Fenugreek potent activity against nitrate-induced diabetes in young and adult male rats

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Received: 14 September 2013 / Accepted: 12 February 2014 / Published online: 11 March 2014
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Abstract Nitrate has described as an endocrine disruptor that promotes onset of diabetes. This study was undertaken to evaluate diabetic effect of high nitrate intake in young and adult male rats and its amelioration by fenugreek administration. The study revealed significant increase in serum glucose and blood glycosylated hemoglobin (HbA1c%), while serum insulin and liver glycogen were decreased among nitrate exposed animals, in particular the young group. A significant reduction in the body weight gain and serum thyroid hormones (T4 & T3) was also recorded. Further reduction in serum levels of urea and creatinine, as well as total protein in serum, liver and pancreas was demonstrated, with elevation in their levels in the urine of all nitrate exposed groups. Meanwhile, the activity of serum transaminases (ALT and AST) was increased, with decline in their activity in the liver tissue. In addition, an elevation in serum total bilirubin, tissues (liver and pancreas) nitric oxide and lipid profile, as well as liver activity of glucose-6-

phosphatase was recorded. Fenugreek administration to nitrate exposed rats was found to be effective in alleviating hyperglycemia and other biochemical changes characterizing nitrate-induced diabetes. So, fenugreek can be considered to possess potent activity against onset of nitrate induced-diabetes.

Keywords Nitrate · Diabetes · Thyroid hormones · Nitric oxide · Fenugreek

Abbreviations

NC	Normal control
FG	Fenugreek
NT	Nitrate
NT + FG	Nitrate + fenugreek
NaNO ₃	Sodium nitrate
HbA1c%	Glycosylated Hb
T3	Triiodothyronine
T4	Thyroxine
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TL	Total lipids
TC	Total cholesterol
TG	Triglycerides
PLs	Phospholipids
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
VLDL-C	Very low density lipoprotein cholesterol
NO	Nitric oxide
G-6-Pase	Glucose-6-phosphatase
TCA	Trichloroacetic acid

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Introduction

Diabetes mellitus is one of the most serious health problems worldwide. It is a chronic metabolic disorder characterized by hyperglycemia, resulting from absolute or relative deficiency of insulin secretion or action (Abou-Seif and Youssef 2004). Recently, several studies indicated an increased incidence rate of diabetes all over the world and further added that genetic factors account for less than half of the diabetic cases (Ahamed and Banji 2012). Other studies reported that the rate of incidence varies widely across different geographic areas and is much higher in the developing countries, suggesting that environmental pollutants may account for the increased disease (Longnecker and Michalek 2000).

Regarding the role of environmental pollutants, it was indicated that nitrate exposure may be a causative factor in this context. Drinking water is the most common pathway through which population is exposed to nitrate (Van Maanen et al. 2000). Studies in this field provided evidence for the association between incidence of diabetes and nitrate in drinking water at concentrations above or less than WHO level of 50 mg/L (Van Maanen et al. 2000). In this concern, most of the studies have focused on the incidence of diabetes in the adult population (Ghasemi and Zahediasl 2013). However, more recent study has described the incidence of diabetes among children living in rural environments with high nitrate concentrations in drinking water (Marienfeld et al. 2007). Thus, a link between nitrate exposure and the risk of diabetes can be emphasized among population of different ages.

Nowadays, much attention has focused on the use of herbs such as fenugreek for the cure of number of diseases (Kaur et al. 2011). Fenugreek (*Trigonella foenum graecum*) is one of the oldest medicinal plants originating in India and North Africa regions (Shirani and Ganesharanee 2009). The Uses of fenugreek dates backed to ancient Egyptians, Greeks and Romans (Moosa et al. 2006). Fenugreek seeds are commonly used as condiment and seasoning in food preparation and are assumed to possess nutritive properties (Renuka et al. 2009). In addition, seeds were used as tonic and lactagogue (Basch et al. 2003) as well as for treatment of weakness and edema of legs (Yoshikawa et al. 1997). Several studies indicated the hypoglycemic and hypolipidemic properties of fenugreek seeds

(Renuka et al. 2009) suggesting that fenugreek may help to control diabetes (Basch et al. 2003).

Based on these observations, the present study was carried out to evaluate the relation between nitrate water pollution and incidence of diabetes among young and adult male rats. In addition to investigate the anti-diabetic effect of fenugreek on nitrate induced diabetic rats.

