# Cataract Development in Sand and Galactosemic Rats Fed a Natural Tomato Extract

Ayalah Pollack,[†] Pnina Oren,[‡] Aliza H Stark,[‡] Ziporah Eisner,[‡] Abraham Nyska,[§] and Zecharia Madar*[,‡]

Department of Ophthalmology, Kaplan Hospital, Rehovot 76100, Israel; Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Nutritional and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel, and Kimron Veterinary Institute and Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Beit Dagan 50250, Israel

This study investigated the effect of a natural tomato extract (TE) on cataract formation in two animal models. A TE containing 5% lycopene was included in the diet of diabetic sand rats at 0.2%, and Sprague Dawley rats were fed a high-galactose diet (30 g/100 g of diet), supplemented with either the lycopene-rich extract at concentrations of 0.2, 0.4, and 0.8% or BHT (0.2%). TE had no significant effect on plasma glucose levels or cataract development in sand rats; however, in rats maintained on a diet rich in galactose, both BHT and TE decreased cataract incidence, and grades were lower than in control animals. In addition, lens protein and reduced glutathione levels were higher and aldose reductase activity was lower than in the control group. The results suggest that antioxidants act as protective agents when oxidative stress is a primary cause of cataract formation but may be less effective in preventing cataracts in hyperglycemic animals.

**Keywords:** *Aldose reductase; cataract; glutathione; lycopene; sand rats*

## INTRODUCTION

The present work evaluates the effect of a lycopene-rich tomato extract (TE) on the development of cataracts in rats. Two models were used, the sand rat (*Psammomys obesus*), a model for type 2 diabetes, and Sprague Dawley rats fed a galactose-rich diet. In its natural environment, the sand rat feeds exclusively on succulent plants of low-energy value/high-salt content and demonstrates normal blood glucose levels. However, long-term feeding of synthetic chow diets results in obesity and a diabetes syndrome that includes insulin resistance, hyperinsulinaemia, markedly decreased glucose tolerance, hyperglycemia, and cataracts (Kalman et al., 1993; Marquie et al., 1984). Feeding high levels of galactose to healthy Sprague Dawley rats leads to galactosemia and increases both galactose in the lens and aldose reductase (AR; EC 1.1.1.21) activity (Gonzalez et al., 1984), whereas sorbitol dehydrogenase activity is decreased (Konoshita, 1986). This shift in enzyme activity, combined with increased available lenticular galactose, favors galactitol accumulation that influences osmotic imbalance with resultant influx of water. Lens hydration causes fiber swelling and vacuolation (Yokoyama et al., 1993). Other metabolic lenticular disturbances, that is, decreases in levels of reduced glutathione (GSH) and ATP, amino acid transport, and protein synthesis, precede the formation of nuclear opacity (Yokoyama, 1993).

Three mechanisms may be involved in cataract formation as a result of hyperglycemia or hypergalactosemia: the polyol pathway, oxidation, and nonenzymatic glycation (Spector, 1995). The polyol pathway has been the most extensively investigated of the three and involves the accumulation of polyols in the eye, leading to lenticular damage.

Considerable evidence suggests the impact of oxidative stress on tissue damage is associated with cataracts (Srivastava and Ansari, 1990). Glucose autoxidation produces hydroxyl radicals from hydrogen peroxide (Yokoyama et al., 1993). The concentration of $H_2O_2$ in the normal lens and aqueous humor is ∼20−30 μmol/L. However, as the level of $H_2O_2$ in the cataractous lens is 2−10-fold higher, it has been concluded that $H_2O_2$ is the major oxidant contributing to cataract formation (Spector, 1995). Free radicals and $H_2O_2$, produced by glucose enediol autoxidation in the presence of free metal, induce oxidation of a lens protein that, in turn, leads to structural alterations and cataract development in the lens. GSH, ascorbate (vitamin C), tocopherol (vitamin E), and carotenoids (Bunce et al., 1990; Taylor, 1989, 1993) are lenticular antioxidant-protective substances. GSH concentrations are lower in cataractous lenses than in their normal counterparts (Bunce et al., 1990). GSH can act directly as an antioxidant by maintaining the reduced state of protein sulfhydryl groups or indirectly as a reverse superoxide reductase to hydrogen peroxide by nonenzymatic reduction of dehydroascorbate to ascorbate (Yokoyama et al., 1993). Oxidative damage may also be increased due to competition for NADPH between AR and glutathione reductase, leading to decreased GSH levels and a lower capacity of the lens to reduce oxidants.

