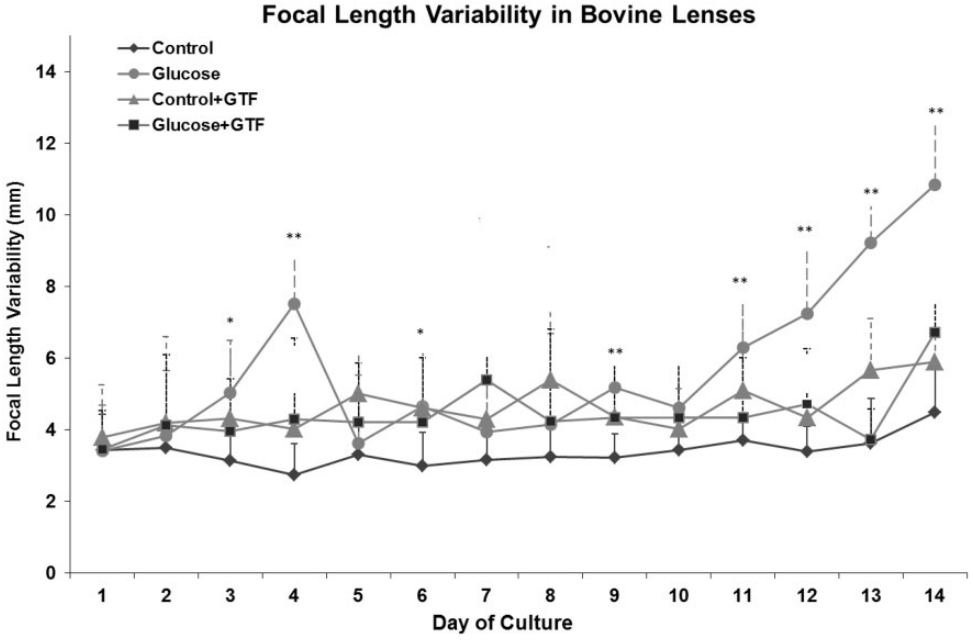

Figure 4 Lens optical quality during 14 days in culture. Focal lens variability of bovine lenses during 14 days in culture under different conditions: Control lenses, lenses incubated in 450 mg% glucose, control lenses incubated with GTF (0.1 mg/mL), lenses incubated in 450 mg% glucose+GTF (0.1 mg/mL)

the culture. GTF prevented the increase in AR activity through all days of incubation.

Figure 8 demonstrates dose-dependent inhibition of GTF on lens AR activity *in vitro*. As can be seen, higher doses of GTF had higher inhibitory effect on lens AR activity.

We examined lens cortical proteins following the different treatments: control lenses, lenses incubated in high glucose conditions, control lenses with GTF, and lenses incubated in high glucose conditions with the addition of GTF. We examined lenses that were incubated for 12 days, when the optical damage could be clearly detected in lenses incubated in high glucose conditions. Samples of soluble cortical proteins were prepared. The cortical proteins were run on SDS-PAGE (sodium dodecyl sulfate - PolyAcrylamide Gel Electrophoresis) and identified by specific antibodies. Figure 9(a) to (c) presents Western Blot analysis of soluble cortical proteins. The amount of total soluble proteins (Figure 9(a)) was significantly reduced in lenses incubated in high glucose conditions. Addition of GTF to the high glucose medium resulted in similar amounts of soluble proteins to those detected for control lenses.

