

Curcumin and Turmeric Delay Streptozotocin-Induced Diabetic Cataract in Rats

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PURPOSE. The purpose of this study was to investigate the effect of curcumin and its source, turmeric, on streptozotocin-induced diabetic cataract in rats.

METHODS. Wistar-NIN rats were selected and diabetes was induced by streptozotocin (35 mg/kg body weight, intraperitoneally) and divided into four groups (group II-V). The control (group I) rats received only vehicle. Group I and II animals received an unsupplemented AIN-93 diet, and those in groups III, IV, and V received 0.002% and 0.01% curcumin and 0.5% turmeric, respectively, in an AIN-93 diet for a period of 8 weeks. Cataract progression due to hyperglycemia was monitored by slit lamp biomicroscope and classified into four stages. At the end of 8 weeks, the animals were killed and the biochemical pathways involved in the pathogenesis of cataract such as oxidative stress, polyol pathway, alterations in protein content and crystallin profile in the lens were investigated, to understand the possible mechanism of action of curcumin and turmeric. Blood glucose and insulin levels were also determined.

RESULTS. Although, both curcumin and turmeric did not prevent streptozotocin-induced hyperglycemia, as assessed by blood glucose and insulin levels, slit lamp microscope observations indicated that these supplements delayed the progression and maturation of cataract. The present studies suggest that curcumin and turmeric treatment appear to have countered the hyperglycemia-induced oxidative stress, because there was a reversal of changes with respect to lipid peroxidation, reduced glutathione, protein carbonyl content and activities of antioxidant enzymes in a significant manner. Also, treatment with turmeric or curcumin appears to have minimized osmotic stress, as assessed by polyol pathway enzymes. Most important, aggregation and insolubilization of lens proteins due to hyperglycemia was prevented by turmeric and curcumin. Turmeric was more effective than its corresponding levels of curcumin.

CONCLUSIONS. The results indicate that turmeric and curcumin are effective against the development of diabetic cataract in rats. Further, these results imply that ingredients in the study's dietary sources, such as turmeric, may be explored for anti-cataractogenic agents that prevent or delay the development of

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Chronic hyperglycemia is a major determinant in the development of secondary complications of diabetes, including diabetic cataract. Studies indicate that hyperglycemia and the duration of diabetes increase the risk of development of cataract.¹⁻³ Cataracts, characterized by cloudiness or opacification of the crystalline eye lens, are the leading cause of blindness all over the world—more so in developing countries.⁴ Apart from aging, other risk factors such as nutritional deficiencies or inadequacies, trace metals, sunlight, smoking, and certain drugs are known to increase the risk of cataract.¹⁻⁴ In view of the widespread prevalence of diabetes in developing countries such as India,⁵⁻⁷ diabetic cataract may pose a major problem in the management of blindness.

Although the etiology of cataract is not fully understood, oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the initiation and progression of various types of cataracts, including diabetic cataract.⁸ Diabetes causes increased oxidative stress in various tissues, as evidenced by increased levels of oxidized DNA, proteins, and lipids, which are thought to play an important role in the pathogenesis of various diabetic complications.⁹ Several studies have suggested that intake of antioxidant-rich foods may slow the progression of cataract.¹⁰⁻¹³

Dietary intervention, particularly the use of traditional foods and medicines derived from natural sources, is the mainstay in the management of diabetes. In this context, there has been a growing interest in recent times in identifying as many dietary/spice sources as possible for their ability to control diabetes.^{12,14,15} Nevertheless, most studies of natural sources have focused only on their ability to maintain blood glucose levels, and have not been investigated for their beneficial effects on secondary complications of diabetes such as cataract, retinopathy, nephropathy, and neuropathy. Therefore, we have been interested in investigating various dietary sources for their potential to prevent the secondary complications of diabetes, such as cataract.¹⁶⁻¹⁸

Rhizome of turmeric (*Curcuma longa L*) is a widely used spice in Indian cuisine. Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), the active portion of turmeric, has been shown to have significant antioxidant activity, both in vitro and in vivo,¹⁹⁻²¹ in addition to its various other health-benefiting properties, such as anti-inflammatory, anticarcinogenic, antiviral, hypolipidemic, and anti-infectious effects.²⁰⁻²³ Earlier, it was reported that lenses obtained from curcumin-treated rats are resistant to 4-hydroxy-2-transnonenal-induced cataract formation in organ culture.²⁴ Although later it was demonstrated that dietary curcumin at a very low level (0.0025%) attenuates galactose-induced cataract in rats, dose dependency was not studied.²⁵ To understand the role of curcumin in preventing or delaying diabetic cataract in greater detail, earlier we studied the effect of curcumin in a galactose-induced cataract model with two levels of curcumin, 0.002% and 0.01%, in the diet.¹⁶ Although curcumin delayed the onset of cataract at both the levels, maturation was delayed by