Materials and methods

Chemicals

Sodium nitrate (NaNO_3) was purchased from El-Gomhouria Co. for Chemicals (Mansoura, Egypt). All other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

This study was performed on young (3-weeks old) and adult (12-weeks old) male Wistar rats obtained from the Institute of Ophthalmic Disease Research, Cairo, Egypt. Rats were housed in ventilated cages maintained at 22–25 °C, with 12 h light/dark cycle and free access to standard food and water. A period of 2 weeks prior to the start of the experiment was allowed for acclimation of animals. All animals received human care in compliance with the guidelines of the Mansoura University, and the protocol conformed to the guidelines of the National Institutes of Health (NRC 1995).

Experimental diets

Fenugreek seeds (*Trigonella foenum graecum*) were purchased from a local herb market at Mansoura City, Egypt. Seeds were washed with clean water and dried in the open air. After drying, seeds were crushed using an electric grinder and the powder (50 g) was added to 950 g of standard diet (the standard diet was obtained from forages market in Mansoura, Egypt. It is composed of casein 15.0 %, starch, 67.0 %, corn oil 8.0 %, salt mixture 4.0 %, vitamin mixture 1.0 % and wood fibre 5.0 %) and mixed with little distilled water (Raju et al. 2001). The mixture was made into pellet form and dried, then kept in clean plastic containers.

The experimental diet was prepared once a week and kept below 5 °C.

Study design

After 2 weeks of acclimation, weight matched rats of each age, young (80 ± 5 g) and adult (160 ± 5 g), were randomly divided into four groups (six animals each) as follows: The first was considered as normal control (NC) group in which animals received standard diet without supplementation. In the second group, animals were fed on standard diet mixed with powdered fenugreek seeds as pellets at a dose of 5 % (w/w) (Raju et al. 2001). Animals of the third group, were fed standard diet and received sodium nitrate (NaNO_3) in drinking water at a dose of 550 mg/L to provide average daily dose of approximately 47.7 mg/kg b.w. (National Toxicology Program 2001). In the fourth group, rats were supplied with NaNO_3 plus fenugreek in the same way and doses, as described in the above groups. Rats were administered their respective doses daily for four months. The applied nitrate concentration in drinking water was chosen based on the previous experimental study for evaluating water nitrate toxicity (El-Wakf et al. 2009). The animals were weighed at the beginning and at the end of the experiment and obtained values were recorded for calculating the body weight gain.

Samples collection

At the end of the study period, rats were kept individually in metabolic cages for 24 h. Urine was collected, centrifuged and stored at -20 °C until analysis. Animals were overnight fasted then sacrificed under ether anesthesia by cervical dislocation. Two blood samples from each rat were collected into clean centrifuge tubes; the first was collected on EDTA for determination of glycosylated hemoglobin (HbA1c%), while the second was allowed to clot, then centrifuged at $855 \times g$ for 15 min at 4 °C and non hemolyzed sera were separated for hormonal and biochemical analysis.

Liver and pancreas were removed quickly, cleaned and cut into pieces. Samples from a known portion of the liver and pancreas were accurately weighed and homogenized for later biochemical analysis. Other specimens of liver were weighed and kept in trichloroacetic acid solution (TCA) 10 % for determination of glycogen content.

Biochemical analysis

Glycogen content was measured at 620 nm using anthrone reagent (Nicholas et al. 1956). Glucose-6-phosphatase (G-6-Pase) activity was assayed based on the incubation of a specific substrate with the enzyme and the determination of the liberated inorganic phosphorus at 640 nm (Rossetti et al. 1993). Serum insulin was estimated using the Diamond Diagnostic kit (Los Angeles, CA, USA) for immunoenzymatic assay as described by Temple et al. (1992), while serum triiodothyronin (T3) and thyroxin (T4) levels were estimated using kits supplied by Roche Elecsys (Indianapolis, IN, USA) (Klee 1996; Wada et al. 1997), respectively. Glycosylated hemoglobin (HbA1c%) was estimated using a Diamond Diagnostic kit (Gonen and Rubenstein 1978).

Biochemical parameters including, glucose, total lipids (TL), total cholesterol (TC), triglycerides (TG), phospholipids (PLs), high density lipoprotein cholesterol (HDL-C), urea, creatinine, total bilirubin (T. bilirubin), total protein (T. protein), transaminases {aspartate aminotransferase (AST) and alanin aminotransferase (ALT)}, and nitric oxide (NO) were determined using instructions of kits supplied by Bio-diagnostic Co. (Mansoura, Egypt). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated using the equations described by Friedewald et al. (1972), as follows: $\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$; $\text{VLDL-C} = \text{TG}/5$.