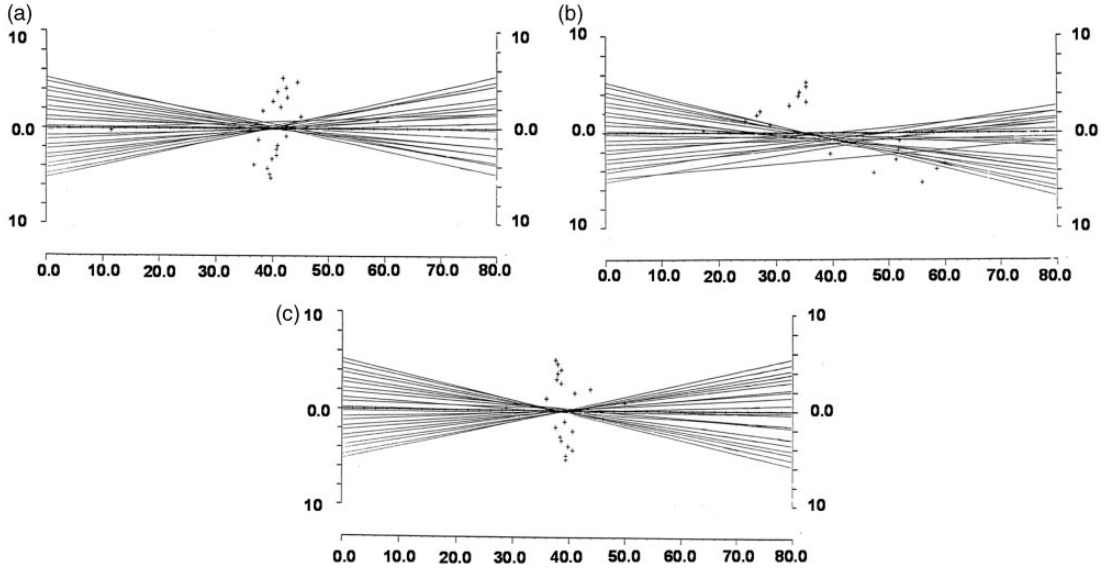

Figure 5 Focal length profile of bovine lenses on day 12 of the culture. Abscissa indicates focal length (mm). Ordinate refers to laser beam distance (mm) from the optic center (0.0) of the lens. The plus signs (+) indicate the focal points for each incident beam position away from the optic axis. The array of 22 lines coming to a focal point represents the direction of the refracted beams for each of the 22 incident beam positions. (a) Focal length profile for control bovine lens. (b) Focal length profile for lens treated with 450 mg% glucose. (c) Focal length profile for lens treated with 450 mg% glucose and 0.1 mg/mL GTF

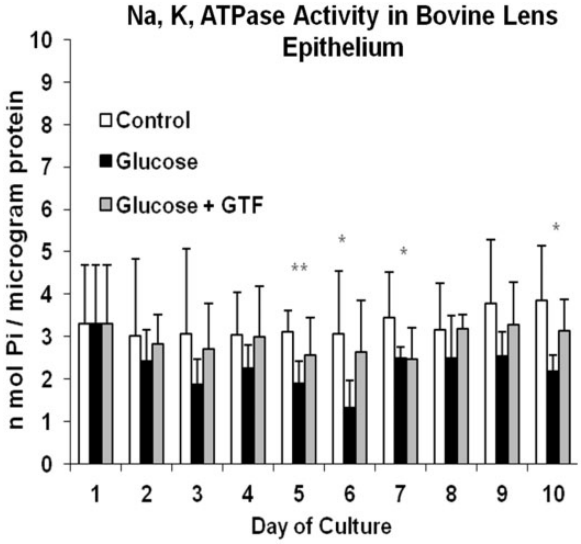

Figure 6 Na^+, K^+, ATPase activity in bovine lens epithelium. Na, K, ATPase activity in bovine lens epithelium during 10 days in culture under different conditions: Control lenses, lenses incubated in 450 mg% glucose, lenses incubated in 450 mg% glucose + GTF (0.1 mg/mL). The specific activity is expressed as nmole Pi/μg protein. Statistical significance between diabetic untreated and the two other groups: *$P < 0.01$ **$P < 0.05$