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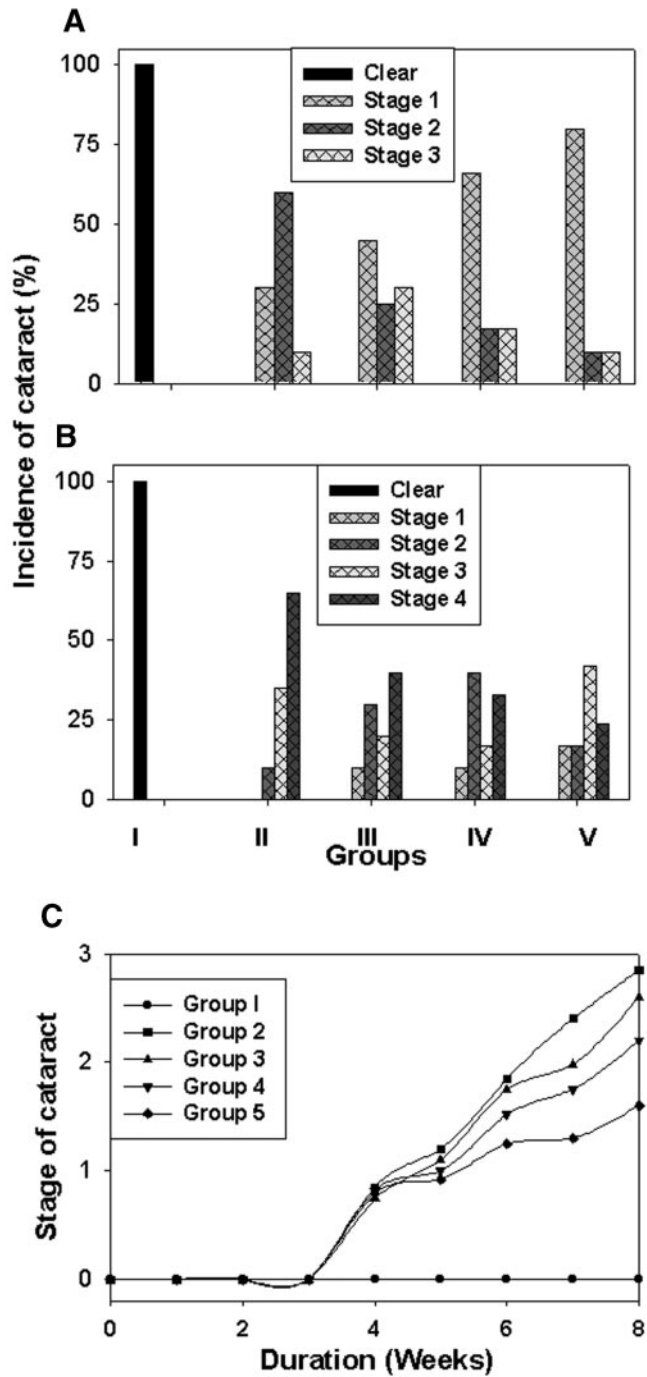


FIGURE 1. The effect of curcumin and turmeric on STZ-induced cataract after 5 (A) and 8 (B) weeks. In the calculation of the incidence of cataract in various stages, total lenses in a group were considered to be 100%. (C) The effect of curcumin and turmeric on the average stage of STZ-induced cataract, as a function of time.

0.002% curcumin, but not by 0.01%. The maturation was, in fact, faster with 0.01% curcumin.¹⁶

Because galactose-induced cataract does not mimic typical diabetic human cataract, we decided to investigate these differential effects of curcumin in another model, streptozotocin (STZ)-induced diabetes. Moreover, the protective effect of turmeric, the source of curcumin, against cataract has not been investigated. Hence, in the present study, we also investigated the effect of turmeric against diabetic cataract and compared it with that of curcumin.

METHODS

Materials

Streptozotocin (STZ), curcumin, NADPH, NADH, 2-thiobarbituric acid (TBA), 1,1,3,3-tetraethoxy propane (TEP), di-glyceraldehyde, lithium sulfate, β -mercaptoethanol, glutathione, BSA, 2,4-dinitrophenylhydrazine (DNPH), and EDTA were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of analytical grade and were obtained from local companies.

Experimental Design and Dietary Regimen

Three-month-old male WNIN rats with an average body weight of 228 ± 13 g (obtained from the National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad) were used in the study. All the animals were fed AIN-93 diet ad libitum. The control (group I; $n = 8$) rats received 0.1 M citrate buffer [pH 4.5] as a vehicle, whereas the experimental rats received a single intraperitoneal injection of STZ (35 mg/kg) in citrate buffer. After 72 hours, fasting blood glucose levels were monitored. Animals having blood glucose levels <145 mg/dL were excluded from the experiment and rest were distributed into four groups (groups II-V). Animals in these groups received either only the AIN-93 diet (group II; $n = 13$) or received the AIN-93 diet containing 0.002% (group III; $n = 9$) or 0.01% (group IV; $n = 9$) curcumin or 0.5% turmeric (group V; $n = 8$) for a period of 8 weeks. Turmeric contains 1% to 2% curcumin and hence 0.5% turmeric corresponds to approximately 0.01% curcumin.