Hyperglycemia is strongly associated with long-term complications of diabetes (Diabetes Control and Complication Trial Research Group, 1993). The hyperglycemic conditions in experimental models are known to

* Author to whom correspondence should be addressed (telephone 972 8 948 9008; fax 972 8 936-3208; e-mail madar@agri.huji.ac.il).
[†] Department of Ophthalmology.
[‡] Institute of Biochemistry.
[§] Kimron Veterinary Institute.

10.1021/jf9900231 CCC: $18.00 
Published on Web 11/25/1999

cause lenticular changes (Konoshita, 1986). These changes, including increased sorbitol levels, altered membrane permeability, loss of GSH, decreased amino acid levels, and decreased protein synthesis, eventually lead to cataract formation. Furthermore, experimental results indicate that AR initiates the process leading to sugar cataracts via the production of sorbitol (Konoshita, 1986; Linklater et al., 1986).

This study focused on the ability of a tomato extract, rich in antioxidants, to influence cataract development in a hyperglycemic animal model (sand rats) and in normoglycemic, galactosemic rats with cataract induction associated with increased polyol production and oxidative stress.

## MATERIALS AND METHODS

**Rats and Diets.** *Sand Rats.* Weanling male sand rats (*P. obesus*, The Hebrew University of Jerusalem, Israel) of 70 ± 9 g body weight were divided into two groups of 15 rats each and, over a 4 week period, fed diets composed of (g/kg diet) the following: soybean protein (200), cornstarch (650), soybean oil (50), cellulose (40), AIN-76 vitamins (20), and AIN-76 mineral (40). Diets were administered in pellet form with or without 0.2% TE rich in lycopene (5%) (Lyc-O-Mato, Makhteshim, Israel). Diet composition for both sand and galactosemic rats was according to AIN recommendations (American Institute of Nutrition Committees, 1987).

*Galactosemic Rats.* Fifty male Sprague Dawley rats (Harlan, Israel), weighing 160−180 g, were divided into five groups of 10. Rats were housed in individual cages maintained at 22 °C with a 12 h light/dark phase with free access to water and food. During the 5 week experimental period, the rats were fed diets composed of (g/kg diet) the following: galactose (300), casein (200), cornstarch (320), corn oil (7), cellulose (5), DL-methionine, vitamins (20), and minerals. Casein, mix-minerals, and cellulose were obtained from ICN Biochemicals (Cleveland, OH). Vitamin mixture and BHT were from Kuffolk (Petach-Tikva, Israel), and cornstarch was from Galam (Rishon-Lezion, Israel). Galactose and DL-methionine were from Sigma, Israel. Soybean and corn oils were obtained from the local market.

Experimental groups received either 0 (control), 0.2, 0.4, or 0.8% concentrations of TE, whereas BHT (0.2%) served as an additional control. Animal care and treatment conformed to The Hebrew University Guide for the Care and Use of Laboratory Animals.

**Glucose Determination.** Following an 18 h fast, blood samples were drawn from the tail tip into tubes prewashed with heparin and sodium fluoride. Glucose was measured according to the glucose oxidase (EC 1.1.3.4) method (Morrison, 1972) using a Beckman glucose analyzer II (Beckman Instruments, Inc., Fullerton, CA).

**Cataract Classification.** Lenses were evaluated (without anesthesia) in a blinded fashion using a slit-lamp (Kowas SL-14). Pupils were dilated by a single topical application of tropicamide (0.5%). Cataracts were classified as previously described (Varma, 1991): grade 0, no vacuoles present, clear lens; grade 1, vacuoles of less than one-third of the lens radius; grade 2, vacuoles located at the periphery of the lens occupying an area of between one-third and two-thirds of the radius from the periphery; grade 3, vacuoles extending up to two-thirds of the radius from the periphery (nuclear opacity may be seen); grade 4, vacuoles cover the entire lens, which appears white to the naked eye. Incidence of cataract appearance was expressed as the percentage of rats developing cataracts in one or both eyes. A grade 1 lens was rated as cataractous. The mean grade of cataract was calculated as the average of grades in all eyes in each group. The clear and cataractogenous lenses were photographed with a Topcon photo-slit-lamp (Kodak).

