

Camel Milk and the Prevention of Glucose Cataract, an Organ Culture Study

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Abstract: *Purpose:* To test if camel milk affects glucose-induced opacity in organ cultured rat and human lenses.

Methods: Whole human and rat lenses were cultured in various media containing either 55 mM glucose, camel milk, or a combination of both glucose and milk. Some lenses were cultured in a media containing neither moiety to establish a control. Absorbance spectra of human and rat lenses were measured daily using a visible/ultraviolet light spectrometer. Lens opacities were graded by a blinded grader from photographs taken daily. Aldose reductase activity, catalase activity, glutathione and receptor for advanced glycation end products levels were assayed.

Results: The optical density and light scattering intensity of human lenses cultured with glucose were higher after two to four days in organ culture compared with lenses cultured without glucose. Camel milk in the culture media attenuated the glucose-induced increase in optical density, light scattering intensity and opacity grade after two to four days for both human and rat lenses. Aldose reductase activity, catalase activity and glutathione levels were restored but the receptor for advanced glycation end products was similar in rat lenses cultured with glucose compared with those cultured with glucose and camel milk. There were no differences between the assayed moieties in human lenses cultured with glucose or glucose plus milk. Since camel milk restored rat lens glutathione levels, it is possible that camel milk may protect the lens from oxidation and significantly reduce the glucose-induced increase in light scattering of human lenses. Structurally and physiologically, rat lenses are distinct from human lenses, therefore, the rat lens data was highly variable when compared with the human lens data, highlighting the importance of using human lenses in future studies.

Conclusions: Camel milk present in the organ culture medium inhibited the glucose-induced opacity in human lenses and restored the amount of glutathione to the same levels of lenses not cultured in glucose. The positive results of the current study leads to future studies to determine the moieties in camel milk that are responsible for cataract inhibition and *in vivo* studies involving camel milk.

Keywords: Camel milk, Cataract, Glucose, Human, Lens, Organ culture.

INTRODUCTION

Cataract is among the major causes of visual impairment and blindness worldwide [1]. A major goal then is to delay the onset of cataracts. Most cataracts appear in elderly individuals, but diabetes is also a major factor; individuals with diabetes are two times more likely to have cataracts than those without diabetes [2]. In this study, we tested if camel milk inhibited cataract in human and rat lenses *in vitro*. We chose to study camel milk since it has proven to be beneficial to human health and contains the antioxidant ascorbate at three times the levels found in cow's milk [3-6]. Epidemiological literature suggests that the risk of cataract can be diminished by diets that are optimized for vitamin C, lutein/zeaxanthin, B vitamins, omega-3

fatty acids, multivitamins, carbohydrates and micronutrients [7]. The health benefits of drinking camel milk, especially for children, has been reviewed [8]. Camel milk consumption lowers the dose required to maintain glycemic control in patients with diabetes [9] and could be an alternative for cow milk allergy in children [10]. Camel milk could also be antimicrobial since the level of lactoferrin, an antimicrobial protein, is higher in camel milk when compared with cows' milk [11]. Camel milk fat globular membrane proteins have been characterized [12,13] and have multiple health benefits for humans [14,15]. For example, lactadherin found in milk prevents symptomatic rotavirus infection in breastfed infants [16].

A method to measure absorbance and light scattering for the first time in organ cultured lenses was developed. It was found that light scattering accounted for all of the absorbance of the human lens above 550 nm which indicates that cellular and molecular structure contributes to the attenuation of light passing through

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the lens rather than the absorbance of unique moieties [17]. Using the new method, we found dexamethasone treated lenses scattered more light when compared with untreated human and rat lenses [17]. Relevant to the present study, almost all of the attenuation of light through glucose treated human lenses was due to light scattering from structural changes [18]. Some of the structural changes in rat lenses cultured with glucose (RLg) may have been caused by oxidative stress since the levels of glutathione, catalase, and aldose reductase were reduced in RLg compared with rat lenses that were cultured in control media (RLc) [18].