Animal Care

Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee. Animals were housed in individual cages in a temperature- and humidity-controlled room with a 12-hour light-dark cycle. All the animals had free access to water. Food intake (daily) and body weights (weekly) were monitored. We adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Slit Lamp Examination and Cataract Classification

Eyes were examined every week using a slit lamp biomicroscope (Kowa Portable, Japan) on dilated pupils. Initiation and progression of lenticular opacity was graded into five categories as follows: clear, clear lenses and no vacuoles present; stage 1, vacuoles cover approximately one half of the surface of the anterior pole, forming a subcapsular cataract; stage 2, some vacuoles have disappeared and the cortex

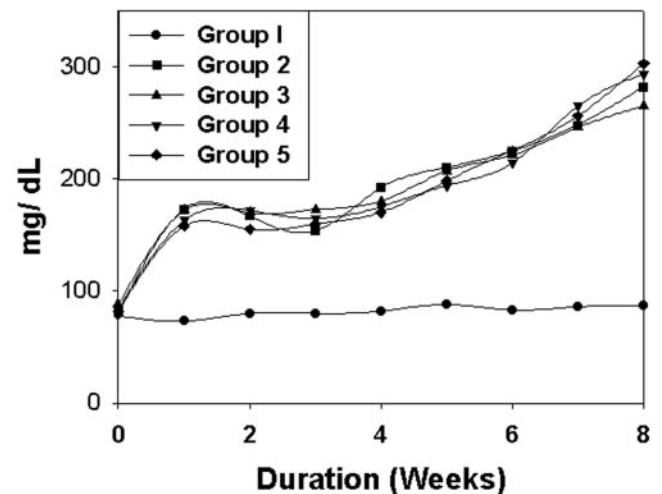


FIGURE 2. The effect of curcumin and turmeric on blood glucose levels in STZ-treated rats. Data are an average of all the animals in a given group.

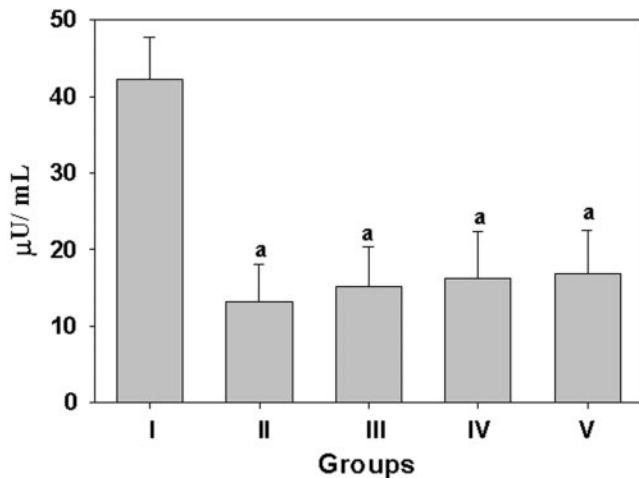


FIGURE 3. The effect of curcumin and turmeric on serum insulin levels in STZ-treated rats. Data are the average \pm SD of results in all the animals in a given group. a, significantly different from group I.

exhibits a hazy opacity; stage 3, a hazy cortex remained and dense nuclear opacity is present; and stage 4, a mature cataract is observed as a dense opacity in both cortex and nucleus.

Blood and Lens Collection and Processing

Blood was collected once a week from the retro-orbital plexus for glucose and insulin estimation. At the end of 8 weeks, animals were killed by CO₂ asphyxiation, and lenses were dissected by the posterior approach and stored at -70°C until further analysis. A 10% homogenate was prepared from three to five pooled lenses in 50 mM phosphate buffer (pH 7.4). All the biochemical parameters were analyzed in the soluble fraction of the lens homogenate (15,000g at 4°C) except for lens malondialdehyde (MDA), which was determined in the total homogenate.

Biochemical Estimations

Serum glucose was measured by the glucose oxidase-peroxidase (GOD-POD) method²⁶ in a commercially available kit (Ozone Biomedicals Pvt. Ltd., New Delhi, India) and serum insulin by an RIA kit (BRIT-DAE, Mumbai, India). Lens MDA, as thiobarbituric acid-reacting substances (TBARS)²⁷; protein carbonyl content²⁸; and reduced glutathione (GSH)²⁹; and the activities of aldose reductase (AR)³⁰ and sorbitol dehydrogenase (SDH)³¹ were determined according to methods described previously. The specific activities of superoxide dismutase (SOD),³² glutathione peroxidase (GPx),³³ and glucose-6-phosphate dehydrogenase (G6PD)³⁴ were assayed according to reported methods. Total, soluble, and insoluble protein was assayed by the Lowry method, with BSA as a standard.