Histological evaluation was carried out over a 5 week period. Enucleated globes were fixed by immersion in Davidson's fluid for 10 h and then stored in 70% ethanol until trimming. The globes were trimmed sagittally on the meridional plane at the optic disk level. Both hemispheres were embedded in paraffin wax, sectioned to a thickness of 5 μm, and stained by hematoxylin and eosin.

**Table 1. Effect of a Lycopene-Rich TE on AR Activity, Protein Level, and GSH Content in Lenses of Sand Rats (*P. obesus*) Fed Experimental Diets for 4 Weeks[a]**

| treatment | AR activity [μmol of NADPH (mg of protein)$^{-1}$ min$^{-1}$] | protein (μg/mg of tissue) | GSH (μg/g of tissue) |
|---|---|---|---|
| − TE | 17.6 ± 3.8 | 12.0 ± 1.8 | 200 ± 60 |
| + TE | 15.9 ± 5.2 | 13.3 ± 2.4 | 340 ± 100 |

[a] Values are means ± SE for 15 rats in each group.

**AR Activity and Protein Content in the Lens.** After week 4 for sand rats and week 5 for galactosemic rats, the animals were anesthetized with Nembutal (80 mg/kg of body weight); right lenses were removed quickly and frozen. At the time of analyses, lenses were weighed and homogenized for 1 min in 5 volumes of 50 mmol/L ice-cold potassium phosphate buffer, pH 7.2, containing 5 mmol of 2-mercaptoethanol/L. The homogenate was centrifuged at 12300*g* for 6 min at 4 °C, and the supernatant was stored at −20 °C. The latter was used to determine lenticular AR activity and protein content. AR activity was ascertained by monitoring NADPH oxidation at 340 nm. The assay mixture (total volume = 1.0 mL including lens supernatant) was composed of 100 mmol/L potassium phosphate buffer, pH 6.2, 0.4 mol/L $Li_2SO_4$, 5 mmol/L DL-glyceraldehyde, and 50 μmol/L NADPH. The reaction, carried out at 25 °C, was initiated by addition of the supernatant containing the enzyme and lasted 6 min. Protein content was determined according to the standard Bradford assay (Bradford, 1976).

**Reduced Glutathione Content in the Lens.** GSH contents were determined using a modification of the fluorophotometric technique using ophthalaldehyde (OPT) as a fluorescent reagent (Gonzalez et al., 1984; Cohen-Melamed et al., 1995). This method exploits the reaction of GSH with OPT at pH 8. Frozen lenses were weighed and homogenized for 1 min in 16 volumes of 0.1 mmol/L sodium phosphate−0.005 mol/L EDTA buffer and 5 volumes of $HPO_3$ (250 mL/L). The total homogenate was centrifuged at 4 °C at 12300*g* for 30 min, and the supernatant was then diluted with 4 volumes of the buffer. The final assay mixture (2.0 mL) contained 100 μL of the dilute tissue supernatant, 1.8 mL of buffer, and 100 μL of OPT solution containing 100 μg of OPT. After thorough mixing and subsequent incubation at room temperature for 15 min, samples were read for fluorescence at 420 nm with activation at 50 nm using a JASCO EP-550 spectrofilament (Spectroscopic Co. Ltd., Tokyo, Japan) and compared with a GSH standard curve.

**Statistical Analyses.** All data are expressed as means and standard errors. Results were analyzed by one-way analysis of variance (ANOVA), and significance differences were determined by Student's *t* test or Duncan's test for multiple comparisons. Cataract grading comparisons were made by Wilcoxon rank sum test. Differences were statistically significant at $P < 0.05$.