Statistical analysis

All data were statistically analyzed by one way analysis of variance (One-way ANOVA) and post comparison was carried out with LSD test, using SPSS statistical package, version 17.00 software. The results were expressed as mean $\pm$ SE and the values of $p \leq 0.05$ were considered statistically significant (Snedecor and Cochran 1980).

Results

As shown in Table 1, administration of fenugreek supplemented diet to normal rats of the two tested ages did not exhibit significant changes in the body weight gain, in comparison to normal control (NC) animals. However, chronic four months sodium nitrate (NaNO_3)

Table 1 Body weight gain (g) of control and different experimental groups of male rats

Tested parameters	Animal groups				Adult groups			
	Young groups							
	NC	FG	NT	NT + FG	NC	FG	NT	NT + FG
Initial body weight (g)	80.53 ± 0.17	80.86 ± 0.39 (+0.41)	80.24 ± 0.16 (−0.36)	80.00 ± 0.00 (−0.66)	160.76 ± 0.33	160.50 ± 0.30 (−0.16)	160.90 ± 0.32 (+0.09)	160.94 ± 0.66 (+0.11)
Final body weight (g)	201.68 ± 2.55	205.79 ± 3.00 (+2.04)	153.89 ^a ± 1.12 (−23.70)	180.63 ^{ac} ± 1.99 (−10.44)	230.00 ± 3.78	231.25 ± 3.50 (+0.54)	209.14 ^b ± 3.16 (−9.07)	219.37 ^{bd} ± 2.21 (−4.62)
Body weight gain (g)	121.15 ± 2.39	124.93 ± 2.63 (+3.12)	73.65 ^c ± 0.99 (−39.21)	100.63 ^{ac} ± 1.99 (−16.94)	69.24 ± 3.89	70.75 ± 3.33 (+2.18)	48.25 ^b ± 2.85 (−30.31)	58.44 ^d ± 1.75 (−15.60)

Values are mean ± SE of 6 animals for each group. The values in parentheses are percentage of change from control

Values bearing superscript are significantly different by ANOVA test at $p \leq 0.05$

NC normal control, FG fenugreek, NT Nitrate, NT + FG nitrate + fenugreek

^a Significant as compared to young control group

^b Significant as compared to adult control group

^c Significant as compared to young nitrate group

^d Significant as compared to adult nitrate group

exposure at dose (550 mg/L) induced significant reduction in the body weight gain in animals of both ages, in particular the young ones compared to their respective control groups. In contrast, feeding nitrate exposed animals on fenugreek diet helped to produce beneficial effect evidenced as significant increase in the body weight gain in all tested animals comparing to nitrate group of the same age.

Fenugreek administration to normal animals of the two ages significantly decreased serum levels of lipids (TL, TG, TC, PLs, LDL-C and VLDL-C), transaminases (ALT, AST) and total bilirubin, whereas serum total protein and HDL-C were increased compared to NC animals. However, no significant changes in serum levels of glucose, HbA1c%, insulin, T3, T4, urea and creatinine were recorded in the same fenugreek fed animals. On the other hand, nitrate exposed rats exhibited significant elevation in serum levels of glucose, HbA1c%, lipids (TL, TG, TC, PLs, LDL-C and VLDL-C), transaminases (ALT, AST), total bilirubin, urea and creatinine, while serum insulin, T3, T4, total protein and HDL-C were significantly decreased in animals of both ages, in particular the young ones compared to their respective control groups. In contrast, administration of fenugreek to all nitrate exposed rats tended to alleviate changes in the above mentioned parameters significantly, comparing to nitrate groups of the same age (Table 2).

As shown from the present findings, administration of fenugreek seed powder to normal rats of the two ages exhibited significant reduction in liver and pancreas levels of lipids (TL, TG, TC and PLs) and NO, accompanied with significant elevation in total protein in comparison to NC animals. Similar elevation in the glycogen content, but no significant changes in ALT, AST and G-6-Pase activities were recorded in the liver of the same group. However, nitrate exposure via drinking water tended to produce significant elevation in liver and pancreas levels of lipids (TL, TG, TC and PLs) and NO, while significant reduction in total protein content was observed in animals of both ages, in particular the young one compared to their respective control groups. Besides, nitrate exposure significantly reduced ALT, AST activities and glycogen content in the liver tissue; while an increase in G-6-Pase activity was recorded. Administration of fenugreek concomitantly with nitrate exposure succeeded to restore alterations in these parameters significantly in all animal groups comparing to their relative nitrate group (Table 3).

