

Supplementation of Type 1 Diabetic Rats with Carrot Powder Lowers Blood Glucose without Improving Cardiac Structure and Function.

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ABSTRACT: Foods and food bioactives have shown to be effective in preventing some human disease conditions. In this study, we examined the effects of carrot powder, rich in carotenoids, as a dietary supplement for the prevention of cardiac anomalies in streptozotocin (STZ) induced type 1 diabetic rats. Male Wistar rats were fed either control or carrot powder containing diet for 3 weeks. Type 1 diabetes was induced with STZ injection (65 mg/kg body weight) in half of the rats in each group. All rats were continued on their respective diet for a further 9 weeks. Cardiac structural and functional parameters were measured using echocardiography at 8 weeks post STZ administration. In comparison to non-diabetic rats, diabetic rats showed significant increase in isovolumetric relaxation time and a significant decrease in systolic function parameter, cardiac output. Left ventricular internal dimension and left ventricular posterior wall thickness were significantly higher in diabetic animals. Blood glucose levels were significantly lower in carrot supplemented diabetic rats when compared with non-treated diabetic rats. Diabetic rats treated and untreated had elevated level of lipid peroxidation. Catalase levels were significantly elevated in the carrot powder supplemented diabetic rats when compared to the control rats. Carrot supplementation lowered blood glucose levels significantly but did not normalize it to control levels. It had no effect on cardiac abnormalities and anti-oxidant status in rats with type 1 diabetes.

Keywords: diabetes mellitus, heart disease, carrot, carotenoids

INTRODUCTION

Dietary intake of fruits and vegetable has been correlated with a lower risk for cardiovascular diseases (CVD) (1, 2). Building on the same concept, alternative approaches including use of dietary bioactives may be pursued as a valuable aid in developing preventive and therapeutic strategies for CVD. Type I diabetes mellitus (type 1 DM) is a disease which occurs due to pancreatic dysfunction resulting in the production of very little or no insulin (3-5), a hormone which facilitates uptake of glucose into cells. On the other hand, type II diabetes (type II DM) occurs when insulin is available (or slightly less available), but cannot facilitate glucose uptake into the cells resulting in an insulin resistant state. Both type I and II DMs are chronic conditions which result in diminished uptake of sugar by cells and less energy production. Dia-

betic patients are also at greater risk of developing a distinct form of heart disease (cardiomyopathy) termed, 'diabetic cardiomyopathy', characterized by abnormalities in the structure and function of the heart without any other risk factors that usually accompany diabetes such as coronary heart disease and hypertension (6). Diabetes induced cardiomyopathy has been shown to be clinically manifested as hypertrophy or dilatation of the left ventricle with systolic and diastolic functional impairment in diabetic patients. Impaired cardiac relaxation in diabetic patients is an early sign of diabetic cardiomyopathy that usually precedes the occurrence of systolic dysfunction (7). It has been demonstrated that diabetic cardiomyopathy is induced through various factors, including hyperglycemia, augmented cardiac fatty acid metabolism and lipid accumulation, enhanced reactive oxygen species generation, abnormal calcium homeostasis, activation of

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the renin-aldosterone-angiotensin system, and mitochondrial dysfunction (8).

Carotenoids are a class of natural, fat-soluble, and highly colored pigments found in plants. The common dietary carotenoids are lycopene, β -carotene, α -carotene, β -cryptoxanthin, lutein, and zeaxanthin. Among these, β -carotene, α -carotene, and β -cryptoxanthin are the precursors of vitamin A which is essential for various physiological functions. Epidemiological data from prospective and case control studies suggested that carotenoids are beneficial in the prevention of heart disease due to their anti-oxidant and anti-inflammatory effects (9-11). Type 1 diabetes is associated with decreased efficiency of endogenous anti-oxidant system and increased oxidative stress which may also subsequently cause reactive oxygen species mediated cardiac impairment (12,13). Accordingly, a combination of dietary carotenoids may be effective in preventing diabetic cardiomyopathy by improving the anti-oxidant status in diabetic conditions. However, there is a lack of studies which have tested the efficacy of carotenoid rich dietary source (as an added supplementation with normal diet) in preventing the development of cardiovascular complications in type 1 diabetes. This scenario suggests the fact that studies are needed to explore the cardioprotective effects of dietary sources of carotenoids rather than any particular single carotenoid (9,14). Therefore, this study examined the effect of carrot powder, one of the rich sources of carotenoids, on streptozotocin (STZ)-induced diabetic cardiomyopathy in rats.