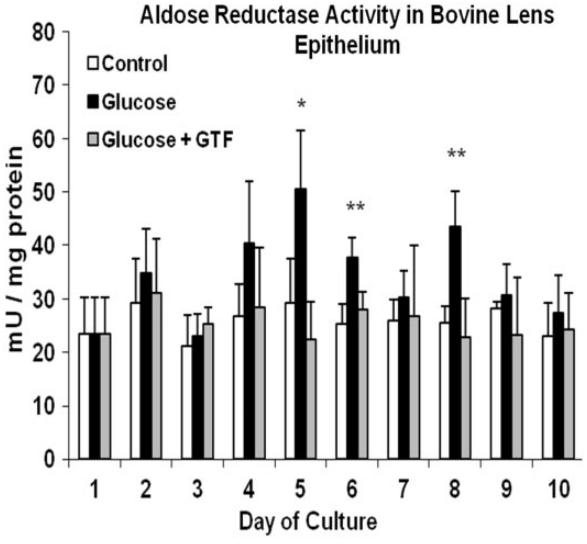

Figure 7 Aldose reductase activity of lenses in culture. Aldose reductase activity in bovine lens epithelium during 10 days in culture under different conditions: Control lenses, lenses incubated in 450 mg% glucose, lenses incubated in 450 mg% glucose+GTF (0.1 mg/mL). Statistical significance between diabetic untreated and the two other groups: *$P < 0.01$ **$P < 0.05$

While no change was detected in the amount of αB proteins for all different treatments (Figure 9(b)), significant changes were detected in the profile of βB1 proteins between control lenses and lenses incubated in high glucose medium (Figure 9(c)). The major band detected for βB1 was degraded in the high glucose treatment whereas the lower band, which was hardly seen in control lenses, was more intense for high glucose treatment. These changes were prevented in lenses incubated in high glucose conditions with the addition of GTF, indicating a protective effect of GTF on high glucose damage induced to βB1 proteins (Figure 9(c)).

Discussion

The relation between diabetes and cataract development was established in many investigations. The lens is most affected by hyperglycemia. Because insulin does not control glucose transport to the lens, in conditions of hyperglycemia blood glucose can freely diffuse into the lens and

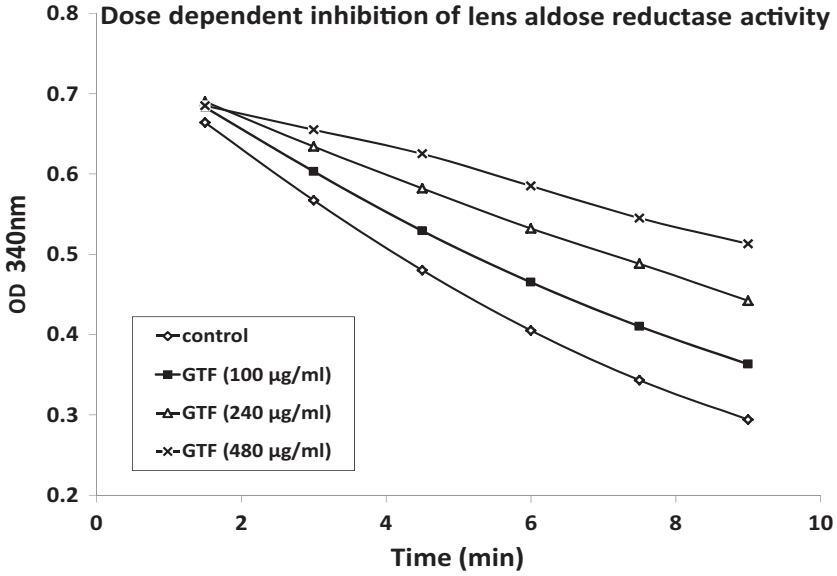

Figure 8 Dose-dependent inhibition of lens aldose reductase activity by GTF. The activity of lens aldose reductase was measured with the addition of increasing doses of GTF (100, 240, 480 µg/mL)

induce glycation of lens proteins. Lens proteins are long living, and there is almost no protein turnover, which brings to accumulated damage induced by external causes like hyperglycemia and oxidative stress.[6–8]

Hyperglycemia, oxidative stress, non-enzymatic glycation, and increased polyol accumulation contribute significantly to the appearance of diabetic cataract.[3,6–8,40–46]

In recent years, many efforts have been invested in exploring the use of natural substances to inhibit the development of cataract: antioxidants, anti-glycation compounds, and AR inhibitors. Recently, considerable attention has been devoted to the search for new therapeutics including food additives and phytochemical substances.[47–49]

The GTF that showed antidiabetic effects in general and anticataract activity in particular (as presented in the current study) is one of the promising natural compounds to treat cataract.