Table 2 Tested serum parameters in control and different experimental groups of male rats

Tested parameters	Animal groups		Adult group			
	Young group		NC			
	NC	FG	NT	NT + FG	FG	NT
Glucose (mg/dL)	107.29 ± 0.38	97.28 ^a ± 0.36 (−9.33)	179.68 ^a ± 1.85 (+67.47)	116.76 ^{ac} ± 0.51 (+8.83)	105.40 ± 1.25 (−5.00)	161.81 ^b ± 2.15 (+53.52)
Hb A1c %	3.07 ± 0.15	3.05 ± 0.08 (−0.65)	4.62 ^a ± 0.18 (+50.49)	3.27 ^c ± 0.03 (+6.51)	3.32 ± 0.09 (−0.60)	4.27 ^b ± 0.13 (+28.61)
Insulin (μU/mL)	1.38 ± 0.06	1.40 ± 0.01 (+1.45)	1.16 ^a ± 0.01 (−15.94)	1.30 ^{ac} ± 0.01 (−5.80)	1.34 ± 0.01 (−0.75)	1.15 ^b ± 0.003 (−14.18)
T3 (ng/mL)	1.11 ± 0.009	1.10 ± 0.3 (−0.90)	0.86 ^a ± 0.02 (−22.52)	1.08 ^c ± 0.004 (−2.70)	1.04 ± 0.005 (0)	0.97 ^b ± 0.01 (−6.73)
T4 (μg/dl)	6.86 ± 0.03	6.79 ± 0.07 (−1.02)	5.28 ^a ± 0.05 (−23.03)	6.23 ^{ac} ± 0.13 (−9.18)	3.96 ± 0.05 (−1.01)	3.27 ^b ± 0.03 (−17.42)
T. protein (g/dl)	7.35 ± 0.15	8.77 ^a ± 0.19 (+19.32)	4.68 ^a ± 0.11 (−36.33)	5.85 ^{ac} ± 0.12 (−20.41)	6.56 ± 0.10 (−10.98)	4.77 ^b ± 0.04 (−27.29)
T. bilirubin (mg/dL)	0.96 ± 0.02	0.75 ^a ± 0.01 (−21.88)	1.37 ^a ± 0.06 (+42.71)	1.06 ^{ac} ± 0.02 (+10.42)	0.89 ± 0.02 (−15.73)	1.14 ^b ± 0.03 (+28.09)
ALT (U/mL)	27.08 ± 0.71	23.65 ^a ± 0.38 (−12.67)	41.92 ^a ± 1.02 (+54.80)	30.46 ^{ac} ± 0.54 (+12.48)	28.85 ± 0.27 (−11.75)	41.21 ^b ± 0.83 (+42.84)
AST (U/mL)	54.31 ± 1.40	50.05 ^a ± 0.29 (−7.84)	66.49 ^a ± 1.25 (+22.43)	56.04 ^c ± 0.38 (+3.19)	57.98 ± 1.28 (−1.55)	68.92 ^b ± 1.29 (+18.87)
Urea (mg/dL)	26.38 ± 0.60	26.09 ± 0.73 (−1.10)	61.85 ^a ± 0.68 (+134.46)	34.73 ^{ac} ± 0.60 (+31.65)	31.93 ± 0.74 (−0.50)	54.55 ^b ± 0.70 (+70.84)
Creatinine (mg/dL)	0.72 ± 0.01	0.71 ± 0.008 (−1.39)	0.91 ^a ± 0.008 (+26.39)	0.78 ^{ac} ± 0.01 (+8.33)	0.85 ± 0.02 (−1.18)	0.96 ^b ± 0.01 (+12.94)
TL (mg/dL)	253.97 ± 2.31	217.82 ^a ± 4.31 (−14.23)	417.21 ^{ac} ± 7.23 (+64.28)	284.77 ^{ac} ± 1.17 (+12.13)	288.06 ± 2.37 (−9.03)	421.65 ^b ± 2.15 (+46.38)
TC (mg/dL)	57.57 ± 0.72	51.36 ^a ± 1.43 (−10.79)	80.61 ^a ± 1.67 (+40.02)	58.73 ^c ± 0.79 (+2.01)	64.53 ± 0.58 (−7.61)	81.60 ^b ± 0.79 (+26.45)
TG (mg/dL)	55.06 ± 1.09	42.41 ^a ± 1.17 (−22.97)	102.46 ^a ± 1.22 (+86.09)	59.11 ^{ac} ± 1.13 (+7.36)	55.24 ± 1.25 (−12.26)	81.32 ^b ± 1.11 (+47.21)
PLs (mg/dL)	74.94 ± 1.18	55.50 ^a ± 1.92 (−25.94)	136.19 ^a ± 2.97 (+81.73)	106.08 ^{ac} ± 0.54 (+41.55)	102.58 ± 2.62 (−12.50)	148.27 ^b ± 2.48 (+44.54)
HDL-C (mg/dL)	34.03 ± 0.62	38.94 ^a ± 1.14 (+14.43)	22.40 ^a ± 1.21 (−34.18)	31.90 ^c ± 0.81 (−6.26)	41.71 ± 0.33 (+11.12)	38.32 ^b ± 0.65 (−8.13)
						NT + FG 114.71 ^{bd} ± 1.74 (+8.83) 3.38 ^d ± 0.06 (+1.81) 1.28 ^{bd} ± 0.01 (−4.48) 0.93 ^{bd} ± 0.01 (−10.58) 3.80 ^d ± 0.09 (−4.04) 5.89 ^{bd} ± 0.15 (−10.21) 1.03 ^{bd} ± 0.03 (+15.73) 30.36 ^d ± 0.47 (+5.23) 58.88 ^d ± 0.97 (+1.55) 34.15 ^{bd} ± 0.76 (+6.95) 0.89 ^{bd} ± 0.01 (+4.71) 325.47 ^{bd} ± 6.43 (+12.99) 64.93 ^d ± 0.74 (+0.62) 56.53 ^d ± 0.79 (+2.34) 132.67 ^{bd} ± 2.02 (+29.33) 41.66 ^d ± 0.43 (−0.12)