MATERIALS AND METHODS

This *in vivo* study protocol (F-11-016) was approved by the University of Manitoba Office of Research Ethics & Compliance and Animal Care Committee. Animal care procedures were based on guidelines described in the Canadian Council for Animal Care.

Experimental design

Three week old weanling male Wistar rats (Charles River Laboratories, St. Constant, QC, Canada) were housed in pairs in flat-bottomed polycarbonate cages in a temperature and humidity controlled room with a 12-hour light and dark cycle. After four days of acclimatization, rats (n=40) were randomly assigned to either carrot enriched (15% w/w) diet (n=20) or carrot free semi-synthetic control diet (n=20) and fed *ad libitum* (Table 1). The supplement dose for the present study was chosen based upon a previous study that investigated anti-oxidant effects of carrot carotenoids (15). Carrot powder used was obtained from Food Product Development Centre (Portage la Prairie, MB, Canada). The control diet contained approximately 35% of calories as fat, 42~44% carbohydrate,

Table 1. Composition of control diet without carrot powder and experimental diet with carrot powder (unit: g/kg)

	Control	Carrot
Casein	218.00	216.00
Corn starch	423.00	403.50
Sucrose	37.50	—
Glucose	15.00	—
Fructose	15.00	—
Non-nutritive cellulose	50.00	0.00
Vitamin mix AIN-93VX	10.00	9.00
Mineral mix AIN-93M	50.00	45.00
Choline chloride	2.75	2.75
Inositol ¹⁾	6.25	6.25
L-Methionine	2.50	2.50
Canola oil ²⁾	170.00	170.00
Carrot powder ³⁾	—	150.00

All values are represented as g/kg diet.

Diet ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA) except canola oil and inositol.

¹⁾Inositol (Bio-Serv, Frenchtown, NJ, USA).

²⁾Capri canola oil (Bunge Canada Ltd., Altona, MB, Canada) containing dimethylpolysiloxane, as an anti-foaming agent.

³⁾Contains approximately 10.5 g protein, 18 g starch, 37.5 g sucrose, 15 g glucose, 15 g fructose, 0.05 g vitamins, 4.5 g minerals, and 47.5 g fiber.

and 20% protein, reflecting the current macronutrient distributions recommendations by the Canadian Diabetes Association-2013 (16). Diet induced changes in metabolism and function were considered to be based on differences in micronutrients such as carotenoids (β -carotene, α -carotene, and lutein; 61.9 mg, 43.1 mg, and 0.62 mg per kg diet, respectively). All animals received fresh food 3 times per week to minimize ingredient decomposition and nutrient loss.

Three weeks into diet adaptation, animals in both group were injected with either a vehicle solution (non-diabetic) or STZ (Tocris Biosciences, Ellisville, MO, USA) at 65 mg/kg body weight (0.2 M acetate buffer, pH 4.5) I.V. to induce type 1 diabetes. Animals were grouped as follows: non-diabetic with control diet (control, n=10); non-diabetic with carrot enrichment (control+carrot, n=10); diabetic with control diet (diabetic, n=10); and diabetic with carrot enrichment (diabetic+carrot, n=10). Animals with fasted blood glucose of 12 mmol/L or greater on third day after STZ injection were considered as diabetic. All the animals were monitored weekly to record body weight. Diabetic animals were not administered with insulin in order to sustain elevated blood glucose during the study period.

Echocardiographic imaging and analyses

Transthoracic echocardiography was performed to measure the clinically relevant cardiac structural and functional parameters *in vivo* at 8 weeks of STZ treatment. Echocardiogram was obtained from 2D guided M-mode and

Pulsed-Wave Doppler imaging modalities with a Sonos 5500 ultrasound system (Agilent Technologies, Andover, MA, USA) equipped with a 12-MHz (S12) transducer as described in our earlier study (17). Two-dimensional M-mode imaging was done to determine systolic functional parameters such as percentage of left ventricular ejection fraction (EF) and cardiac output (CO), as well as the structural parameters such as interventricular septal wall thickness (IVSD), left ventricular posterior wall thickness at diastole (LVPWD) and left ventricular internal dimensions at diastole (LVID). Pulsed-Wave Doppler modality imaging was analyzed to determine the diastolic functional parameter isovolumetric relaxation time, which is the interval between aortic valve closure and mitral valve opening. Values obtained from 3 consecutive cardiac cycles were averaged to obtain final values. LVID, LVPWD, and IVSD were expressed as ratio to body weights to normalize the variations between body weights of control and diabetic rats.