SDS-PAGE and Size Exclusion Chromatography of Lens Proteins

The subunit profile and cross-linking of soluble proteins were analyzed on 10% polyacrylamide gels in the presence of SDS under reducing conditions. Crystallin distribution in the soluble protein fraction was performed by size-exclusion chromatography on a 600×7.5 mm column (TSK-G4000 SW; Tosoh Co., Tokyo, Japan) in an HPLC system (Class-VP; Shimadzu, Kyoto, Japan). The column was equilibrated with 0.1 M sodium phosphate buffer (pH 6.7) containing 0.1 M sodium chloride at a flow rate of 1 mL/min. Soluble protein samples (20 μL of 1 mg/mL solution) were loaded onto the column, and protein peaks were detected at 280 nm.

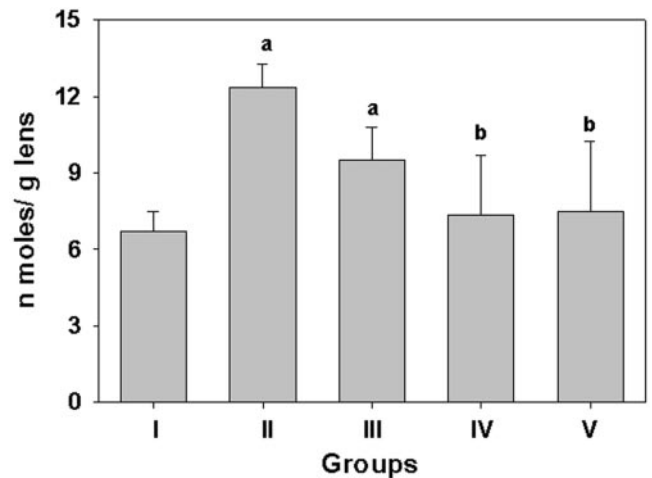


FIGURE 4. The effect of curcumin and turmeric on lipid peroxidation in the lens. Data are the mean \pm SD ($n = 4$). a, significantly different from group I; b, significantly different from group II.

Statistical Analysis

One-way ANOVA was used for testing statistical significance between groups of data, and individual pair difference was tested by means of Duncan's multiple-range test. Heterogeneity of variance was tested by the nonparametric Mann-Whitney test. $P < 0.05$ was considered significant.

RESULTS

Food Intake and Body Weights

There was an increase in the food intake in all the diabetic groups (II-V) compared with the control group (group I; data not shown). Despite the increased food intake, the body weight of group II animals was decreased (194g), when compared with the control subjects (385g). However, the decrease in body weight due to hyperglycemia was not ameliorated either by treatment with curcumin or turmeric (data not shown).

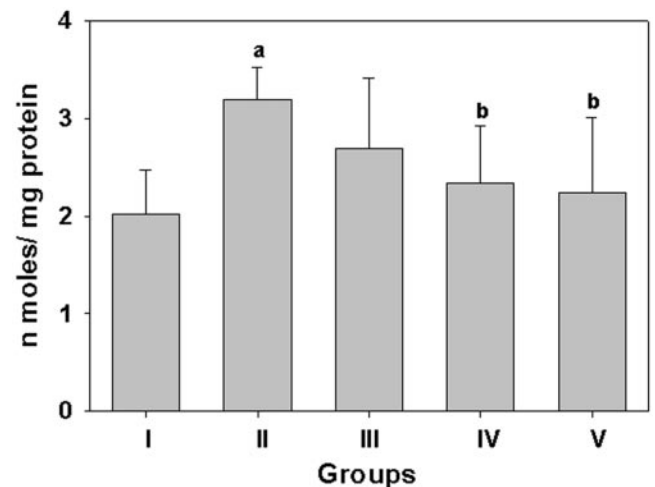


FIGURE 5. The effect of curcumin and turmeric on protein carbonyl content in the lens. Data are the mean \pm SD ($n = 4$). a, significantly different from group I; b, significantly different from group II.

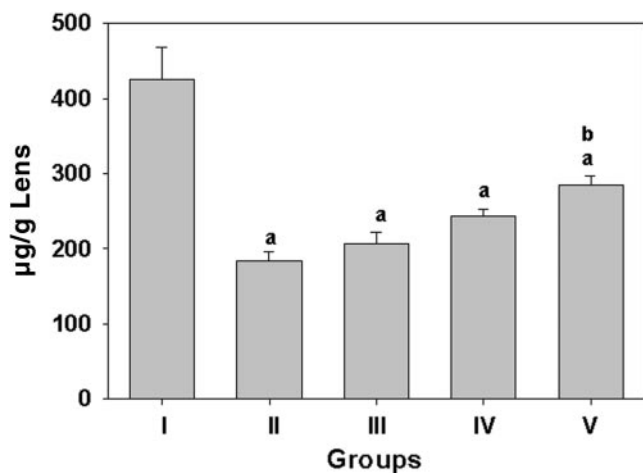


FIGURE 6. The effect of curcumin and turmeric on GSH levels in the lens. Data are mean \pm SD ($n = 4$). a, significantly different from group I; b, significantly different from group II.