Table 2 continued

Tested parameters	Animal groups		Adult group						
	Young group								
	NC		FG	NT	NT + FG	NC	FG	NT	NT + FG
LDL-C (mg/dL)	12.53 ± 0.56		7.00 ^a ± 0.32 (−44.13)	37.72 ^a ± 0.32 (+201.04)	15.00 ^{ac} ± 0.34 (+19.71)	11.43 ± 0.72	8.92 ^b ± 0.37 (−21.96)	27.02 ^b ± 1.25 (+136.40)	11.62 ^d ± 0.66 (+1.66)
VLDL-C (mg/dL)	11.01 ± 0.22		8.48 ^a ± 0.23 (−22.98)	20.49 ^a ± 0.24 (+86.10)	11.83 ^{ac} ± 0.23 (+7.45)	11.05 ± 0.25	9.69 ^b ± 0.22 (−12.31)	16.27 ^b ± 0.22 (+47.24)	11.30 ^d ± 0.16 (+2.26)

Values are mean ± SE of 6 animals for each group. The values in parentheses are percentage of change from control

Values bearing superscript are significantly different by ANOVA test at $p \leq 0.05$

NC normal control, FG fenugreek, NT nitrate, NT + FG nitrate + fenugreek

^a Significant as compared to young control group

^b Significant as compared to adult control group

^c Significant as compared to young nitrate group

^d Significant as compared to adult nitrate group

Feeding fenugreek diet to normal rats of both ages did not produce any significant changes in the levels of urine total protein, urea, creatinine and NO. However, nitrate exposure exhibited significant elevation in all these parameters in both tested age groups, in particular the young ones compared to their respective control groups. In contrast, feeding fenugreek diet to nitrate exposed animals significantly reduced the values of these parameters in all tested animals, if compared to nitrate group of the same age (Table 4).

Discussion

Recently, several investigators have described a possible relation between nitrate exposure and incidence of diabetes mellitus (Van Maanen et al. 2000; Marienfeld et al. 2007; Ghasemi and Zahediasl 2013). Diabetes mellitus is a pathologic condition resulting in severe metabolic imbalance. Hyperglycemia is a major contributor to such metabolic imbalance (Ananda et al. 2012). In this regard, the present study confirmed incidence of hyperglycemia in all tested rats following nitrate exposure especially among the young animals. This finding may be due to the individuals at early life having low gastric acidity, this leads to increased toxic substances including nitrite production that increased susceptibility to nitrate toxicity at this age (Gatseva et al. 1996).