Although the beneficial effects of GTF both *in vivo* and *in vitro* are well established, the precise composition and structure of GTF have not been elucidated. Two main reasons can explain this fact: There is no established procedure for extraction and purification of GTF, and every research group has its own method to isolate the active material. In addition, as mentioned in many articles on GTF, the more purified fractions are not stable and tend to lose their activity within several days.[50,51] This instability contributed to the complexity of determining the molecular structure of GTF.

Most research groups (including our group) who studied GTF used the same procedure for the first steps of extraction and purification. All these groups found that GTF is a small cationic organic substance (around 1000 MW) and that it contains several amino acids. The differences among the groups are present in the more advanced steps of purification.

Mertz postulated that the GTF molecule contains chromium ion; nicotinic acid; and glycine, cysteine, and glutamic acid.[17,52,53] Other researchers stated that GTF extract contained quinoline derivatives[54] or phosphatidylinositol glycans.[55]

Mertz and his colleagues and additional investigators correlated GTF to chromium ion.[56,57] In contrast to this speculation, many research groups who used new and advanced equipment for detection of trace elements, including our laboratory, have not found any chromium in GTF.[58–60] In addition, no activity has been found for chromium complexes prepared as synthetic models for GTF.

We developed a method to extract an active and stable GTF from yeast. Our procedure includes filtration through several membranes, cationic and anionic exchange columns, and HPLC. The partially purified extract has a molecular weight below 1000 Da, is cationic, and soluble in water. It is stable to high and low pH and is active for almost a year when kept refrigerated. GTF is also stable to proteolytic enzymes, so it can be administrated orally, whereas insulin, which is a protein, should be injected.[22,23,29,61]

We examined GTF prepared in our laboratory in diabetic animals and found a significant decrease in hyperglycemia and hyperlipidemia in these animals.[22,23,29,61]

In the present study, we showed that while STZ-injected diabetic rats developed cataracts in their eye lenses within two months, diabetic rats treated with 15 daily doses of GTF showed a remarkable reduction in cataract development (Figure 1, Table 1).

It was shown in the literature[34] that STZ rats developed lens opacity from the third week of injection. This process increased with time. Full opacification was detected after eight weeks. We also found a similar pattern of cataractogenesis in STZ-injected rats.

Onkaramurthy *et al.*[62] examined the effects of methanolic extract of *Chromolaena odorata* Linn. leaves (ACO) in reducing hyperglycemia and cataractogenesis in STZ-induced

