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Black and Green Teas Equally Inhibit Diabetic Cataracts in a Streptozotocin-Induced Rat Model of Diabetes

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Green and black teas were given at 1.25% in the drinking water to streptozotocin-induced diabetic rats for 3 months. Normal and diabetic control groups were also studied. As expected, diabetic animals had significantly increased glucose in lens and plasma. Lens and red blood cell sorbitol were significantly increased as a result of the aldose reductase pathway activation. Plasma and lens lipid thiobarbituric acid-reactive substances and protein glycation were also significantly elevated. Both teas significantly inhibited diabetic cataracts and caused significant reductions in the biochemical pathway implicated in the development of the pathology. After corrections for glucose, it was found that the teas retard the development of diabetic cataracts by a hypoglycemic effect that in turn inhibits the biochemical indicators of pathology. There were significant correlations between glucose, cataract score, and these indicators. Green tea but not black tea caused a significant decline in triglycerides in the diabetic animals. Tea may be a simple, inexpensive means of preventing or retarding human diabetes and the ensuing complications. Tea also should be investigated as an adjunct therapy for diabetes treatment.

KEYWORDS: Tea; diabetes; cataracts; glucose; lipid peroxides; glycation; sorbitol

INTRODUCTION

Diabetes mellitus is now considered to be a worldwide epidemic and without primary prevention, the epidemic will continue unabated. The cost of diabetes in the United States was estimated to be $44 billion in 1997 (*1*). Cost-effectiveness of any prevention or treatment modality is of prime importance, especially to underdeveloped countries. It has been convincingly demonstrated by the Diabetes Control and Complications Trial for insulin-dependent diabetics (*2*) and the Kumamoto study for non-insulin-dependent diabetics (*3*) that tight control of blood glucose significantly prevented or delayed diabetic complications such as nephropathy, neuropathy, and retinopathy. Although the hallmark of diabetes is hyperglycemia, the pathology of diabetes is caused by reactive oxygen species that activate multiple pathways including nonenzymatic glycation of proteins and subsequent advanced glycation end-products leading to structural and functional changes, the aldose reductase pathway causing sorbitol accumulation, and oxidative stress resulting in protein, DNA, and lipid damage (*4*). Diabetic complications reflect a single hyperglycemia-induced process of overproduction of superoxides by the mitochochodrial electron-transport chain that causes β cell dysfunction and ultimately type 2 diabetes (*5*).

Tea has a long history as a folk remedy, but the beneficial medicinal properties have only been elucidated in the past 20 years. Tea is the most widely consumed beverage in the world, second only to water. The annual world per capita consumption is 40 L (*6*). Black tea is manufactured from green tea by fermenting the leaves of *Camellia sinensis.* Despite the fact that green tea is becoming more popular in the Western world, black tea accounts for 80% of the worlds' tea production (*7*). The major ingredients in teas are a group of polyphenols known as catechins. Black tea contains simple as well as oxidized and polymerized catechins. All of these tea compounds are antioxidants. Epigallocatechin gallate, a major compound in green tea, is the most powerful antioxidant in an in vitro model of lipoprotein oxidation (*8*). Black tea has an equivalent amount of polyphenol antioxidants to green tea (*9*, *10*). Black tea or green tea at a high dose (5 g of tea solids/kg of body weight) is hypoglycemic in the streptozotocin (STZ)-induced diabetic rat (*11*). Green tea given to normal rats at a high dose decreased fasting plasma glucose, insulin, and triglycerides (*12*).