In previous studies, nitrate- induced hyperglycemia has been attributed to the diminished utilization of glucose by tissues (Ragab and Ashry 2004). However, insulin deficiency could be a reason in this context. The etiology can be related to the fact that nitrate when ingested can be converted to nitrite in the gut. Gut bacteria have nitrite reductase activity that can generate nitric oxide (NO) from nitrite (Duncan et al. 1995). NO is a potent cellular signal used in a variety of regulatory physiological pathways. However, increased generation of NO can lead to tissue damage, which can be a direct effect mediated by NO itself or indirect effect mediated by reactive nitrogen species, such as peroxynitrite (ONOO)[−] produced by reaction of NO and superoxide anions (O₂)[−] (Beckman and Koppenol 1996). In diabetes, increased NO generation can lead to pancreatic β-cells apoptosis (Oyadomari et al. 2001) associated with insulin deficiency.

Insulin deficiency could be also related to nitrate—induced hypothyroid state. There is evidence that

Table 3 Liver and pancreas tested parameters in control and different experimental groups of male rats

Tested parameters	Animal groups		Adult group					
	Young group							
	NC	FG	NT	NT + FG	NC	FG	NT	NT + FG
Liver								
TL (mg/g wet tissue)	74.79 ± 1.58	65.55 ^a ± 2.27 (−12.35)	119.47 ^a ± 4.11 (+59.74)	87.77 ^{ac} ± 1.36 (+17.36)	79.06 ± 1.07	72.38 ^b ± 1.35 (−8.45)	111.75 ^b ± 1.83 (+41.35)	85.34 ^{bd} ± 1.36 (+7.94)
TC (mg/g wet tissue)	17.17 ± 0.28	13.27 ^a ± 0.41 (−22.71)	29.35 ^a ± 0.99 (+70.94)	21.48 ^{ac} ± 0.49 (+25.10)	17.15 ± 0.32	13.47 ^b ± 0.23 (−21.46)	23.17 ^b ± 0.46 (+35.10)	20.98 ^{bd} ± 0.36 (+22.33)
TG (mg/g wet tissue)	23.61 ± 0.93	19.10 ^a ± 0.30 (−19.10)	39.63 ^a ± 1.12 (+67.85)	29.46 ^{ac} ± 0.32 (+24.78)	25.76 ± 1.05	22.26 ^b ± 0.69 (−13.59)	39.17 ^b ± 1.02 (+52.06)	29.32 ^{bd} ± 0.35 (+13.82)
PLs (mg/g wet tissue)	28.83 ± 1.05	25.76 ^a ± 0.64 (−10.65)	44.08 ^a ± 1.36 (+52.90)	31.79 ^{ac} ± 0.66 (+10.27)	31.88 ± 0.87	28.95 ^b ± 0.63 (−9.19)	42.98 ^b ± 0.91 (+34.82)	32.38 ^d ± 1.13 (+1.57)
T. protein (g/g wet tissue)	1.49 ± 0.02	1.76 ^a ± 0.03 (+18.12)	1.29 ^a ± 0.02 (−13.42)	1.40 ^{ac} ± 0.01 (−6.04)	1.42 ± 0.02	1.56 ^b ± 0.02 (+9.86)	1.24 ^b ± 0.01 (−12.68)	1.34 ^{bd} ± 0.01 (−5.63)
ALT (μU/g wet tissue)	46.04 ± 0.23	45.88 ± 0.40 (−0.35)	40.19 ^a ± 0.30 (−12.71)	45.28 ^c ± 0.24 (−1.65)	75.02 ± 0.33	74.98 ± 0.27 (−0.05)	65.55 ^b ± 0.55 (−12.62)	74.33 ^d ± 0.48 (−0.92)
AST (μU/g wet tissue)	48.06 ± 0.30	47.91 ± 0.74 (−0.31)	41.33 ^a ± 0.49 (−14.00)	45.23 ^{ac} ± 0.27 (−5.89)	51.92 ± 0.78	51.73 ± 0.46 (−0.37)	45.58 ^b ± 0.42 (−12.21)	50.18 ^{bd} ± 0.60 (−3.35)
Glycogen (mg/g wet tissue)	6.58 ± 0.05	7.12 ^a ± 0.06 (+8.21)	5.86 ^a ± 0.06 (−10.94)	6.14 ^{ac} ± 0.07 (−6.69)	6.75 ± 0.07	7.05 ^b ± 0.04 (+4.44)	6.05 ^b ± 0.07 (−10.37)	6.30 ^{bd} ± 0.06 (−6.67)
G-6-Pase (μmol/min/g)	0.11 ± 0.004	0.10 ± 0.005 (−9.09)	0.26 ^a ± 0.008 (+136.36)	0.15 ^{ac} ± 0.003 (+36.36)	0.12 ± 0.005	0.12 ± 0.007 (0)	0.24 ^b ± 0.008 (+100)	0.14 ^{bd} ± 0.006 (+16.67)
NO (μmol/g wet tissue)	11.93 ± 0.13	10.67 ^a ± 0.23 (−10.56)	24.35 ^a ± 0.19 (+104.11)	19.06 ^{ac} ± 0.12 (+59.77)	10.95 ± 0.27	10.12 ^b ± 0.20 (−7.58)	15.58 ^b ± 0.41 (+42.28)	12.63 ^{bd} ± 0.45 (+15.34)
Pancreas								
TL (mg/g wet tissue)	121.96 ± 1.61	100.79 ^a ± 0.90 (−17.36)	197.05 ^a ± 1.96 (+61.57)	140.50 ^{ac} ± 0.35 (+15.20)	152.53 ± 0.60	126.99 ^b ± 0.71 (−16.74)	198.09 ^b ± 0.78 (+29.87)	155.62 ^{bd} ± 0.68 (+2.03)
TC (mg/g wet tissue)	15.68 ± 0.49	13.22 ^a ± 0.53 (−15.69)	36.64 ^a ± 1.35 (+133.67)	16.38 ^c ± 0.48 (+4.46)	16.28 ± 0.46	13.73 ^b ± 0.54 (−15.66)	28.39 ^b ± 0.91 (+74.39)	16.95 ^d ± 0.43 (+4.12)
TG (mg/g wet tissue)	71.93 ± 1.51	57.84 ^a ± 1.26 (−19.59)	104.28 ^a ± 3.11 (+44.79)	88.01 ^{ac} ± 1.94 (+22.36)	88.80 ± 2.36	80.51 ^b ± 1.88 (−9.34)	108.85 ^b ± 1.64 (+22.58)	92.12 ^d ± 1.94 (+3.74)
PLs (mg/g wet tissue)	31.75 ± 0.75	27.06 ^a ± 0.28 (−14.77)	50.27 ^a ± 0.47 (+58.33)	34.64 ^{ac} ± 0.58 (+9.10)	32.09 ± 0.30	28.95 ^b ± 0.63 (−9.78)	49.18 ^b ± 0.72 (+53.26)	32.28 ^d ± 0.19 (+0.59)