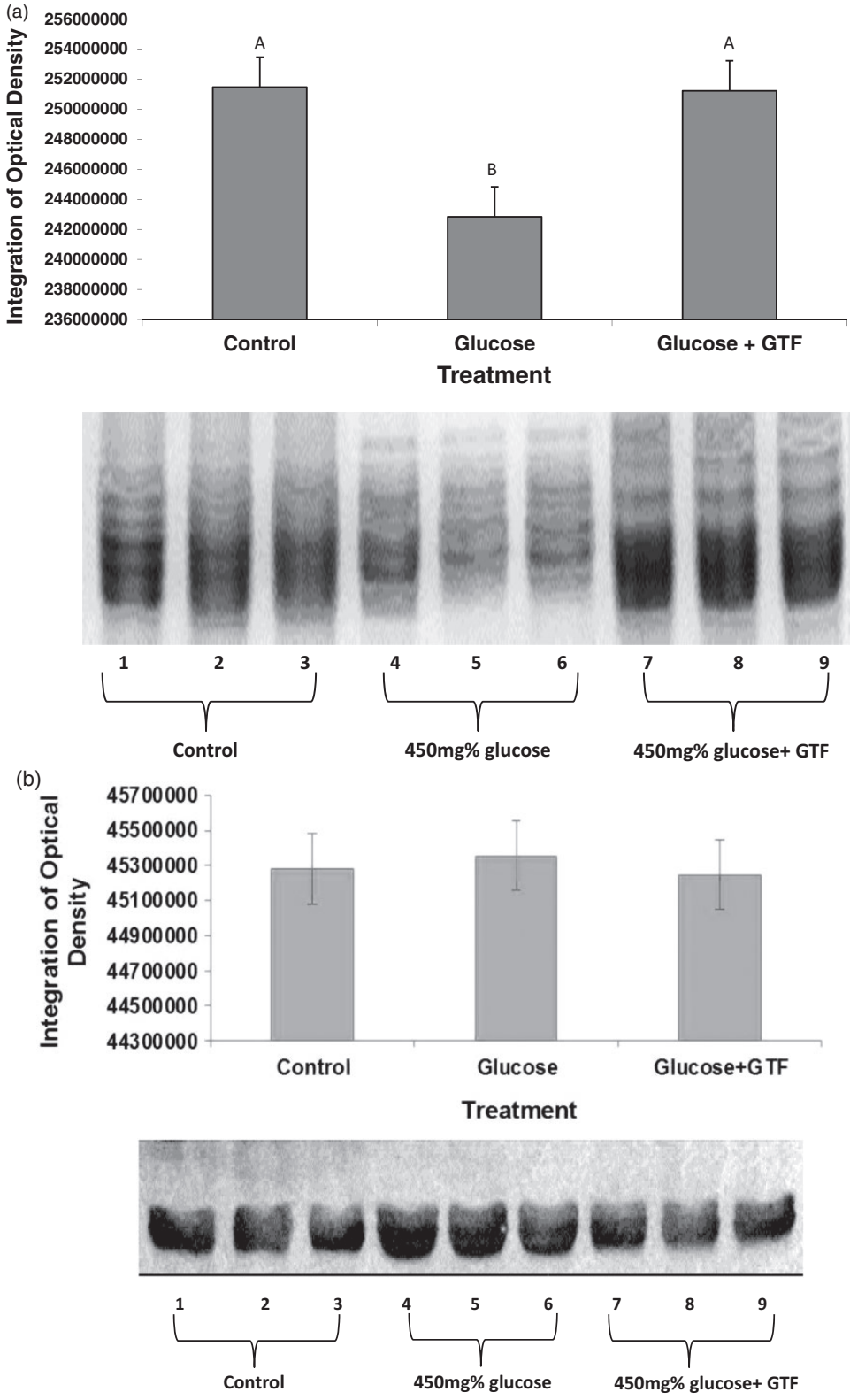

Figure 9 (a) Total soluble proteins from lens cortex, on day 12 of culture following different treatments: 1–3: Control lenses, 4–6: lenses incubated with 450 mg% glucose, 7–9: lenses incubated with 450 mg% glucose + GTF (0.1 mg/mL). Soluble cortical proteins were separated using SDS-PAGE. The gel was stained for proteins with Coomassie blue. (b) Western blot analysis of αB proteins from lens cortex, on day 12 of culture, following different treatments: 1–3: Control lenses, 4–6: lenses incubated with 450 mg% glucose, 7–9: lenses incubated with 450 mg% glucose + GTF (0.1 mg/mL). The proteins were detected by specific antibodies. (c) Western blot analysis of βB1 proteins from lens cortex, on day 12 of culture, following different treatments: 1–2: Control lenses, 3–6: lenses incubated with 450 mg% glucose, 7–9: lenses incubated with 450 mg% glucose + GTF (0.1 mg/mL). The proteins were detected by specific antibodies