Spectroscopic measurement offers two major advantages over grading of lenses from photographs. Photographic grading only quantifies opacity into 4 to 5 grades, whereas spectroscopic measurement provides a continuous grade and is not subjective. Additionally, one can discern from the absorbance measurements of chromophores whether compositional changes occur; light scattering can also measure changes in lens structure whereas, photographic grading cannot. Another general advantage of spectroscopy is that the sample is not altered or destroyed by spectroscopic measurement so subsequent metabolomic assays can be performed. Finally, another advantage of spectroscopic measurement is unlike metabolomics assays, it can be made continuously on the same lens which is helpful in determining the optimum time point to measure metabolic changes using destructive assays.

Thus, to utilize the advantages listed above, in this study, the opacity of whole human and rat lenses cultured with and without glucose, with and without camel milk and camel milk plus glucose were measured to test the ability of camel milk to ameliorate glucose-induced lens opacity.

METHODS

The methods used in this study were identical to those described previously [17,18] except contralateral human lenses were incubated with 55 mM glucose, camel milk and glucose plus camel milk.

Unpasteurized camel milk was obtained from Desert Farms, Santa Monica, California, USA. The milk was shipped on ice and stored at 2.8°C and used within three weeks. One lot was used for all of the studies. The supplier indicated that 240 mL of milk contained: 4.5g total fat (3g saturated), 15 mg cholesterol, 150 mg sodium, 390 mg potassium, 11 g of carbohydrate, 5g

protein and the following percent of the required amount of vitamins and minerals based on a 2,000 calorie diet: vitamin A 4%, vitamin C 0%, vitamin B1 70%, calcium 30 %, iron 2%, vitamin D 6%, thiamin 70 %, niacin 8 %, phosphorous 15 %, magnesium 4 % and zinc 4%.

Lens Dissection

Clear human lenses were obtained from the Kentucky Lions Eye Bank (Louisville, Kentucky, USA) which had been previously dissected from deceased donors. Donors with diabetes or opaque lenses were excluded. All human lenses were collected with written informed consent. This study was approved by the University of Louisville Institutional Review Board. All experiments were performed in accordance with the Declaration of Helsinki. Human lenses were removed from the globe by an anterior approach after removal of the cornea for transplantation. The iris and ciliary body were carefully removed from the lens by cutting the zonules with curved microscissors.

Male Sprague Dawley rats which were approximately eight weeks old and weighing 250 to 274g-each, were euthanized with CO₂ and their globes were immediately removed. The animal protocols were approved by the University of Louisville Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research. Lenses were dissected using a posterior approach. A small, shallow incision was made at the optic nerve with a scalpel blade and the sclera was cut into four sections with straight micro scissors and peeled back to the iris. The dissection was aided by an illuminated head magnifier (SE MH1047L, Amazon Inc, Seattle Washington, USA). The iris and ciliary body were carefully removed from the lens by cutting the zonules with curved microscissors.

Whole Lens Organ Culture

Immediately after dissection, human and rat lenses were carefully placed into 10 mL and 2 mL of medium, respectively, using curved blunt forceps. The incubation medium was freshly prepared by mixing minimum essential medium (MEM) Eagle with Earle's BSS without L-glutamine or phenol red (Lonza, Walkerville, MD, USA) with L-Glutamine-Penicillin, streptomycin and amphotericin B (Sigma Chemical Company, St Louis, MO, USA) at concentrations of 2 mM/mL, 100 U/mL, 0.1 g/mL, and 0.25 µg/mL,

respectively. The medium used for glucose treated lenses also contained 55 mM glucose. The medium containing camel milk had the same ingredient concentrations as stated above but with 50 % fresh camel milk (Desert Farms, Santa Monica, California, USA). The lenses were incubated at 37 °C in a 5% CO₂ atmosphere. The lens culture medium was replaced every day. Lenses were incubated for a period of four days. An incubation period of four days was chosen because after this period of time, control lenses became increasingly opaque [17, 18] and it was one of the earliest times in which there was a significant difference in the optical properties of lenses cultured with glucose compared to those cultured without glucose [18].