Table 3 continued

Tested parameters	Animal groups							
	Young group				Adult group			
	NC	FG	NT	NT + FG	NC	FG	NT	NT + FG
T. protein (g/g wet tissue)	2.33 ± 0.04	3.20 ^a ± 0.18 (+37.34)	0.86 ^a ± 0.04 (−63.09)	2.03 ^{ac} ± 0.09 (−12.88)	2.03 ± 0.10	2.78 ^b ± 0.13 (+36.95)	0.91 ^b ± 0.06 (−55.17)	1.80 ^d ± 0.07 (−11.33)
NO (μmol/g wet tissue)	11.96 ± 0.19	10.28 ^a ± 0.09 (−14.05)	14.76 ^a ± 0.31 (+23.41)	12.32 ^c ± 0.39 (+3.01)	11.87 ± 0.04	10.33 ^b ± 0.10 (−12.97)	14.30 ^b ± 0.25 (+20.47)	12.19 ^d ± 0.11 (+2.70)

Values are mean ± SE of 6 animals for each group. The values in parentheses are percentage of change from control

Values bearing superscript are significantly different by ANOVA test at $p \leq 0.05$

NC normal Control, FG fenugreek, NT nitrate, NT + FG nitrate + fenugreek

^a Significant as compared to young control group

^b Significant as compared to adult control group

^c Significant as compared to young nitrate group

^d Significant as compared to adult nitrate group

nitrate (via increased NO production) may inhibit iodide transport into thyroid gland, causing decreased iodide accumulation into the gland coupled with hypothyroid state (Costamagna et al. 1998). The latter is proposed to directly affect insulin secretion from β -cells and to cause diabetes. Accordingly, the present findings of decreased thyroid hormones (T4 and T3), together with increased NO levels in pancreas and urine are suggested to be etiologic factors in occurrence of diabetes among nitrate exposed rats of both ages. Onset of diabetes was confirmed by increased level of serum glucose and HbA1c%, coupled with decreased serum insulin level in all nitrate exposed rats. The study also showed increased hepatic G-6-Pase activity that responsible for liberation of glucose from glycogen (Schaftingen and Gerin 2002). So, levels of serum glucose increase and hepatic glycogen decrease, indicating disturbance of carbohydrate metabolism following nitrate exposure. However, all recorded disturbances seemed higher in the young group, whose nitrate exposure starts earlier in life. A finding which goes in harmony with prior research indicating that individuals at early life having low gastric acidity that favors conversion of nitrate to more toxic substances being linked to increased susceptibility to nitrate toxicity at this age (Gatseva et al. 1996).