Blood and tissue collection

At the end of 9 week post STZ administration, all animals were anesthetized with ketamine/xylazine 6.25 mg/100 g and 1.25 mg/100 g weight by intraperitoneal. Blood was collected from inferior vena cava by opening the thoracic cavity and heart was immediately excised. Plasma was separated and stored at -80°C for further analysis. Heart was removed and rinsed in saline solution before separating into atria, right ventricle, left ventricle, and septum tissue. Tissues were flash frozen in liquid nitrogen and stored at -80°C .

Serum glucose measurement

Serum glucose was measured using a Genzyme DiagnosticsTM (Charlottetown, PEI, Canada) colorimetric assay kit. The quinoneimine dye produced in the reaction between hydrogen peroxide, hydroxybenzoate and 4-aminopyridine was measured at its absorbance of 505 nm. This test had a coefficient of variation (CV) of 3.1%.

Oxidative stress measurement

In order to determine the level of oxidative stress, lipid peroxidation product levels in plasma and left ventricular (LV) tissue collected at the time of sacrifice were measured by estimating the amount of malondialdehyde using the oxiselect thiobarbituric acid reactive substance (TBARS) assay kit (Cell Biolabs, San Diego, CA, USA) as described by us earlier by following the manufacturer's instructions (18). Values were expressed as nmol/mL for plasma and nmol/mg protein for LV tissue.

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities

Anti-oxidant enzyme status, activities of SOD, CAT, and

GPx was determined in LV tissue by using the Cayman chemical assay kits (Cayman Chemicals, Ann Arbor, MI, USA). LV tissue was homogenized in lysis buffer, centrifuged at 10,000 rpm, and supernatant was used for the assay. Protein concentrations of the samples were estimated using Biorad DCTM Protein Assay kit (Bio-Rad Laboratories, Mississauga, ON, Canada) and samples with equal amount of protein was used for the assay. For SOD, after addition of reaction mixture (supplied in the kit), samples were incubated for 20 min and absorbance was read at 450 nm using a 96-well microplate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany). For CAT and GPx, after the addition of reaction mixture, the absorbance was read at 540 nm. All assays were run in duplicate.

Statistical analyses

All data were analyzed by two-way analysis of variance (ANOVA) using SAS 9.3 (SAS Institute Inc., Toronto, ON, Canada) for main effects of diet and disease. Tukey test was used for the posthoc analyses. *P*-value <0.05 was considered statistically significant. Data were expressed as mean \pm standard error of the mean (SEM).

RESULTS

General characteristics

There was an independent effect of disease on body weights of diabetic rats. Body weights were significantly lower in diabetic rats when compared to non-diabetic rats. There was no effect of diet or an interaction between diet and disease. For blood glucose levels, there was significant main effect of disease. Blood glucose was increased by 5 fold when compared to non-diabetic rats. There was a significant interaction between diet and disease. Carrot supplementation significantly lowered blood glucose levels in diabetic rats (Table 2).

Cardiac structure and function

M-mode echocardiography analysis at 9 weeks post STZ administration showed that there was significant main effect of disease on normalized (to body weight) diastolic LVID, IVSD, and LVPWD. All three parameters were significantly increased in diabetic rats. There was no independent effect of diet or an interaction between diet and disease (Fig. 1). There was no main effect of diet or disease on EF (Fig. 2A). There was significant main effect of disease on CO. CO was significantly reduced in diabetic rats, when compared to non-diabetic rats. For IVRT, there was a significant main effect of disease. IVRT was significantly increased in diabetic rats. There was no significant main effect of diet or an interaction between diet and disease on CO and IVRT (Fig. 2B and 2C).

Table 2. Data showing changes in body weight and blood glucose level in control and diabetic rats supplemented with and without carrot powder

	Control	Control+Carrot	Diabetic	Diabetic+Carrot
Body weight (g)	467.56±24.0 ^a	494.44±29.38 ^a	339.88±11.65 ^b	364.47±7.96 ^b
Blood glucose (mmol)	6.52±0.6 ^c	7.63±0.80 ^c	34.70±1.37 ^a	29.23±2.88 ^b

Data are means±SEM, n=8~10 for body weight and n=3~5 for blood glucose. Different letters (a-c) indicate significantly ($P<0.05$) different from each other.