Lens Optical Property Measurements

Immediately after dissection, lenses were photographed and lens absorbance and light scattering were measured every 24 hours as described [18].

Human Lens Assays

Human lenses were homogenized in 0.5 ml of phosphate buffered saline (Sigma Chemical Company, St Louis, MO, USA) and centrifuged to obtain a clear supernatant. Aliquots of the homogenate (55 µL) were used to measure: glutathione (Kit CS0260, Sigma-Aldrich Inc., St Louis MO, USA), catalase (Kit CAT100, Sigma-Aldrich Inc., St Louis MO, USA), aldose reductase (United States Biological, Salem MA, USA) and a receptor for advanced glycation end products (RAGE) (ELISA Kit RAB1040, Sigma-Aldrich Inc., St Louis MO, USA) according to the manufacturer's instructions.

Averages were compared using the Student's t-test. Data were reported as averages ± the standard error of the mean.

RESULTS

Human Lens Studies

The demographics of ten human lenses obtained from five donors are listed in Table 1. A broad absorbance peak near 600 nm appeared in the glucose cultured human (HLg) spectra that was not present in the human control (HLc) and glucose plus camel milk cultured human (HLgm) spectra (Figure 1A). The average level of light scattering was significantly more than two times higher ($P < 0.001$) in HLg compared with HLc (Figure 2C). The optical density (O.D., Figure 2B), and light scattering (Figure 2C) of HLgm was not significantly different ($P > 0.05$) from that of HLc.

When the baseline absorbance at 750 nm was subtracted from the O.D. at 450 nm (Δ O.D.) the Δ O.D. was higher in HLg above day two in organ culture compared with HLc, and the Δ O.D. for HLgm were below that of HLc (Figure 2A).

Human lens opacity was graded into four categories by a blinded grader based on the opacity of the lens (Figure 3A). HLg were almost completely opaque after three days in organ culture (Figure 2D) and more opaque than HLc above zero days in organ culture. After one day, the grade for HLgm reached a maximum that was below the maximum opacity of HLg (Figure 2D).

There was little difference between aldose reductase activity (Figure 4A), the level of the receptor for advanced glycation end products (RAGE) (Figure 4B), Catalase activity (Figure 4C) or glutathione levels (Figure 4D) between HLg and HLgm.

Rat Lens Studies

The O.D. of rat lenses was greater in wavelengths below 750 nm in the average spectrum of RLg

Table 1: Demographics and Parameters Related to Human Lens Donors

Donor Parameter	Control Average ± Standard Deviation	Glucose/Camel Milk Cultured Average ± Standard Deviation
Average Age (years)	59 ± 7	53 ± 9
Age range	48 to 65	39 to 61
Male (%)	80	60
Race (%)	Caucasian 80, Black 20	Caucasian 100
Average Time from death to enucleation (hours)	11 ± 4	7 ± 3
Average lens weight (g)	0.23 ± 0.04	0.22 ± 0.02
Number of lenses	16	10