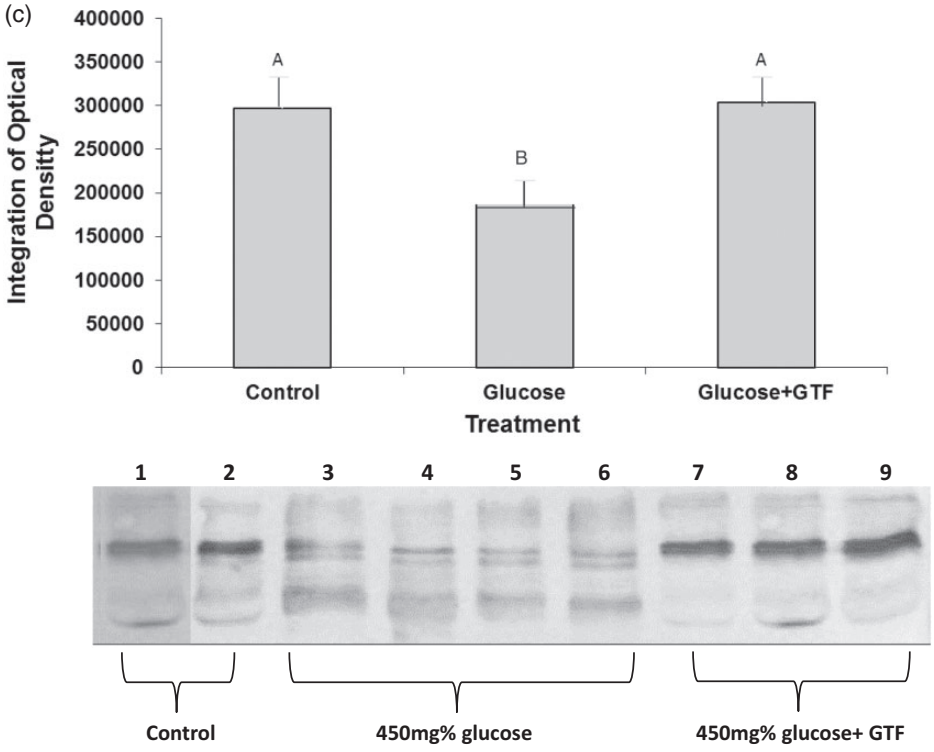

Figure 9 Continued

diabetic rats. They found both hypoglycemic and anticataractogenic effect of their extract, similar to our findings on GTF. The authors attributed the anticataractogenic activity of ACO to its hypoglycemic and antioxidant action.

In addition to the hypoglycemic and anticataractogenic activity of GTF in the animal level, we found a direct effect of the material on preventing lens cataract by adding GTF directly to the incubation medium.

Our lens organ culture is an efficient system for *in vitro* study on the damages induced by extracellular causes to the lens. This system already proved its value in experiments with UV irradiation.[63–69] In the current study, we used this system to detect the damage induced by high glucose concentrations on the lens incubated *in vitro* for 14 days and to study the potential effect of GTF added to the medium on preventing this damage.

Adding GTF to lenses in high glucose conditions conserved lens optical quality and prevented cataract formation. Lens AR activity, increased in high glucose conditions, was decreased by the addition of GTF. Our *in vivo* study on the activity of AR in rat lenses (Figure 3) showed that GTF decreased the elevated AR activity detected in lenses removed from hyperglycemic rats, in concert with our *in vitro* results (Figure 7).