Onset and Progression of Cataract

The onset of cataract was observed after 4 weeks by slit lamp examination. Whereas all the lenses in group I were clear and normal, in group II, 30% of the lenses were in stage 1, 60% in stage 2, and 10% in stage 3 of cataract formation, and none of them was clear after 5 weeks (Fig. 1A). However, group III animals had 45% of the lenses in stage 1, 25% in stage 2, and 30% in stage 3, whereas in groups IV and V, most of the lenses were in stage 1 and only a few were in stages 2 and 3 (Fig. 1A). These results clearly indicate that treatment with turmeric and curcumin delayed progression of hyperglycemia-induced cataract. At the end of 8 weeks, most of the lenses (65%) in group II showed development of mature (stage 4) cataract (Fig. 1B). Remarkably, the percentage of mature cataract lenses decreased to 43%, 33%, and 25%, respectively, in groups III, IV, and V (Fig. 1B). The data thus suggest that curcumin and turmeric delayed maturation of diabetic cataract due to slow progression. Because lenses were in different stages of cataract in a given group at the given time, we averaged the stages at a given time in a given group, to track the onset and progression of cataract in an empiric manner in all the groups (Fig. 1C). The data suggest that, although onset of cataract due to STZ-induced hyperglycemia was not affected, progression and maturation were delayed significantly in a dose-dependent manner with curcumin treatment. The effect was even more pronounced with turmeric treatment.

The Molecular Basis for the Delay of Cataract

To investigate the possible mechanisms by which curcumin and turmeric delay the STZ-induced diabetic cataract, various biochemical parameters related to cataractogenesis such as

oxidative stress/antioxidant system, the polyol pathway, protein oxidation, protein content, and crystallin distribution were studied. In addition, we have also estimated blood glucose and insulin levels. As expected, blood glucose levels were elevated, and insulin levels were lowered significantly in group II compared with those in group I (Figs. 2, 3). Nonetheless, treatment with curcumin or turmeric did not reverse the changes in blood glucose and insulin levels (Figs. 2, 3), indicating that curcumin and turmeric treatment had no effect on the state of STZ-induced hyperglycemia.

Oxidative Stress and the Antioxidant System

The increased TBARS levels in group II compared with the control group indicate increased lipid peroxidation in the lens due to hyperglycemia (Fig. 4). Protein carbonyl content, a measure of oxidative damage to proteins, was found to be increased in group II compared with group I, suggesting enhanced protein oxidation under hyperglycemic conditions (Fig. 5). Further, decreased levels of reduced glutathione (GSH) in group II (Fig. 6) suggest and support that there is increased oxidative stress in diabetic cataractous lenses. However, treatment with curcumin and turmeric prevented the alterations, not only in TBARS, but also in protein carbonyls, despite elevated glucose levels (Figs. 4, 5). Although GSH levels were not returned to normal by curcumin and turmeric feeding, they improved significantly with turmeric treatment when compared with levels in untreated diabetic animals (Fig. 6). Together, these data clearly demonstrate that turmeric and curcumin prevent hyperglycemia-mediated lenticular oxidative stress and may thus be one of the mechanisms by which turmeric and curcumin delay the progression and maturation of diabetic cataract. Increased specific activity of GPx and the marginal decrease in G6PD observed in group II compared with group I, further substantiate the role of oxidative stress in cataractogenesis due to hyperglycemia (Table 1), though no change in SOD activity was observed. Turmeric and curcumin treatment prevented the altered activities of antioxidant enzymes (Table 1). Nevertheless, the mechanism of effect of curcumin and turmeric on the enzyme activities needs further investigation.

Polyol Pathway

While the specific activity of AR, a key enzyme of the polyol pathway was significantly higher in group II animals than in group I, sorbitol dehydrogenase activity was not significantly altered (Table 2). Treatment with curcumin and turmeric resulted in normalization of AR activity (Table 2). Based on these results, it appears that curcumin and turmeric are effective against osmotic stress caused by hyperglycemia. Although curcumin has been shown to inhibit AR in vitro, the IC_{50} for in vitro inhibition was far above the level fed to the animals in the present study (Suryanarayana P, Reddy GB, unpublished data, 2004). Because an oxidative milieu is known to activate AR, normalized activities may be due to the improved antioxidant

TABLE 1. The Effect of Curcumin/Turmeric on Activities of SOD, GPx, and G6PD in Rat Lenses