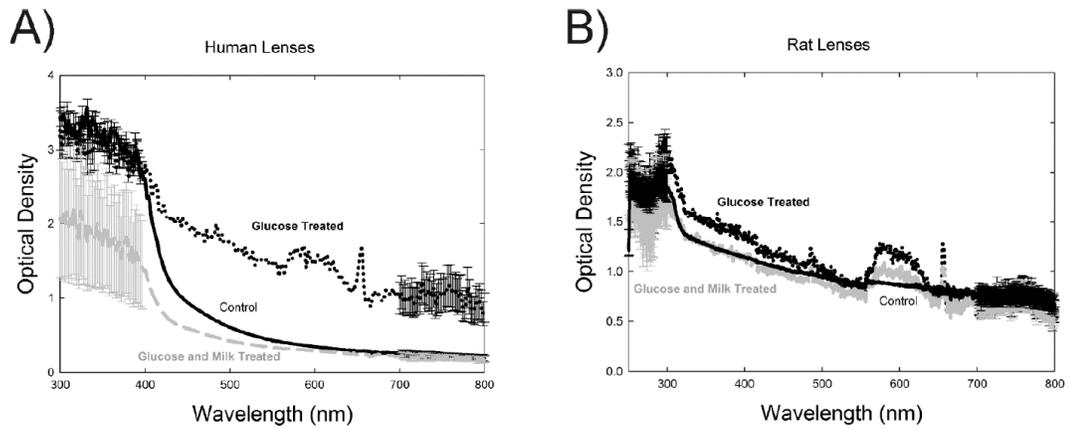


Figure 1: Average absorbance spectra of **A)** human lenses and **B)** rat lenses after three days in organ culture media. (—) Untreated lenses. (---) Treated with glucose and milk. (dotted) Treated with glucose. Error bars are the standard error of the mean and are shown only from 700 to 800 nm and 250 to 300 nm so as not to obscure the spectra. The error was greatest at lower wavelengths where very little of the light was transmitted. The spectra of human untreated, glucose treated and glucose and milk treated lenses were averaged from 10, 5 and 5 spectra, respectively. The spectra of rat untreated, glucose treated and glucose and milk treated lenses were averaged from 19, 8 and 9 spectra, respectively.