Human evidence for the beneficial effect of tea on diabetes is primarily based on epidemiological studies. In 2003, epidemiological evidence from the SU.VI.MAX study with almost 3000 subjects has shown that increased tea drinking is significantly associated with lower levels of serum glucose and triglycerides (*13*). A Saudi study with over 3000 subjects found that increased black tea consumption was significantly and inversely related with lower levels of blood glucose (*14*). The only human intervention study was with oolong tea, which is intermediate between green and black tea. In this study the tea was given to type 2 subjects for 1 month. Fasting glucose and fructosamine, the long-term indicator of glucose control, were significantly decreased with the tea (*15*). The common belief is that green tea is more beneficial to health than black tea. We

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Table 1. Cataract Rating, Plasma, Red Blood Cell (RBC), and Lens Parameters in Normal and Diabetic Rats[a]

group	plasma glucose (mM)	lens glucose (nm/mg)	cataract rating (0–4)	plasma glycated lysine (μm/mg)	lens glycated lysine (μm/mg)	RBC sorbitol (μM)	lens sorbitol (nm/mg)	plasma TBARS (μM)	lens TBARS (pm/mg)	plasma cholesterol (mM)	plasma triglycerides (mM)
normal (N)	0.65 ± 0.17a	19 ± 7a	1.13 ± 0.98a	0.48 ± 0.08a	0.60 ± 0.12a	1.10 ± 0.73a	1.05 ± 0.16a	0.45 ± 0.12a	4.67 ± 1.52a	0.764 ± 0.224a	4.37 ± 2.30a
diabetic control (D)	2.07 ± 0.51b	64 ± 22b	3.02 ± 0.23b	0.65 ± 0.08b	1.15 ± 0.46b	4.72 ± 1.68b	31.3 ± 8.1b	0.79 ± 0.20b	12.8 ± 4.9b	1.04 ± 0.36a	7.37 ± 2.06a
diabetic + green tea (DGT)	1.41 ± 0.54c	48 ± 13c	2.61 ± 0.63c	0.52 ± 0.13ac	0.90 ± 0.25c	2.68 ± 0.99c	22.4 ± 4.5c	0.60 ± 0.18c	8.55 ± 2.94c	0.99 ± 0.28a	5.13 ± 1.49b
diabetic + black tea (DBT)	1.49 ± 0.67bc	48 ± 16c	2.24 ± 0.83c	0.51 ± 0.10ac	0.81 ± 0.24c	3.62 ± 0.82b	25.4 ± 4.1c	0.61 ± 0.16c	8.46 ± 3.89c	1.25 ± 0.21a	6.43 ± 1.85ab

[a] Data are mean ± SD. For N ($n = 8$), D ($n = 9$), and DBT and DGT ($n = 8$). In columns, groups with different letters are significantly different, $p < 0.05$.

recently compared black and green tea in a hamster model of atherosclerosis and found that both significantly inhibited early atherosclerosis and decreased both lipids and lipid peroxidation (*16*). We report here the first comparison of black and green teas in an STZ-induced rat model of diabetes.

RESEARCH DESIGN AND METHODS

Animal Protocol. Male Sprague−Dawley weanling rats were obtained from Charles River Laboratories. The animals were housed individually in plastic colony cages in a climate-controlled animal facility. The animals were cared for in accordance with the University of Scranton's Institutional Animal Care and Use Committee. They were fed with normal rat chow and water ad libitum during an initial adaptation period of 1 week. STZ from Upjohn Co. was used to induce diabetes. Animals were given STZ at 65 mg/kg (25 mg/mL in 0.1 M sodium acetate buffer, pH 4.4) intraperitoneally (ip) twice during a 6-day interval. After 1 week, the 25 hyperglycemic animals (glycosuria by a urine glucose strip) were then randomly divided into three groups averaging 117 g. There were eight animals were in the normal group (N), nine animals in the diabetic control group (D), and eight animals each in the green tea group (DGT) and black tea group (DBT). All animals were given rat chow and artificial sweetener (Sweet N'Low, 10 g/L) in the drinking water.

The teas were prepared at 1.25% (standard tea for human consumption) using boiling water, and then the sweetener was added. The teas were World Blend from Lipton, Inc., and their polyphenol composition has been measured by HPLC (*10*). Teas were prepared every other day and stored in the refrigerator. All water bottles were changed daily, and the animals were allowed liquid ad libitum, which was monitored weekly.