Enzyme	Group I	Group II	Group III	Group IV	Group V
SOD	39.6 \pm 3.54	40.8 \pm 5.74	39.7 \pm 5.62	38.4 \pm 8.53	40.3 \pm 9.08
GPx	17.0 \pm 0.83	23.4 \pm 1.62*	21.1 \pm 1.31†	19.9 \pm 2.21†	20.8 \pm 1.01†
G6PD	5.2 \pm 0.36	4.2 \pm 0.55*	4.2 \pm 0.40	4.5 \pm 0.18	5.0 \pm 0.52†

The data are the mean \pm SD ($n = 4$). SOD activity is expressed as units/min per 100 mg protein, and the activity of GPx and G6PD is expressed as micromoles of NADPH oxidized/h per 100 mg protein and micromoles of NADP reduced/h per 100 mg protein, respectively.

* Significantly different from group I.

† Significantly different from group II.

TABLE 2. The Effect of Curcumin/Turmeric on Activities of Polyol Pathway Enzymes, AR, and SDH in Rat Lenses

Enzyme	Group I	Group II	Group III	Group IV	Group V
AR	22.6 ± 0.70	29.2 ± 3.23*	26.8 ± 0.86†	22.4 ± 1.81†	23.1 ± 0.64†
SDH	3.6 ± 0.53	4.0 ± 1.24	3.9 ± 0.01	3.5 ± 1.01	3.1 ± 0.94

The data are the mean ± SD ($n = 4$).

* Significantly different from group I.

† Significantly different from group II. AR activity is expressed as micromoles NADPH oxidised/h per 100 mg protein and SDH activity as micromoles NADH oxidized/h per 100 mg protein.

status of the cell after curcumin and turmeric feeding rather than to a direct inhibition of AR by curcumin.

Protein Aggregation and Insolubilization

Whatever the underlying mechanism, alterations in protein profile, and insolubilization of otherwise soluble protein, have been considered to be the ultimate change that results in lens opacification. Therefore, we analyzed the total, soluble, and insoluble protein content in all the groups. There was a significant decrease in both total and soluble protein in group II compared with the control group (Table 3). Feeding the rats curcumin and turmeric improved the total and soluble protein levels. The ability of curcumin/turmeric to prevent the loss of soluble protein of lens in STZ-treated rats was remarkable. However, the improvement in the total and soluble protein content was significant in groups IV and V but not in group III, indicating that curcumin only at 0.01% levels and its corresponding levels of turmeric (0.5%) prevented the loss of total protein content, which is in agreement with the delay of maturation of cataract in those groups (Fig. 1).

To investigate possible alterations in crystallin profile due to diabetes-induced cataract and the influence of turmeric and curcumin, the soluble proteins were analyzed by size-exclusion chromatography. The distribution profile evidenced by HPLC showed a decrease in β - and γ -crystallin abundance in addition to the appearance of a high molecular weight (HMW) aggregate peak in the void volume in group II compared with group I (Fig. 7, Table 4). However, there was no significant difference in the α -crystallin peaks (Fig. 7, Table 4). The decrease in β - and γ -crystallins suggests that protein modification and degradation in diabetic cataract lens may be involved in the formation of HMW aggregates due to either cross-linking or aggregation or some other changes (Fig. 7, Table 4). The SDS-PAGE pattern of soluble protein (Fig. 8) showing an increased proportion of cross-linked and aggregated proteins in the diabetic rats (group II) compared with control (group I) rats substantiates the HPLC data. Some amount of cross-linked and/or aggregated proteins were also observed in the control group (Fig. 8, bottom arrow), most probably due to age-related changes, as these animals were more than 5 months old at the end of the experiment. Further, these modifications were enhanced markedly in the untreated diabetic group. Curcumin- and turmeric-fed diabetic rats showed a crystallin profile similar to that in the control rats (Figs. 7, 8; Table 4).

DISCUSSION

At present, the only treatment for cataract is surgery. It has been estimated that a delay in cataract onset by 10 years could reduce the need for cataract surgery by as much as half.³⁵ Any strategy that prevents or slows the progression of cataract has a significant health impact. In this study, a diet including curcumin and turmeric delayed the progression of diabetic cataract in rats. Although, multiple mechanisms may contribute to these effects, the antioxidant effect of curcumin and turmeric appears to be the predominant mechanism of action.

Although, there have been major advances in the control of hyperglycemia (diabetes) through dietary changes, hypoglycemic agents, insulin, and islet transplantation, the long-term complications of diabetes, such as cataract, remain serious problems. Various mechanisms have been proposed to explain the pathophysiology of diabetic complications. These mainly include oxidative stress, increased polyol pathway or osmotic stress, increased formation of advanced glycation end products, activation of protein kinase C, and increased hexosamine pathway flux.³⁶ Although there is cross talk between these pathways, results in several studies suggest that oxidative stress is a major determinant in diabetic complications.^{9,16,36} Therefore, agents or compounds that exert multiple actions, such as antioxidant, antidiabetic/hypoglycemic, AR inhibitory, and antiglycation properties could be more effective than agents with a single action.