## RESULTS

Treatment with a lycopene-rich TE did not significantly affect glucose concentrations in the blood of fasted sand rats: controls, 16.6 ± 1.1 mM; TE-fed rats, 16.7 ± 0.9 mM. In addition, no significant differences in cataract development were determined in sand rats fed the TE. Cataracts appeared in 66% of the rats after 4 weeks of lycopene-rich TE treatment and in 78% of controls. The mean grade of observed cataracts also tended to be lower in the TE-treated group (2.7 ± 0.1) than in the nontreated sand rats (3.1 ± 0.1). Table 1 describes the effect of lycopene on AR activity and GSH

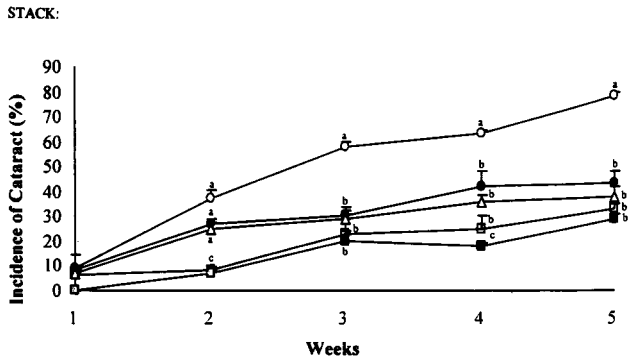


**Figure 1.** Incidence of cataracts in galactogenic rats fed diets containing different concentrations of lycopene-rich TE: control (○); 0.2% BHT (■); 0.2% TE (○) 0.4% TE (△); 0.8% TE (□). Values are means ± standard error for 10 rats per group. Mean values with different superscripts indicate significant differences among groups at $P < 0.05$.

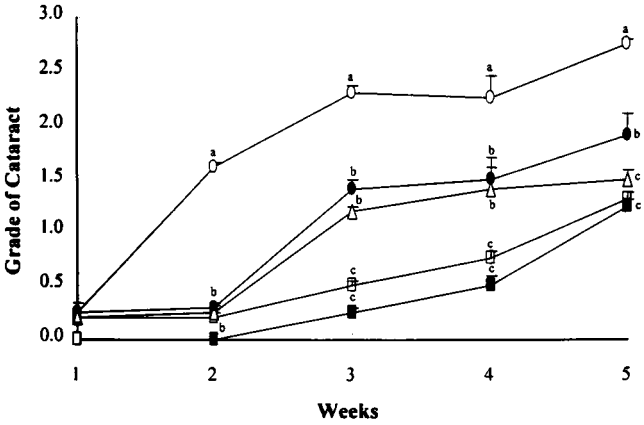


**Figure 2.** Grade of cataract in galactosemic rats fed lycopene-rich TE. Results are expressed as means ± SE of 10 rats per group. Symbols are similar to those described in Figure 1.

and protein levels in the lenses. There were no significant differences between the control and TE-treated rats.

Cataract incidence and grade in galactosemic rats fed different amounts of TE are presented in Figures 1 and 2. The incidence of cataract appearance was consistently lower in the groups receiving all diets rich in antioxidants (Figure 1). After 2 weeks, cataracts developed in 37% of the control group, whereas low cataract incidences (27, 25, and 8%) were observed in the experimental groups receiving 0.2, 0.4, and 0.8% TE, respectively. Cataract incidence in the BHT group was 10%. After 5 weeks on TE-enriched diets, cataracts developed in 43, 38 and 33% of the rats, compared to 78% in the control group. A low cataract incidence of 29% also was observed in animals fed with BHT.

The mean grades of cataract are given in Figure 2. Values are significantly lower in galactosemic rats treated with antioxidant than in the control group. At 3 weeks, the control group showed a mean grade of 2.3 ± 0.06, whereas grades of 1.4 ± 0.10, 1.2 ± 0.01, and 0.5 ± 0.04 were seen in rats fed TE. Rats fed BHT had an average cataract grade of 0.25 ± 0.05 at this experimental time point. In week 5, a grade of 2.75 ± 0.05 was measured in the control group. TE at 0.2, 0.4, and 0.8% yielded grades of 1.9 ± 0.2, 1.50 ± 0.07, and 1.3 ± 0.07, respectively. BHT yielded a grade of 1.24 ± 0.07.