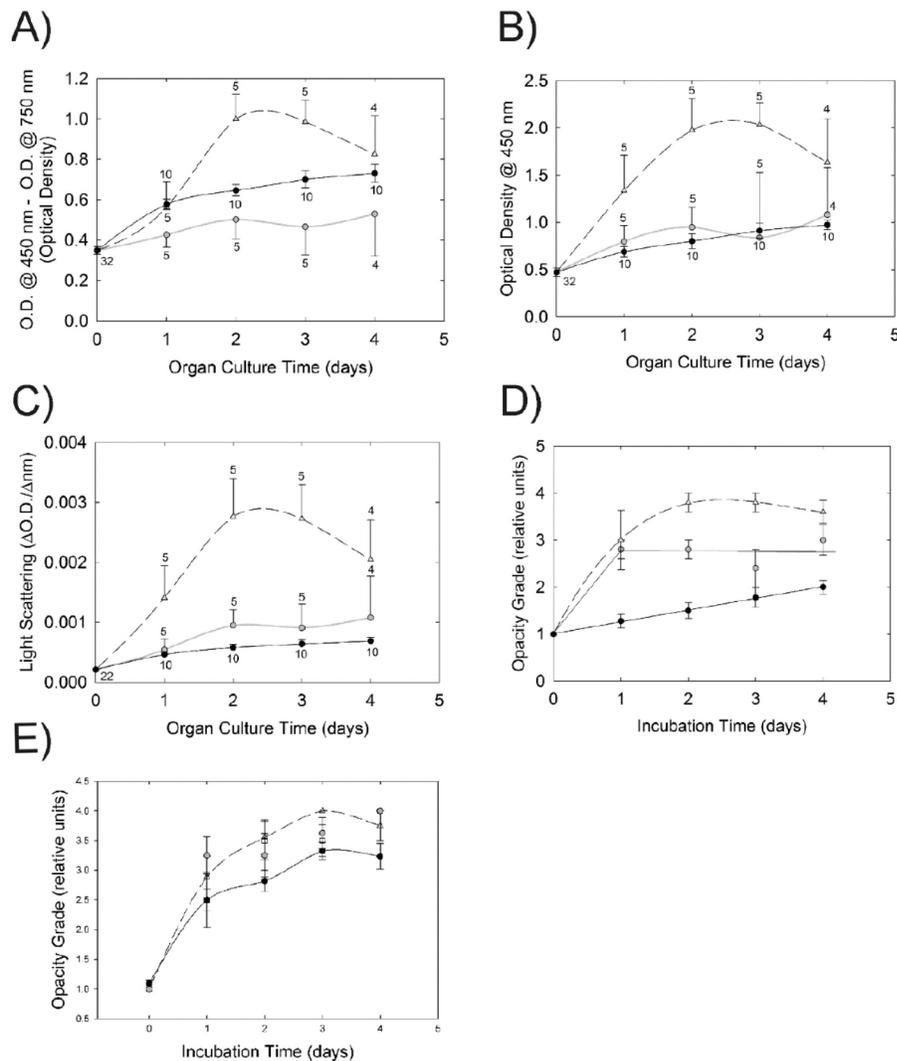


Figure 2: The optical properties of human lenses (**A**, **B**, **C** and **D**) cultured in control medium (●—●), medium with glucose and milk (○—○), and media containing glucose (△—△). Numbers are the number of lenses measured. Error bars are the standard error of the mean. Opacity grades from pictures of (**D**) human and (**E**) rat lenses as shown in Figure 3.

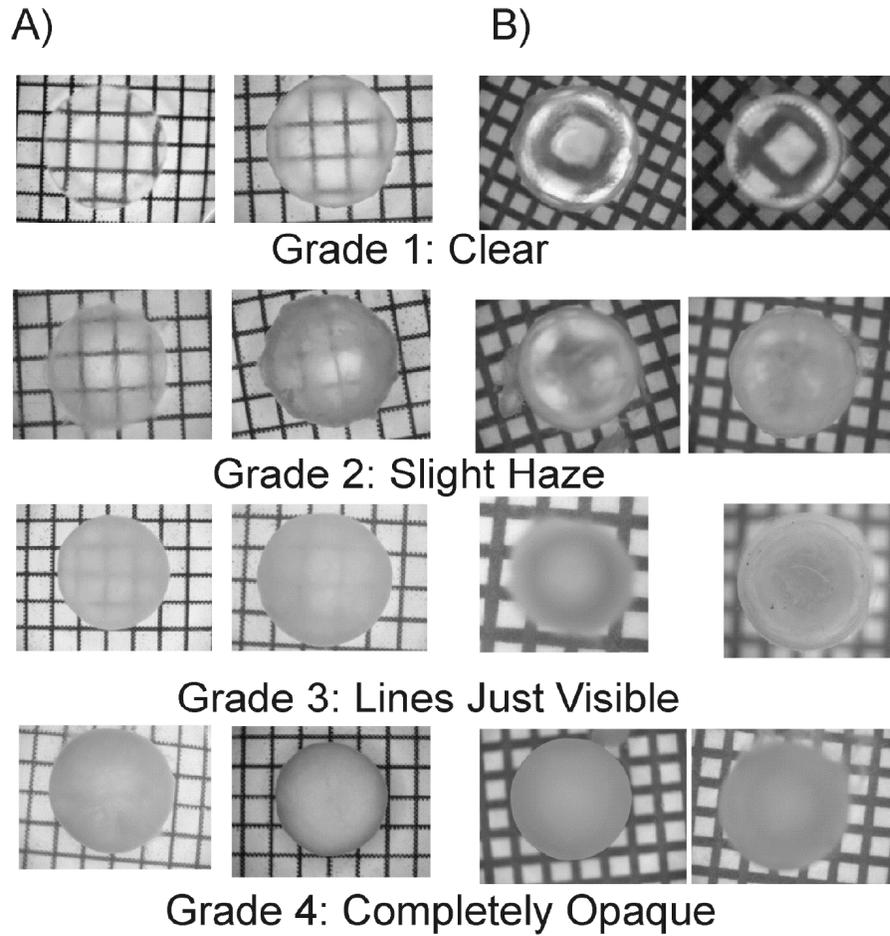


Figure 3: Grading guide used to grade A) human lenses and B) rat lens opacity in Figure 2D and E.