Cataracts were checked on the last day of the study with a slit lamp ophthalmoscope using the modified Sippel rating (*17*) of 0−4: Sippel stage 0 rating = 0 normal lens; stage 1A rating = 0.5 thin band of vacuoles in the periphery; stage 1B rating = 1 vacuole occupies up to one-third of the lens; stage 1C rating = 1.5 vacuoles occupy two-thirds of the lens; stage 2 rating = 2 vacuoles reach the center of the lens and liquification of vacuoles begins; stage 3 rating = 3 vacuoles have liquefied and uniform opalescence develops; stage 4 rating = nuclear opacity begins. The examination was done in a blinded fashion with two trained assistants. The results of each analyst were averaged to obtain the cataract rating. At the end of the study after an overnight fast, the animals were sacrificed by pentobarbital, 0.13 mg/kg ip, and the plasma, red blood cells, and individual lenses of each animal were isolated. Red blood cells (200 μL) were lysed with 1 mL of 6% $HClO_4$. Lenses were weighed and homogenized in 1 mL of Millipore Q purified water. All samples were stored at −80 °C until analysis.

Biochemical Analysis. Glucose was measured by a colorimetric enzyme kit (Sigma). Sorbitol was determined with a standard enzyme fluorometric method that measures NADH. Protein was assayed with brilliant blue G colorimetrically (Sigma). Thiobarbituric acid-reactive substances (TBARS) were measured using malondialdehyde as a standard and a fluorometric assay of the thiobarbituric acid derivative. Glycation was assayed with a standard methodology involving protein hydrolysis with trichloroacetic acid and colorimetric analysis of the product glycated lysine with thiobarbituric acid. Hydroxymethylfurfural was the standard. Plasma total cholesterol and triglycerides were measured with enzymatic kits (Sigma). The biochemical data were analyzed with SigmaStat (Jandel Scientific, San Rafael, CA), using a Student's t test for normally distributed data or a rank sum test for non-normally distributed data. Cataract ratings were compared by a chi-squared test.

RESULTS

The diabetic groups had significantly less body weight compared to the normal group ($p < 0.0001$). There were no differences among the diabetic groups. Diabetic animals consumed significantly more liquid than the normal group, $p < 0.001$. The means were 51 (N), 179 (D), 173 (DGT), and 174 mL (DBT). There were no differences among the diabetic groups. Lenses of the diabetic groups weighed less than those of the normal group ($p < 0.03$), and there were no differences among them. There was no significant difference in plasma protein between the groups. Protein concentration in the lens was significantly lower in all but one of the diabetic groups compared to the normal group ($p < 0.03$); average lens protein: N, 0.226 ± 0.026; D, 0.192 ± 0.053; DGT, 0.197 ± 0.040 mg/mg of lens, respectively. DBT was not significantly different from the normal group, DBT 0.209 ± 0.05 mg/mg of lens. The rest of the data is shown in **Table 1**. Except for the lipids, the D groups' concentration of the biochemicals examined in both the plasma and lens was always significantly higher than the N group's ($p < 0.001$). Both teas significantly decreased glucose, and, with the exception of the DBT red blood cell sorbitol, also inhibited the pathological pathways of diabetes in lens, plasma, and red blood cells. Both teas normalized plasma glycated protein. Lipids were elevated in the D group compared to N, but not significantly. Green tea caused a significant decrease in plasma triglycerides when compared to D.

The average Sippel cataract rating of the D group was almost 3 times that of the N group, $p < 0.001$, with significant improvements in the tea groups. A more revealing comparison is made by looking at the frequency (percent) of lenses in each cataract grade (**Figure 1**), which is significantly improved by both teas ($p < 0.05$). The teas shifted the frequency of cataract scores from high values to low values. A graph of plasma glucose and cataract score is shown in **Figure 2**. There is a high degree of correlation; Pearson's product moment correlation coefficient $r^2 = 0.666$, $p < 0.0001$. **Figure 3** shows the relationship between lens glucose and cataract score, $r^2 = 0.615$, $p < 0.0001$. In fact, plasma and lens glucose are highly correlated, $r^2 = 0.825$, $p < 0.0001$. All of the lens parameters, that is, glycated protein, sorbitol, and TBARS, are significantly correlated with the cataract score ($p < 0.01$). Red blood cell sorbitol and plasma TBARS are significantly correlated with plasma glucose. This shows the interrelationship of the pathways and lens pathology.