Another related finding was a reduction in the body weight gain, as seen herein and in previous trials (Al-Ayed 2000). The possible explanation is that nitrate can exert its effect on the body weight through increasing protein catabolism. Urea is the principle end product of protein catabolism. Therefore, the increased urea concentration in both serum and urine, with decreased serum and tissue protein may indicate enhanced protein catabolism associated with weight loss.

In parallel, a recent study demonstrated several lipid alterations in nitrate induced diabetes (El-Wakf et al. 2011). The mechanism behind this may be related to increased mobilization of fatty acids from peripheral deposits due to lack of insulin, since insulin normally inhibits lipolysis (Al-Shamaony et al. 1994).

Further metabolic changes have also been reported which seemed to occur via liver and kidney dysfunction associated with nitrate exposure (Zaki et al. 2005). Liver dysfunction is reflected by elevation in serum bilirubin concentration, as well as ALT and AST serum activity, with a decline in their activity in the liver tissue. Bilirubin is a metabolic breakdown

Tested parameters	Young group		Adult group	
	NC	NT	NC	NT
T. protein (g/dl)	0.004 ± 0.00	0.17 ^a ± 0.003 (+41.50)	0.004 ± 0.00	0.15 ^b ± 0.009 (+36.50)
Urea (g/dl)	31.50 ± 0.54	46.85 ^a ± 0.65 (+48.73)	35.50 ± 0.30	41.61 ^b ± 0.70 (+17.21)
Creatinine (mg/mL)	5.51 ± 0.33	12.56 ^a ± 0.36 (+127.95)	9.32 ± 0.11	13.59 ^b ± 0.44 (+45.82)
NO (μmol/L)	12.87 ± 0.31	11.76 ± 0.25 (-8.62)	12.30 ± 0.32	21.61 ^b ± 0.76 (+75.69)

Values bearing superscript are significantly different by ANOVA test at $p \leq 0.05$

^d Significant as compared to adult nitrate group

Other mechanisms may be suggested via inhibiting carbohydrate metabolic enzymes such as G-6-Pase, as shown herein and in earlier studies (Raju et al. 2001). Furthermore, the hypoglycemic action may be related to the high amounts of dietary fibers, as the seeds contain around 50 % pectin that forms a colloid suspension when hydrated, and this can decrease the

rate of gastric emptying and slow glucose absorption from small intestine (Buyken et al. 1998). Another aspect of fenugreek benefits is its ability to increase the body weight gain which could be mediated via better utilization of nutrients in the diet (Xue et al. 2007) and the normalized serum and tissue protein, as seen herein.

Additionally, fenugreek seeds have shown to lower serum and tissue lipids (TL, TG, TC and PLs). Fenugreek hypolipidemic action may be an outcome of the achievement of normal glucose level which may reduce degradation of already accumulated lipids and inhibit lipolysis. Besides, fenugreek contains the steroidal saponins that are transformed in the gastro-intestinal tract to sapogenins which increase biliary cholesterol excretion, leading to lowered cholesterol levels (Stark and Madar 1993).

Along with this, fenugreek has also shown to prevent alterations in the liver and kidney function associated with diabetes (Devi et al. 2003). This can be confirmed by the present results showing normalized level of bilirubin and activity of AST and ALT, as well as level of creatinine and protein urea following fenugreek administration. A finding which may be an outcome of maintaining normal levels of glucose and lipids by fenugreek, thereby inhibiting the cytotoxic effects linked to elevations in both glucose and lipids.

In conclusion, fenugreek seeds powder seemed to possess antidiabetic activity, as evidenced by improving alterations in glucose, HbA1c%, insulin and lipids, as well as other diabetic aspects induced by nitrate. Thus, indicating the efficacy of feeding fenugreek seeds to maintain normal metabolic homeostasis in the present diabetic state and probably in other diabetic models. As a result, fenugreek can be considered as a good candidate for future studies on diabetes.

Acknowledgments The authors appreciate the financial support from Faculty of Science; Mansoura University; Egypt.

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