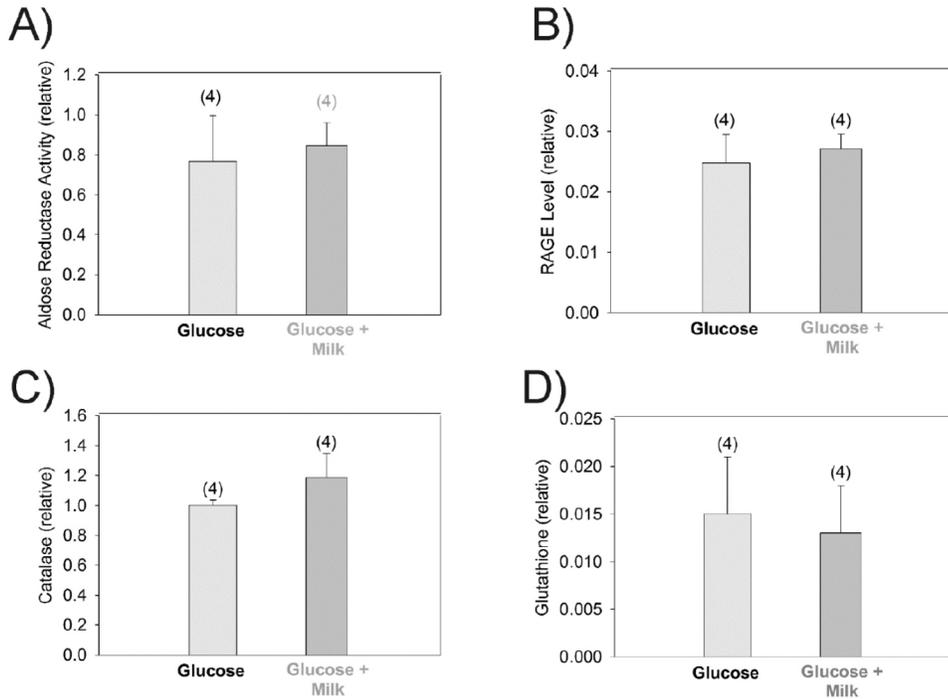


Figure 4: Assays on treated human lenses at four days in organ culture. Error bars are the standard error of the mean. Numbers are the number of lenses measured.