Curcumin, the major yellow phenolic curcuminoid present in turmeric, has been reported to have a wide range of biological activities. Earlier, we found that curcumin delays galactose-induced cataract in rats only at very low amounts (0.002%) in the diet; higher levels ($\geq 0.01\%$) are not effective but rather have deleterious effects.¹⁶ Although the galactose-ataract model has been widely used, it should be noted that the mechanism of galactose-induced cataract is different from typical diabetic cataract.^{37,38} In contrast to sorbitol, galactitol is not further metabolized by sorbitol dehydrogenase in galactosemia and may represent osmotic changes of sugar cataract than oxidative stress.³⁸ It is possible that the mechanism of action of curcumin may be different under various conditions. Hence, we set out to investigate the role of curcumin in the prevention or delay of STZ-induced diabetic cataract. In addition, we studied the effects of turmeric, the source of curcumin, an area that had not been investigated earlier.

TABLE 3. The Effect of Curcumin/Turmeric on Protein Content of Rat Lenses

Parameter	Group I	Group II	Group III	Group IV	Group V
Total protein (mg/g lens)	495 ± 37.3	385 ± 27.8*	398 ± 53.1	472 ± 48.3†	468 ± 17.5†
Soluble protein (mg/g lens)	359 ± 33.8	180 ± 40.5*	237 ± 50.3	297 ± 39.1†	305 ± 20.4†
Soluble protein (%)	72.5	46.9	59.7	62.9	65.1

The data are the mean ± SD ($n = 4$).

* Significantly different from group I.

† Significantly different from group II.

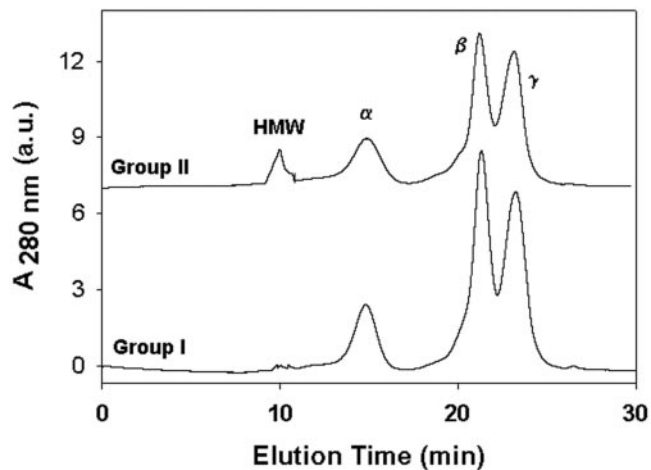


FIGURE 7. The distribution profile of crystallins in the soluble fraction in lenses in groups I and II. Soluble protein was loaded on a gel filtration column, and protein peaks were detected at 280 nm. Peaks representing an HMW aggregate and the α -, β - and γ -crystallins, are indicated at their respective positions. a.u., arbitrary units for absorbance at 280 nm.

In contrast to the results of galactose model,¹⁶ in the present study, curcumin delayed the progression and maturation of cataract in a dose-dependent manner. In addition, these results substantiate the fact that the mechanism of cataract formation and/or the actions of curcumin under different conditions are dissimilar. Oxidative stress may be a predominant mechanism in STZ-induced hyperglycemia and hence curcumin is more effective in the STZ model. The increased TBARS and protein carbonyls along with the decreased GSH and altered activities of antioxidant enzymes in the present study suggest increased oxidative stress in diabetic conditions. The accumulation of carbonyl groups in proteins is generally attributed to oxidative damage and is thought to contribute to general protein dysfunction.³⁹ A decrease in GSH and increase in lipid peroxidation was observed in various types of cataract, including diabetic cataract. As curcumin proved to be a potential antioxidant,¹⁹⁻²³ it is possible that the delay of STZ-induced cataract by curcumin is predominantly due to its antioxidant activity. Furthermore, curcumin- and turmeric-influenced changes in polyol pathway enzymes, particularly AR, in STZ-induced hyperglycemia, further suggest that a combination of AR inhibition and antioxidant potential could be more effective than either of them alone.