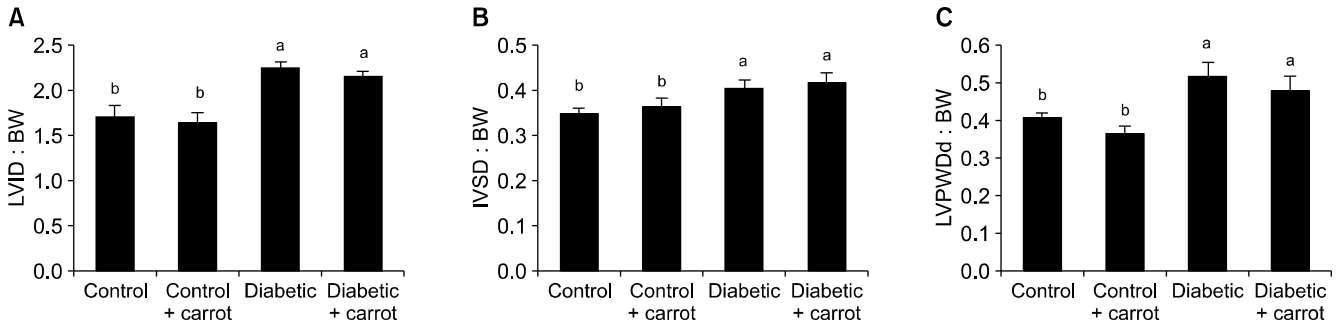


Fig. 1. Cardiac structural measurements of control and diabetic rats supplemented with and without carrot powder. All parameters are represented as ratio of body weight (BW). (A) Left ventricular internal diameter (LVID), (B) interventricular septal wall diameter (IVSD), and (C) left ventricular posterior wall diameter (LVPWD). Data are means±SEM (n=8~10). Different letters (a,b) indicate significantly ($P<0.05$) different from each other.

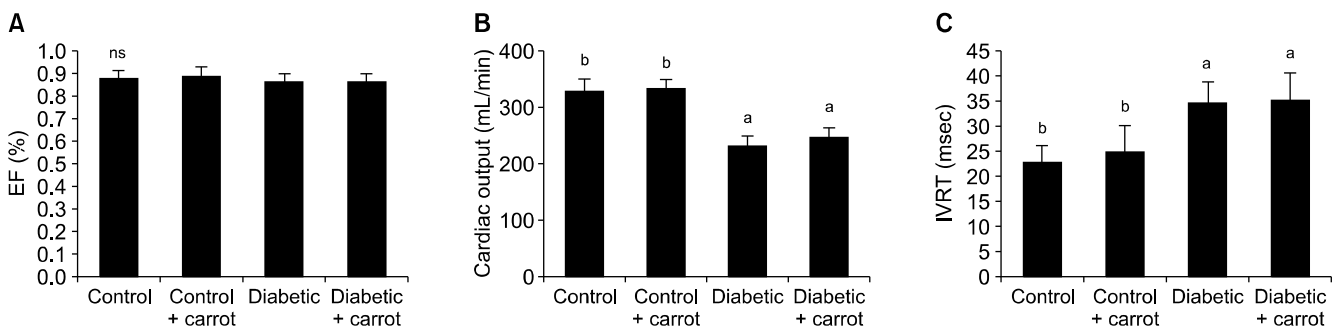


Fig. 2. Cardiac functional measurements of control and diabetic rats supplemented with and without carrot powder. (A) Ejection fraction (EF), (B) cardiac output (CO), and (C) isovolumic relaxation time (IVRT). Data are means±SEM (n=8~10). Different letters (a,b) indicate significantly ($P<0.05$) different from each other. ns, not significant.