Differences between the control group and the groups receiving antioxidants were not only in cataract incidence and grade but also in the induction of vacuoles within a particular grade. The vacuoles were numerous and appeared dense in the control group, whereas in the experimental groups, they were few and sparse. In a galactosemic rat with a grade 3 cataract (Figure 3), the vacuoles extended from the periphery toward the center covering the peripheral two-thirds of the lens. The vacuoles appear crowded and cover the entire area, and nuclear opacity is evident. Although in the galactosemic rat fed lycopene the vacuoles also extended centrally up to two-thirds from the periphery (grade 3), they were sparse and did not occupy the entire area.

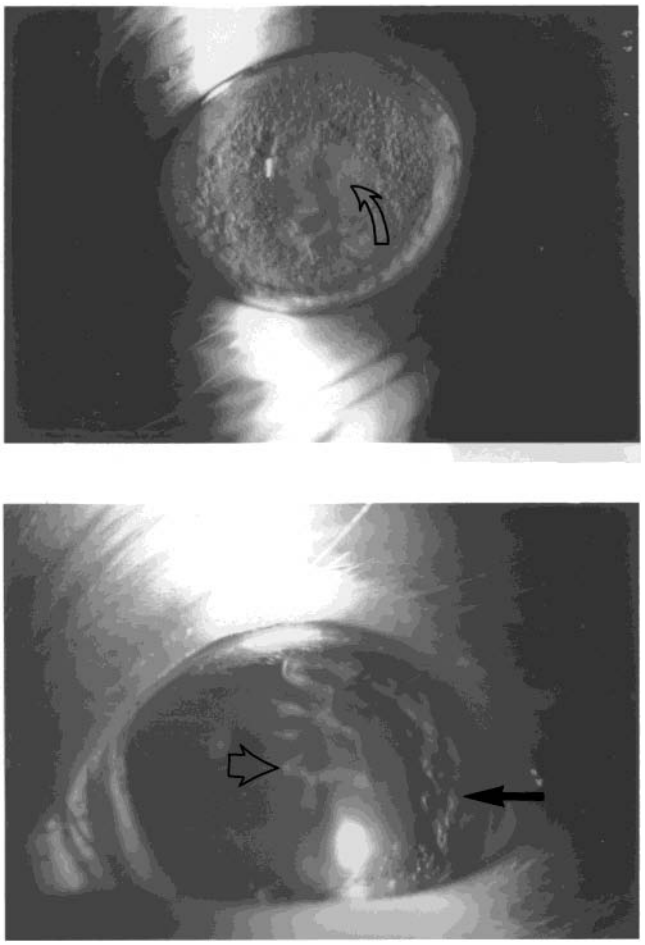

**Figure 3.** (Top) Grade 3 cataract in an animal fed 30% galactose (control group). The vacuoles, present at the periphery of the lens, are dense and occupy two-thirds of the lens radius from the periphery. Nuclear opacity is seen at the center (located by the arrow). (Bottom) Grade 3 cataract in an animal fed 30% galactose plus 0.8% TE (experimental group). The vacuoles are large and sparse, extending up to two-thirds of the lens radius form the periphery. No nuclear opacity is observed (located by the arrow).

The effect of BHT and TE on AR activity, protein level, and GSH content in lenses of galactosemic rats is shown in Table 2. AR activity in the lenses of galactosemic rats fed antioxidants for 5 weeks was significantly lower than in the control group. Compared with control galactosemic rats, the lower AR activity of BHT- and lycopene-treated galactosemic rats was accompanied by an almost 2-fold higher lens protein level and an almost 3-fold higher lenticular GSH content.

## DISCUSSION

This study investigated the effect of a tomato extract containing lycopene, a carotenoid considered to have

**Table 2. Effect of Different Concentrations of Lycopene-Rich TE on AR Activity, Protein Level, and GSH Content in Lenses of Galactosemic Rats Fed Experimental Diets for 5 Weeks[a]**

| treatment | AR activity [$\mu$mol of NADPH (mg of protein)$^{-1}$ min$^{-1}$] | protein ($\mu$g/mg of tissue) | GSH ($\mu$g/g of tissue) |
|---|---|---|---|
| control | $11.9 \pm 1.4^{a}$ | $18.0 \pm 3.4^{a}$ | $70 \pm 10^{a}$ |
| BHT (0.2%) | $8.3 \pm 0.8^{a}$ | $35.1 \pm 4.1^{b}$ | $280 \pm 30^{c}$ |
| TE (0.2%) | $8.1 \pm 1.3^{a}$ | $29.1 \pm 4.2^{ab}$ | $190 \pm 20^{b}$ |
| TE (0.4%) | $6.8 \pm 1.3^{b}$ | $30.3 \pm 1.8^{ab}$ | $210 \pm 30^{bc}$ |
| TE (0.8%) | $4.2 \pm 0.7^{b}$ | $35.7 \pm 5.4^{b}$ | $270 \pm 20^{c}$ |