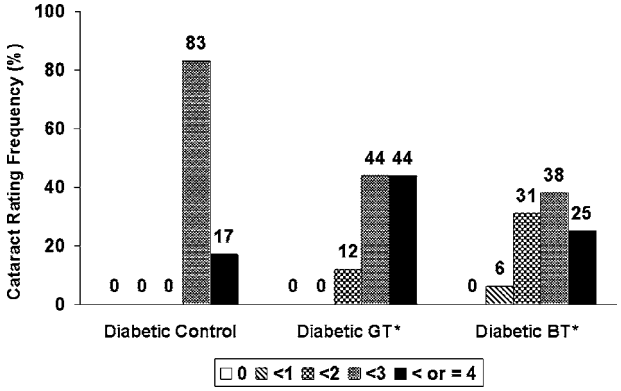

Figure 1. Frequency of cataract ratings (0–4) within the different groups (GT, green tea; BT, black tea). *, significantly different from diabetic group, $p < 0.05$.

DISCUSSION

The weight data indicated that diabetes caused a reduction in body weight compared with the normal group, which was expected. The greater liquid consumption of the D groups was also a signal phenomenon in diabetes. Lens protein was found to be lower in diabetes, which can be attributed to a leaky lens in diabetes. We used common, sensitive but nonspecific assays for lipid peroxides and glycation, but these methods are accurate enough to definitively show a significant difference between the N and D control groups. Thus, they are suitable for comparison within the D groups.

There is in vitro evidence teas could inhibit the biochemical indicators of pathology independently of a glucose-lowering effect: for instance, inhibition of aldose reductase (*18*), protein glycation (*19*), or lipid peroxidation (TBARS), the latter by an antioxidant effect (*16*). This hypothesis could be tested in this study by normalizing to glucose (dividing the biochemical

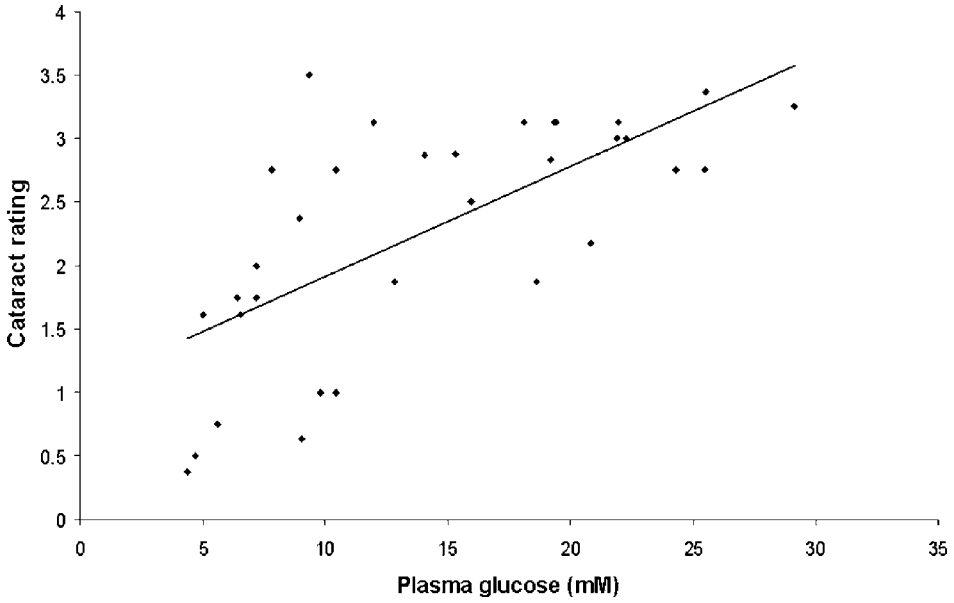

Figure 2. Correlation of plasma glucose with average cataract rating (left and right lens) of each animal.