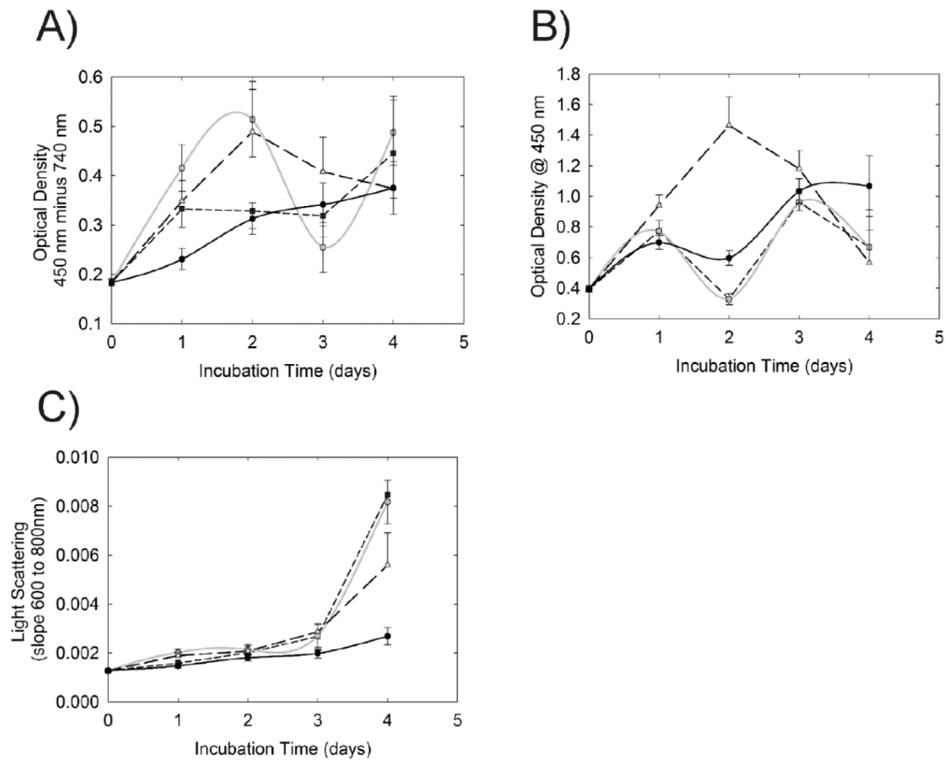


Figure 5: The optical properties of rat lenses organ cultured in control media (●—●), media with glucose and milk (o—o), media containing glucose (■—■) and media containing milk (△---△). Error bars are the standard error of the mean. The number of lenses measured for lenses cultured for 0, 1, 2, 3 and 4 days were respectively: untreated lenses 71, 18, 19, 19, 13; glucose treated 71, 9, 9, 9, 4; glucose plus milk treated 71, 8, 8, 8, 4; milk treated 71, 9, 9, 9, 3.

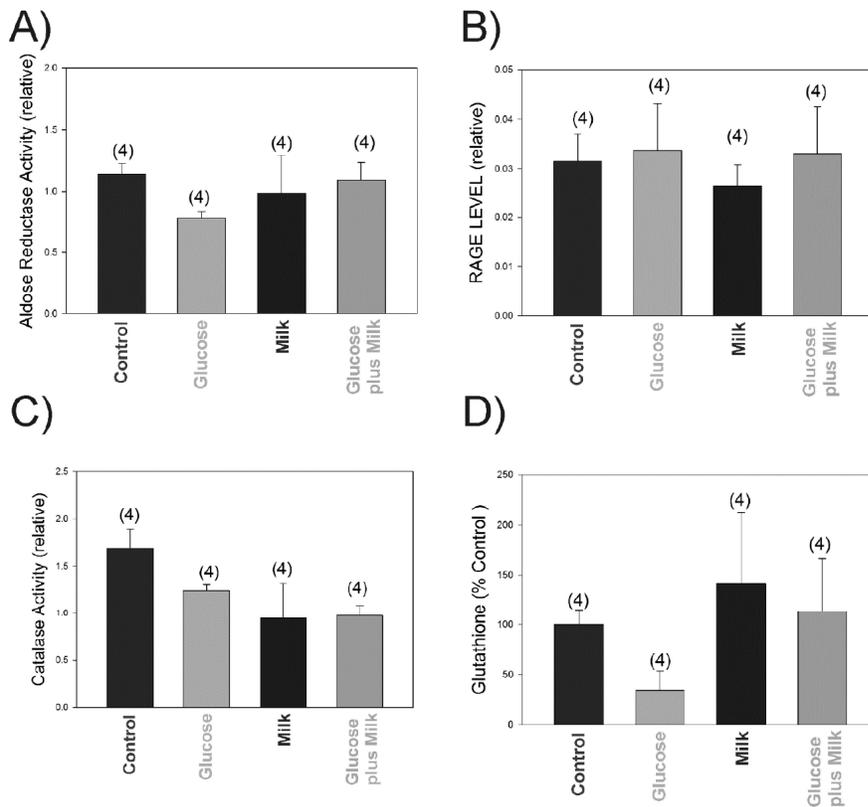


Figure 6: Assays on treated human lenses at four days in organ culture. Error bars are the standard error of the mean. Numbers are the number of lenses measured. Two rat lenses from the same group were pooled.