It is widely known that hyperglycemia causes increased glucose concentration in the eye lens, which activates AR.[7,42,43,70] This enzyme, induced by high glucose concentrations, reduces glucose to sorbitol by using the reduction power of NADPH. Since sorbitol cannot leave the lens, it creates an osmotic effect which causes a mass entrance of water, swelling of the cells, and a chain of deleterious effects in the lens, like damages to Na/K ATPase pump, and other biochemical and structural changes, leading to cataractogenesis.[71]

It is assumed that cataractogenesis directly depends on AR concentration: Mice having lower amounts of the enzyme will not develop cataract despite of very high glucose levels in their blood.[72] Transgenic mice having higher AR concentrations develop cataract in much higher rates.[73] In addition, since AR reduces glucose to sorbitol using NADPH as the reducing power, higher glucose concentrations as in diabetes cause a depletion in NADPH reserves and in the reduction capacity in the lens, bringing to development of oxidative stress.[70,74] Oxidative stress is considered one of the early causes in the pathway of cataract development.[3,5–9]

AR inhibitors include plant extracts[9–11,49] or synthetic compounds.[75] Some of the synthetic agents are used in the clinic. Flavonoid compounds[75,76] and their derivatives from traditional medicinal herbs[77] and *Brassica juncea*[78] are also used as inhibitors of AR. However, none of these compounds have been found satisfactory.

We demonstrated in the current study that when the lenses were incubated in high glucose conditions the activity of Na/K ATPase decreased and addition of GTF to the medium reversed the enzyme activity.

Na/K ATPase is an enzyme found in the plasma membrane of all animal cells. This enzyme pumps sodium out of cells and potassium into cells, both against their concentration gradients, using energy derived from ATP[79]; Na/K ATPase is a key enzyme in many pathophysiological events related to diabetes, including cataract development.[80]

Mature lens fibers have no organelles or nuclei. The older cells differentiate into long fibers, losing their cellular organelles and filling their cytoplasm with high concentrations of soluble proteins, the crystallins, accounting for lens' transparency. The long-lived lens fibers are interconnected by gap junctions, among themselves and with the layer of epithelial cells at the lens surface, permitting intercellular diffusion of ions, metabolites, and water. The activity of the pump is higher in the epithelium than other parts of the lens.[81]

Functioning Na/K ATPase in epithelial cells is most important to keep lens fiber homeostasis.[82] When such homeostasis fails, cataract occurs.

In diabetics facing hyperglycemia, the conversion of glucose to sorbitol by AR is responsible for inducing an osmotic load within the lens, causing increased osmolarity, entrance of water, swelling, fibers breakdown, and opacification.[83]

The lens does not depend on insulin for its glucose uptake and the intracellular glucose concentration reflects the extracellular environment. The crystallin proteins of the lens may be glycosylated both *in vivo* and *in vitro*, in response to the high glucose concentrations.

Stevens[84] interpreted the decrease in Na/K ATPase activity in lens epithelial layer in diabetic conditions as the result of glycation of the pump proteins, in a similar mechanism detected in erythrocytes and pancreatic β cells of diabetic patients.[85]

The level of control of the pump by insulin might play a role in the complications of diabetes.[86] Insulin can increase the sensitivity of the pump to cellular Na + concentrations, to activate it or to induce its synthesis. Failure of insulin to activate Na/K ATPase pump may result in diabetes complications.

We showed in our previous studies[29,30] that GTF activated key proteins along insulin signaling pathway, exhibiting insulin mimetic potential. Based on these findings we anticipate an insulin-like activity of GTF also on Na/K ATPase pump.

Cataractogenesis also results in major changes in lens proteins. As seen in Figures 2 and 9(a) in the current study, there was a decrease in total protein amounts both in lenses removed from diabetic rats and lenses incubated in high glucose medium, which may indicate that part of the soluble lens proteins were glycated and aggregated as insoluble proteins.

Valavala *et al*.[78] evaluated the effect of extract derived from *B. juncea* leaves (BJLE) on cataract development in STZ diabetic rats. They measured the effect of the extract given every day for eight weeks. The extract inhibited cataract development and decreased total and soluble protein content.

In our current study, we found a decrease in total soluble proteins and a decreased amount of βB1 crystallins in lenses incubated in high glucose conditions (while no change was detected for αB crystallins).

The crystallins comprise about 90% of lens soluble proteins and are responsible for lens' transparency.[87] When diabetic cataract develops, there is a change in the structure of lens crystallins which can be detected when following their electrophoretic movements.