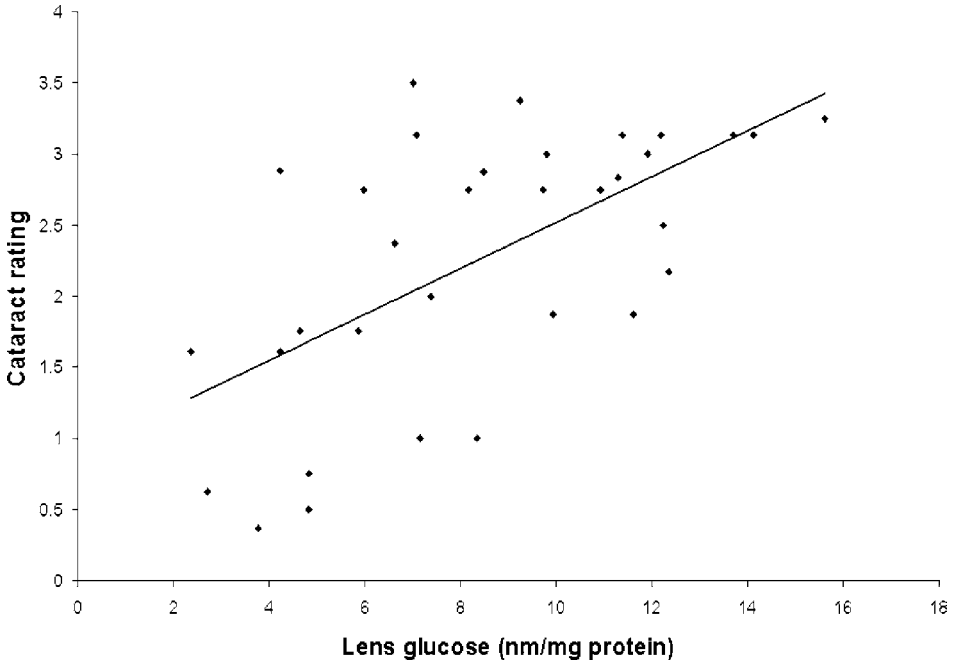

Figure 3. Correlation of lens glucose with average cataract rating (left and right lens) of each animal.

parameter by the corresponding glucose value). Compared to the control diabetic group, there is no significant inhibition of aldose reductase, glycated protein, or TBARS in the tea groups after performance of this normalization. Thus, the mechanism of teas' benefit for diabetes is a hypoglycemic effect among the diabetic mechanisms investigated. This is significant because hyperglycemia is the initial event in the pathogenesis of diabetic complications.

The means by which teas act on glucose has been investigated using in vitro techniques. For instance, the green tea catechin epigallocatechin gallate was shown to repress hepatic glucose production by modulating the redox status of the cell (*20*). Black and green teas were shown to have in vitro insulin-enhancing activity, with the majority of the green tea activity due to epigallocatechin gallate (*21*). Intestinal glucose uptake is mainly accomplished by the sodium-dependent glucose transporter, SGLT1. The transport activity of SGLT1 was markedly inhibited by green tea polyphenols (*22*).

This paper is the first study to examine three mechanisms of diabetic pathology and show a relationship to a diabetic complication, cataracts. In this animal model of type 1 diabetes, both green and black teas appear to be of equal efficacy for improving the diabetic state by means of a hypoglycemic effect, which in turn inhibits the biochemical indicators of diabetic pathology. The calculated daily 65 kg human dose based on the cube root relationship of the human/animal weight ratio is 1096 mL, or 4.6 8-oz cups/day. This is similar to the 6.3 cups/day dose used in the type 2 human diabetes oolong tea study (*15*). The effect of teas on plasma glucose in our study was a reduction of 28−32%, which was equal to the 29% reduction found for oolong tea in the diabetic human study. In an epidemiological study of tea drinking and senile cataracts, there was a 61% risk reduction in cataracts from drinking 5 cups of tea/day (*23*). Black and green tea represent a potential inexpensive, nontoxic, and, in fact, pleasurable hypoglycemic agent. Teas should be investigated further for possible prevention therapy and adjunct therapy in human diabetes.

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