compared with RLc and rat lenses incubated with both glucose and camel milk (RLgm) (Figure 1B). As with human lenses (Figure 1A), a broad absorbance peak near 600 nm appeared in the spectra of RLg that was not present in the spectra of RLc but was not absent in RLgm (Figure 1B). At day two in organ culture, the O.D. at 450 nm (Figure 5B) and Δ O.D. (Figure 5A) were lower for RLc and rat lenses cultured with camel milk (RLm) when compared with RLg and RLgm. There was no significant difference ($P > 0.05$) between the O.D. and Δ O.D. for all rat lens cohorts at incubation times other than at two days. Except at an incubation time of four days, there was no difference in the light scattering between any of the cohorts (Figure 5C). However, on day four, the O.D. of RLc was significantly ($P < 0.05$) less than the other cohorts.

After four days in organ culture, aldose reductase activity (Figure 6A), catalase activity (Figure 6C) and glutathione levels (Figure 6D) for RLg, were attenuated compared with RLc. The level of RAGE was relatively unchanged for all rat lens cohorts (Figure 6B).

Rat lenses were graded by a blinded grader into four categories based on the opacity of the lens (Figure 3B). RLg were almost completely opaque after two days in organ culture (Figure 2E) and slightly more opaque than RLc. RLgm and RLm were about the same opacity grade as RLg (Figure 2E).

DISCUSSION

The major finding of the current study was that camel milk present in the organ culture medium inhibited glucose-induced opacity, especially in human lenses. Because the etiology of diabetic cataract is multifactorial, complex, and not completely established, it is difficult to speculate on the mechanism whereby camel milk inhibits glucose cataract. Hyperglycemia is undoubtedly the initiating factor for diabetic cataract [26]. It is likely to involve osmotic stress and/or oxidation related metabolic changes [26,27]. Based on the author's studies, elevated levels of calcium are also likely to contribute to glucose cataract [28]. It is reasonable to speculate that osmotic stress causes mitochondria to produce reactive oxygen species which, in turn, oxidize membrane lipids [22,23], increasing membrane permeability [29-32] causing an increase in intracellular calcium [28], which may activate lipases and proteases [33,34]. All of these factors contribute to changes in the gross and molecular structure of lenses resulting in increased light scattering, the major factor for the attenuation of

light in glucose treated lenses [18]. Although not a major factor in the attenuation of light through the lens, camel milk in the medium of organ cultured human lenses decreased the absorbance bands between 550 and 700 nm that were induced by glucose. The finding from the current study that camel milk restored RLgm glutathione levels to that of RLc, suggests that the high ascorbate content of camel milk [3-6] may protect the lens from oxidative insult. The factors responsible for these observations are speculative. One possibility is that camel milk proteins coat the capsule protecting the lenses from the damaging effects of glucose. Future studies to determine the moieties in camel milk that are responsible for cataract inhibition are planned. The positive results from this initial *in vitro* study suggests that costlier and time consuming *in vivo* studies to determine the efficacy of camel milk *in vivo* are warranted.

The utility of using human lenses to test the efficacy of drugs and agents is promising. Because rat lenses become opaque relatively quickly (one day) they are less promising tools for organ culture experiments. Rat lenses differ significantly from human lenses so the use of human lens models is significantly advantageous over animal models [19, 20]. Furthermore, it has been suggested that rat lenses are more susceptible to oxidation than human lenses due to their higher unsaturated glycolipid content [21-25].

In conclusion, camel milk present in the organ culture medium suppressed glucose-induced opacity, especially in human lenses, and restored the rat lens glutathione level to that of untreated rat lenses while causing a decrease in catalase activity. Because opacity is the ultimate end point for cataractous lens studies, quantitative assessment of absorbance and light scattering in lenses is essential for model studies.

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