Western blot analysis done on βB1 proteins (Figure 9(c)) showed that the higher band intensity was decreased and the lower band intensity increased (when compared to control lenses), indicating a degradation process in βB1 proteins in high glucose conditions. This degradation could be prevented by addition of GTF to the high glucose medium.

Reddy *et al*.[88] evaluated the effect of hyperglycemia on the crystallins in STZ diabetic cataractous rat lenses. Chronic diabetes significantly decreased the protein levels of α-, β-, and αA-crystallins in both soluble and insoluble fraction of lens. Although there was a decrease in total lens crystallins following high glucose conditions in both our and Reddy's studies, there is a difference in the effect among the different groups of crystallins. These differences might be partially based on the types of lenses (rats versus bovine) and between the conditions of the studies (*in vivo* in Reddy's study versus *in vitro* in our study).

The β-crystallins differ in their number of subunits and formation of oligomers. They are deeply modified during aging and diabetes. Deamidation of β-crystallins may enhance their precipitation, contributing to cataract formation.[89] Lampi *et al*.[90] showed that there is degradation in βB crystallins of bovine lenses during the development of diabetic cataract. The authors suggested that most of the modifications were caused by induced changes in N terminal residues of the βB crystallins. When these changes in β-crystallin N-terminal extensions are enhanced, as in diabetic conditions, it may significantly alter crystallin interactions and lead to the formation of cataract.[91]

The decreased levels of soluble crystallins can be explained by altered protein synthesis or increased post-translational modifications of crystallins in diabetic cataractous lens.[88] The hyperglycemic conditions make the crystallins highly susceptible to degradation[92] as crystallins have been long shown to be targeted by multiple types of post-translational modifications in the lens. Furthermore, several studies reported the increased glycation and augmented concentration of AGEs (advanced glycation end products) in diabetic lens that might lead to cataract formation.[93,94]

Hyperglycemia also causes an increased glycation of lens proteins.[95,96] Glycated compounds are more sensitive to oxidation. The oxidized glycated compounds can react with lens's proteins and induce deleterious effects to these proteins.[6,95,96] In addition, glycated proteins are prone to the creation of cross-linking between groups, causing production of protein aggregates in the lens, leading to decreased light transfer through it.[95,96]

Several studies investigated the influence of natural antidiabetic extracts that reduce cataract and protect lens proteins. As described above, Valavala *et al*.[78] examined BJLE extract on cataract development in STZ diabetic rats. They found a positive effect on total and soluble protein content in diabetic rats' lenses. Diabecom, an ethnic Indian antidiabetic herbal drug, was tested for its *in vitro* effects on bovine lenses in organ culture. It caused a decreased activity of lens AR and decreased lens proteins glycation.[78] Suryanarayana *et al*.[97] examined the effect of *Emblica officinalis* extract (used

as herbal medicine against diabetes) in *in vitro* assay of AR and found an inhibitory effect of this extract on AR activity. Muthenna *et al*.[98] found that a certain fraction isolated from cinnamon extract could delay diabetic cataract by decreasing AGEs formation in diabetic rats. Grama *et al*.[99] studied the effect of nanocurcumin on cataract development in STZ diabetic rats. He showed that curcumin partially prevented the decrease in total and soluble lens's proteins induced by high glucose. This inhibition was attributed to nanocurcumin effects on protein glycation and aggregation, polyol accumulation, crystallins solubility, and oxidative processes.

Although additional studies have been done on phytochemicals and herbal extracts,[100] no single compound has been found so far to serve as anticataract medication in humans.

Our results presented in the current manuscript show that GTF, which was found antidiabetic both *in vivo* and *in vitro*, and exhibited also an anticataract activity, can serve as a source for developing a novel antidiabetic drug in general and an anticataract treatment in particular.

Authors' contribution: NM and AD were the principal investigators. RC and AE participated in the research during their graduate studies.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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