The decrease in total and soluble protein content in group II lenses compared with those in group I lenses in this study could be due partly to leakage of proteins and insolubilization. Curcumin and turmeric treatment not only prevented the decrease in total proteins but also prevented cross-linking/aggregation and distribution of soluble proteins. The crystallin profile of soluble protein suggests that the quality of the existing

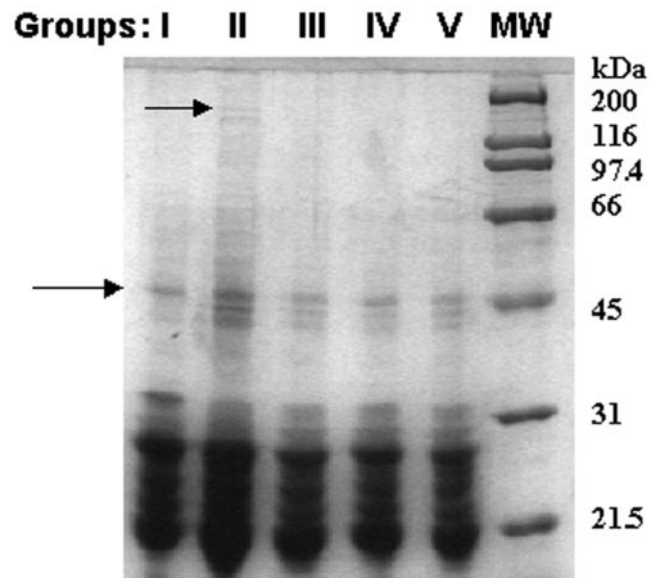


FIGURE 8. The effect of curcumin and turmeric on protein cross-linking and the aggregation of the soluble fraction of the lens. Soluble protein was loaded onto a polyacrylamide gel. *Right:* molecular weight standards; *arrows:* cross-linked proteins.

soluble protein was also different between groups II and I. The decrease in β - and γ -crystallin peaks in diabetic group (group II) may be the result of protein degradation or modification due to oxidative stress. Furthermore, a small but reproducible HMW peak that appeared in the void volume in group II suggests formation of HMW aggregates. The HMW aggregates probably were formed on cross-linking of degraded or modified proteins. However, the possibility of other changes contributing to insolubilization cannot be ruled out. Nevertheless, all these protein modifications were prevented by curcumin and turmeric.

One of the important observations of this study was that both turmeric and curcumin delayed the progression and maturation of cataract, despite elevated levels of glucose. These results thus provide a clue, for the first time, that turmeric or curcumin may act downstream to glucose-mediated changes. Although a study reported the ability of curcumin to lower the glucose levels in alloxan-induced diabetic rats,⁴⁰ other studies have reported an antiglycation ability of curcumin that prevents cross-linking of skin collagen in STZ-induced diabetic rats and an improvement in metabolic status in terms of lipid peroxidation and urinary excretion of electrolytes, despite no effect on hyperglycemic status or body weight.^{41,42} The results of these studies^{41,42} support the findings of the present study. Further, our histologic and immunohistochemistry data on the pancreas suggest that feeding of curcumin or turmeric had no effect on changes due to STZ treatment (Kumar PU, Suryanarayana P, Reddy GB, unpublished data, 2004). These find-

TABLE 4. The Distribution of Crystallins in Soluble Protein Fraction

Peak	Group I	Group II	Group III	Group IV	Group V
HMW Peak	100	286	160	122	129
α -Crystallin	100	93	94	96	94
β -Crystallin	100	79	85	88	90
γ -Crystallin	100	85	86	92	98

The relative percentage of HPLC peaks in untreated and treated diabetic groups was calculated, considering area under the curve for the respective peak in Group I as 100%. Data are the average of three HPLC runs for the area under the curve.

ings thus have an important bearing on the management of secondary complications of diabetes for two reasons:

1. Although the impact of glycemic control in the prevention of diabetic complications has been established by studies such as the UK Prospective Diabetes Study and Diabetes Control and Complications Trial, perfect glycemic control is not always possible.
2. The Memory of glucose toxicity and the persistent progression of hyperglycemia-induced complications during subsequent period of normal glucose homeostasis⁴³⁻⁴⁵ suggest that exclusive management of glucose can no longer be viewed as sufficient for the control of long-term complications. Hence, agents that can prevent diabetic complications, irrespective of glycemic control, would have advantages in the management of secondary complications.

Another interesting finding in this study is that turmeric was more effective in delaying STZ-induced cataract than the corresponding level of curcumin (0.01%) that turmeric contains. The pronounced effect of turmeric may be due to other ingredients besides curcumin. It is for the first time that we report that turmeric, at the levels that are close to average daily intake,^{46,47} can be effective in preventing diabetic cataract in rats. The dried rhizome of *C. longa* is a rich source of phenolic compounds, the curcuminoids. Three main chemically related curcuminoids were isolated from turmeric: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. In addition to curcuminoids, turmeric contains protein, fat, minerals, carbohydrates, and essential oils.^{46,47}

Finally, although there is certainly a need to identify other natural sources for their preventive or therapeutic use in diabetic complications, in this study we demonstrated yet another beneficial property of an otherwise well-studied natural dietary agent. Turmeric and curcumin provide a viable food-based, as well as pharmacologic, approach to the treatment of complications in diabetes. Therefore, delaying of cataract by treatment with turmeric or curcumin merits further attention.

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