[a] Values are means ± SE for 10 rats in each group. Mean values in the same column with different superscripts indicate significant differences among groups at $P < 0.05$.

antioxidative properties, and determined its role in cataract prevention in two animal models. Following treatment of sand rats with TE at the concentration used in this study, no significant retardation of cataract appearance or biochemical changes related to cataract were observed. It should be emphasized that hyperglycemia is a major cause of cataract development in sand rats, which accelerates protein glycation along with activation of the polyol pathway. In previous studies in sand rats, hypoglycemic agents significantly delayed cataract development (Madar and Hazan, 1993; Ohrloff et al., 1994). However, one cannot exclude the possibility that lycopene concentrations greater than those used in this study might affect cataract formation in sand rats.

The addition of both antioxidants (BHT or TE) to the diet of galactosemic rats delayed cataract formation as reflected by morphological and histological examinations. Antioxidants in the diet did not influence plasma glucose levels (data not shown), indicating that mechanisms independent of glucose levels were responsible for the inhibition of cataract development. Biochemical parameters related to cataractogenesis were improved by TE administration (Table 2). Inhibition of cataract formation in galactosemic rats receiving TE was associated, at least in part, with reduced AR activity and polyol formation. The possibility that TE directly inhibited the AR enzyme cannot be discounted.

Autoxidation also occurs in hypergalactosemia and results in the formation of hydrogen peroxide, superoxide, and free radicals. These agents reduce soluble protein levels by oxidation of the lens protein or oxidation of the membrane proten, which increases lens−membrane permeability and consequent protein leakage (Gonzalez et al., 1984). In rats fed antioxidants, as seen in this study, the lens and membrane proteins were protected from oxidative damage and yielded higher protein content at the termination of the experiment.

GSH is one of the more effective lenticular antioxidants offering significant protection against oxidative damage (Ohrloff et al., 1994). The role of GSH is to maintain biological molecules in their reduced state; its administration to galactosemic rats prevented cataract formation. Accordingly, in control galactosemic rats, GSH content was significantly reduced, indicating extensive oxidation (Table 2) accompanied by increased cataract incidence. Addition of dietary supplements considered to have high levels of antioxidant activity, such as lycopene or BHT, reduced "oxidative stress", and lenticular GSH levels were significantly higher (Table 2). The work of Srivastava and Ansari (1988) supports the hypothesis that in the galactosemic model cataract formation is not solely due to polyol accumulation and oxidative damage is a major cause in the advancement of cataractogenesis.

In the control group, galactosemia led to increased AR activity, whereas in the antioxidant groups, AR activity decreased. Possibly, in cells exposed to both sugar and $H_2O_2$, NADPH is preferentially utilized by glutathione reductase for reduction rather than by AR for polyol production (Konoshita, 1986; Yokoyama et al., 1993). Antioxidants, such as vitamin E, glutathione, and BHT, have been reported to delay or prevent sugar- or diabetes-related cataract even when high levels of polyols persisted in the lens. This effect of antioxidants on sugar-induced cataract development could result from the protection of cell-membrane integrity. Increased free radical formation and AR activity may be the outcome of feeding high concentrations of galactose that result in increased oxidative damage, a possible contributing factor in sugar-induced cataractogenesis. The oxidants would be responsible for membrane distress and alter their permeability. Lycopene or other compounds present in the TE may act as a protective agent to prevent oxidation and thereby restore cell membrane integrity.