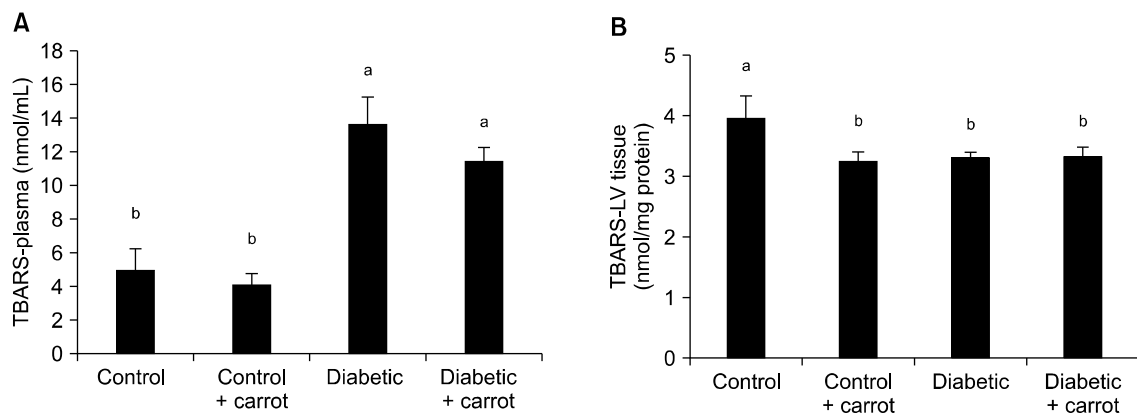


Fig. 3. Oxidative stress marker thiobarbituric acid reactive substance (TBARS) levels in control and diabetic rats supplemented with and without carrot powder. (A) TBARS levels in plasma samples. (B) TBARS levels in left ventricle (LV) tissue samples. Data are means±SEM (n=4~8). Different letters (a,b) indicate significantly ($P<0.05$) different from each other.

Oxidative stress biomarker

There was a significant main effect of disease on plasma

TBARS levels at 9 weeks post STZ administration. There was no independent diet effect or a diet disease inter-

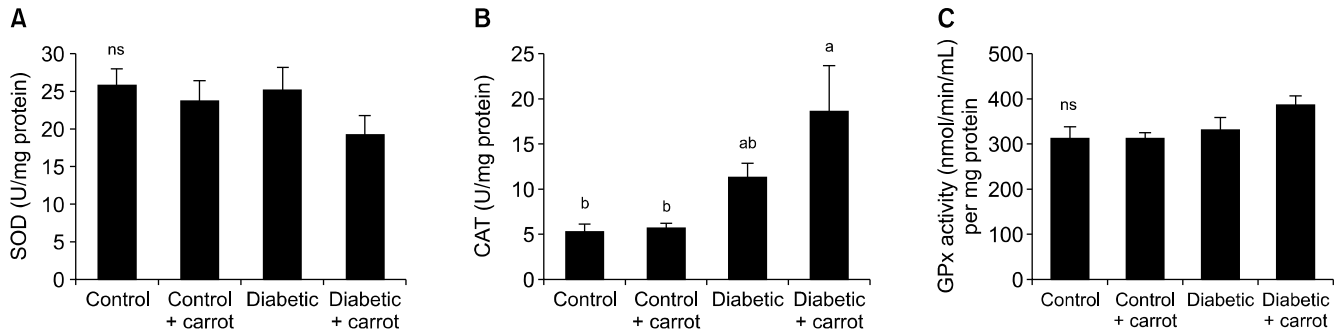


Fig. 4. Anti-oxidant enzyme activities in cardiac left ventricular tissue. (A) Superoxide dismutase (SOD), (B) catalase (CAT), and (C) glutathione peroxidase (GPx) activities in control and diabetic rats supplemented with and without carrot powder. Data are means \pm SEM (n=4~8). Different letters (a,b) indicate significantly ($P<0.05$) different from each other. ns, not significant.

action on plasma TBARS levels (Fig. 3A). Analysis of LV tissue showed that there was a significant main effect of disease. TBARS levels were not changed with disease or diet in LV tissues of diabetic rats (Fig. 3B).

Anti-oxidant enzyme activities

There was no effect of disease ($P<0.0553$) or diet on SOD activity levels (Fig. 4A). There was a significant main effect of disease on CAT activity levels. CAT activity levels were significantly increased in heart tissues of diabetic rats. No effect of diet or interaction between diet and disease was observed (Fig. 4B). There was no effect of disease or diet on GPx levels (Fig. 4C).

DISCUSSION

Dietary approaches have been recommended to prevent the initiation and progression of chronic diseases such as diabetes and cardiovascular disease (19-21). Many observational studies suggest that carotenoids may help prevent chronic diseases like cancer, cardiovascular disease, and UV radiation related skin and eye diseases by reducing oxidative damage (22-24). Diabetes is a metabolic disease that results in hyperglycemic condition that directly affects major organs like heart and eye. Type 1 diabetes characterized by the insulin deficiency and oxidative stress has been considered to be a major factor involved in the initiation and progression of cardiac abnormalities associated with diabetes (25). A recent study showed that mango peel rich in bioactives such as carotenoids reduced diabetic complications in type 1 diabetic rats (26). In addition, multiple anti-oxidant treatment (ascorbic acid, alpha tocopherol, acetate, β -carotene, N-acetyl cysteine, and selenium) for 12 weeks improved oxidative stress, apoptosis and ventricular function in similar diabetic rats (27). Herein, we report the effects of a unique dietary approach for prevention of diabetes induced the cardiac abnormalities.

