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Specific Bioactive Compounds from Ginger, Tea, and Apple Prevent Diabetes-Related Cataract Via Inhibition of Aldose Reducatse

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Abstract

Chronic hyperglycemia is an important risk factor involved in the onset and progression of secondary complications of diabetes. Aldose reductase (AR) has been implicated in the etiology of diabetic eye diseases, diabetic cardiomyopathy and/or nephropathy. High glucose levels activate AR, which is one of the key rate limiting enzymes to use NADPH to reduce glucose to sorbitol in the polyol pathway. The depletion of NADPH is correlated to the production of GSH which increases intercellular oxidative stress. Since inhibition of AR plays an important therapeutic value in preventing and or alleviating diabetic complications. At present there are many AR inhibitors such as Sorbinil, Dilantin, Epalrestat, Tolrestat, Zopolvestat and Minalrestat. However, most of these compounds have serious side effects such as Steven-Johnson syndrome and hypersensitivity reaction. In recent years nutraceuticals have garnered interest for their potential as dietary and natural health promoting properties. Hence, the purpose of this study was to investigate the inhibitory activity of phloretin from apple, (–)-epigallocatechin 3-gallate (EGCG) from green tea and [6]-gingerol from ginger against aldose reductase as indicator or their potential in the alleviation of diabetic complications. These three compounds were selected out of 9 bioactive compounds based on their activity in cell culture assays. Human retinal pigment epithelial (HRPE) cells were subjected to different concentrations of glucose (10–100 mM) for 1 and 4 days during which cell viability and aldose reductase activity were evaluated. Results show that cell viability decreased to 93 ± 3%, 83 ± 6%, 65 ± 4% and 39 ± 3% at 10, 25, 50 and 100 mM of glucose on day 1 with further drastic decrease in cell viability to 73 ± 5%, 61 ± 3%, 35± 2%, and 11 ± 6%, respectively, on day 4 compared to the untreated cell. The activity of aldose reductase was found to increase 5 folds at 10 mM

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from day 1 to day 4, whereas at 25, 50 and 100 mM concentrations of glucose the AR activity was increased to almost 2 folds. The specific activity of aldose reductase was found to be 8.92 ± 1.6 U/mg protein at 100 mM glucose on day 4. The apparent Michaelis constant (Km) of the substrate glyceraldehyde and NADPH was estimated to be 4.5 mM and 68.96 μM, respectively. Pre-treatment of cells with phloretin, EGCG and [6]-gingerol at 25 μM improved cell viability to 72 ± 4%, 77 ± 5% and 78 ± 4%, at day 1 respectively, whereas EGCG further improved cell viability on day 4 to 89 ± 5%, but phloretin and [6]-gingerol could marginally increase to 76 ± 3%, 81± 4% when compared to the untreated cells. The three compounds could inhibit AR activity up to 90 % at 12.5 μM of phloretin, EGCG and [6]-gingerol with IC50 values of 4.1 μM, 3.7 μM, and 2.4 μM, respectively. The enzyme inhibition kinetics showed non-competitive mode of inhibition for phloretin and EGCG, since it did not alter the Km but the maximum velocity (Vmax) decreased in the presence of the compounds, whereas [6]-gingerol indicated uncompetitive type of inhibition against AR, where it decreased both Km and Vmax upon binding. The above cell culture findings were further validated in mouse model. Male C57BL/6J mice 5 weeks old, were divided into eight groups of 11 mice each. The animals had free access to food and water and were fed with either a standard laboratory diet (8604 Teklad Rodent diet, Harlan, TM) or a high fat diet (HFD) (TD 110716, Teklad Research Rodent Diet, Harlan, TM). Animals were randomly assigned to the different treatment groups (N = 11 / group): (i) Normal diet, (ii) HFD, (iii) HFD + EGCG 25 mg/kg, (iv) HFD + EGCG at 75 mg/kg, (v) HFD + phloretin 25 mg/kg, (vi) HFD + phloretin 75 mg/kg, (vii) HFD + [6]-gingerol 25 mg/kg, (viii) HFD + [6]-gingerol 75 mg/kg. All test solutions were prepared freshly every day prior to use and were administered intraperitoneal injection (i. p.), once daily and three times in a week over a period of sixteen weeks. Body weight was recorded every day, while blood glucose levels were determined weekly with a commercially available micro-draw blood monitoring system. After sixteen weeks, the animals were sacrificed; heart, eyes and kidney were examined for AR activity. The results shows that the HFD group had developed diabetes, with blood glucose levels 260 ± 27 mg/dL, whereas the control with normal diet showed about 130 ± 20 mg/dL. The groups treated with EGCG, phloretin and [6]-gingerol at 75 mg/kg significantly decreased blood sugar levels to 128 ± 8, 125 ± 15 and 132 ± 9 mg/dL, respectively. The groups treated with EGCG, phloretin and [6]-gingerol at 25 mg/kg also decreased the blood sugar levels to 198 ± 15, 180 ± 16 and 170 ± 19 mg/dL, respectively. The eye lens of the mice from HFD group had developed cataract and the AR activity increased to 4 fold compared to the group fed with normal diet. All the compounds at 75 mg/kg, prevented/delayed the formation of cataract by 80%, whereas dosing at 25 mg/kg showed signs of cataract but AR activity was decreased to two folds when compared to the HFD group. The HFD enhanced the aldose reductase activity to two and three folds in the heart and kidney. Data from this study suggest the promising potential of these dietary compounds in providing cytoprotection and inhibiting a key enzyme associated with the onset of diabetes-related complications. Upon validation of this benefit in vivo, such dietary compounds could be of interest to dietary supplements and pharmaceutical industry as complementary treatment of secondary complications in diabetic patients.