In conclusion, the present study showed no inhibition of cataract development in hyperglycemic sand rats fed 0.2% TE. In contrast, the same concentration of TE in Sprague Dawley rats fed high-galactose diets led to a marked inhibition of cataract formation. It appears that the lycopene-rich extract is more efficient in inhibiting cataractogenesis in models of oxidative stress than in diabetic or hyperglycemic models. When mechanisms other than hyperglycemia are involved in cataractogenesis, as in the galactosemic rat model, the quenching activity of antioxidants appears to delay cataracts and contribute to the formation of inactive, electronically excited molecules and reduce "oxidative stress".

## LITERATURE CITED

American Institute of Nutrition Committees. Guidelines for describing diets for experimental animals. *J. Nutr.* **1987**, *17*, 78.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Bunce, G. E.; Konishita, J.; Horwitz, J. H. Nutritional factors in cataract. *Annu. Rev. Nutr.* **1990**, *10*, 233−254.

Cohen-Melamed, E.; Nyska, A.; Pollack, A.; Madar, Z. Aldose reductase (EC 1.1.1.21) activity and reduced-glutathione content in lenses of diabetic sand rats (*Psammomys obesus*) fed with acarbose. *Br. J. Nutr.* **1995**, 607−615.

Diabetes Control and Complication Trial Research Group. The effect of intensive research on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **1993**, *329*, 977−986.

Gonzalez, A. M.; Sochor, M.; McLean, P. The effect of an aldose reductase inhibitor (sorbinil) on the level of metabolites in lenses of diabetic rats. *Diabetes* **1984**, *32*, 482−485.

Kalman, R.; Adler, H. H.; Lazarovici, G.; Bar-On, H.; Ziv, E. The efficiency of sand rat metabolism is responsible for development of obesity and diabetes. *J. Basic Clin. Physiol. Pharmacol.* **1993**, *4*, 57−68.

Konoshita, J. D. Aldose reductase in the diabetic eye ILIII Edward Jackson Memorial Lecture. *Am. J. Ophthalmol.* **1986**, *102*, 685−692.

Linklater, H.; Dzialosynski, T.; McLeod, H.; Sanford, S.; Treventhick, J. Modeling cortical cataractogenesis. 8. Effect of butylated hydroxytoluene (BHT) in reducing protein

leakage from lenses of diabetic rats. *Exp. Eye Res.* **1986**, *43*, 305−314.
Madar, Z.; Hazan, A. Effect of miglitol and acarbose on starch digestion, daily plasma glucose profiles and cataract formation. *J. Basic Clin. Physiol. Pharmacol.* **1993**, *4*, 69−81.
Marquie, G.; Duhault, J.; Jacotot, B. Diabetes mellitus in sand rats (*Psammomys obesus*). *Diabetes* **1984**, *33*, 438−443.
Morrison, B. Use of the Beckman glucose analyzer for low and high glucose values. *Clin. Chim. Acta* **1972**, *42*, 192−193.
Ohrloff, C.; Hockwin, O.; Olson, R.; Dickman, S. Glutathione peroxidase, glutathione reductase and superoxide dismutase in the aging lens. *Curr. Eye Res.* **1994**, *3*, 109−115.
Spector, A. Oxidative stress induced cataract: Mechanism of action. *FASEB J.* **1995**, *9*, 1173−1182.
Srivastava, S. K.; Ansari, N. H. Prevention of sugar-induced cataractogenesis in rats by butylated hydroxytoluene. *Diabetes* **1988**, *37*, 1505−1508.
Taylor, A. Associations between nutrition and cataract *Nutr. Rev.* **1989**, *47*, 225−234.
Taylor, A. Cataract relationships between nutrition and oxidation. *J. Am. Clin. Nutr.* **1993**, *12*, 138−146.
Varma, S. D. Scientific basis for medical therapy of cataracts by antioxidants. *Am. J. Clin. Nutr.* **1991**, *53*, 3355−3455.
Yokoyama, T.; Sasaki, H.; Giblin, F. J.; Reddy, V. A physiological level of ascorbates inhibits galactose cataract in guinea pigs by decreasing polyol accumulation in the lens epithelium: A dehydroascrobate mechanism. *Exp. Eye Res.* **1993**, *58*, 207−218.

Received for review January 12, 1999. Accepted October 25, 1999. This work was supported in part by a grant from Lyco-Red Natural Products Industries Ltd. Beer-Sheva, Israel.

JF9900231