For the first time, we examined the effects of dietary

supplementation of carrot powder on STZ-induced type I diabetes and cardiac abnormalities in rats. STZ is a glucosamine-nitrosourea that causes pancreatic β cell damage, insulin deficiency and subsequent cardiac impairment. Our results show that carrot powder supplementation resulted in a mild ($\sim 13\%$) but significant lowering of blood glucose levels in diabetic rats; prolonged treatments may have resulted in a better outcome. Therefore, this result indicates that carotenoid rich carrot powder has a potential to lower blood glucose levels in diabetic conditions. These results are consistent with a recent report that showed that non-fermented and fermented carrot juice supplementation resulted in a moderate reduction of blood glucose in a rat model of type 2 diabetes-low dose STZ-injected high fat fed rats (28). It should also be noted that there was a small increase (6.52 vs 7.63 mmol) in blood glucose level in control rats with carrot powder administration. However, this increase was not statistically significant. Nonetheless, future studies should look into this phenomenon to make sure longer term consumption doesn't increase blood sugar significantly.

Transthoracic echocardiography analysis showed that at 8 weeks STZ-induced type 1 diabetic rats developed characteristic cardiac impairments associated with diabetes, which is in consistent with earlier report (29). Although EF was unaffected, CO was significantly reduced in diabetic rats indicating that at 8 weeks these animals developed systolic dysfunction. Furthermore, diabetic rats had a significant prolongation of IVRT. Carrot powder treated diabetic rats showed no improvement in CO or lowering of IVRT compared to control diabetic rats. Diabetes induced cardiac functional defects are also associated with cardiac structural abnormalities (30). Diabetic rats developed LV dilatation and increase in LV wall thickness that was not prevented or arrested with carrot powder diet. This shows that in this experimental setting, carrot powder did not prevent the development of cardiac dysfunction and structural changes. The ineffectiveness of carrot powder to prevent cardiac abnor-

malties might be due to its lack of a strong effect in reducing the hyperglycemic state. It is possible that a prolonged treatment time or a higher concentration of carrot powder may have improved cardiac structure and function, and this possibility could be explored in future studies.

Increased ROS is a salient feature of diabetes, which has also been recognized to have direct effect on the myocardium leading to diabetic cardiomyopathy (6,31,32). In support of this view, many studies have reported that increased oxidative stress is involved in the pathogenesis and progression of diabetic cardiomyopathy (33,34). Increased oxidative stress has been attributed to the reduction in the functioning of endogenous anti-oxidant machinery (35), and anti-oxidant treatments have been reported to reduce diabetic cardiomyopathy in animal models (32). Anti-oxidant properties of carotenoids have been implicated in the prevention of heart disease (10, 11). Our results showed that STZ-injected animals did have increased oxidative stress, measured in plasma as TBARS levels; however, carrot powder supplementation did not significantly lower oxidative stress. This finding is similar to earlier studies where carrot juice failed to lower TBARS levels and β -carotene failed to reduce oxidative stress in metabolic syndrome model (36,37). However, analysis of LV tissue did not show any significant changes in TBARS levels in the diabetic group and carrot fed groups. Although, cardiac SOD and GPx levels were not changed, a trend towards increase observed in catalase activity with carrot powder supplementation in diabetic animals could be compensatory to increased oxidative stress in diabetic animals. An earlier study has shown that CAT activity increased with carrot supplementation in diabetic animals (28). However, a lack of strong antioxidant activity might be one of the reasons for the ineffectiveness of carrot powder supplementation to prevent the development and progression of cardiac complications in this animal model.

In summary, this study demonstrates that carrot powder supplementation prevented hyperglycemia in type 1 diabetic rats, but it had no impact on oxidative stress and cardiac abnormalities. Nevertheless, these findings suggest that the inclusion of carrot or carotenoid rich foods in the diets may help in blood glucose management in type I diabetic patients without causing any changes to their cardiovascular parameters.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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