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Potential Health Benefits of Bread Supplemented with Defatted Flaxseeds under Dietary Regimen in Normal and Type 2 Diabetic Subjects

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Flaxseed is a rich source of dietary fibers and lignans. Uncontrolled diabetes may induce complication represented by dyslipidemia, high oxidative stress and kidney dysfunction. In the present research the beneficial effect of two months consumption of bread supplemented with defatted flaxseeds (DFB) together with dietary regimen was evaluated in normal and type 2 diabetic patients. Protective effect towards diabetic complications was studied through evaluation of plasma lipid profile, lipid peroxidation, liver and kidney function. The nutritional status of normal and diabetic patients was evaluated through assessing body mass index and nutrients' intake. Results showed reduction of body mass index in both normal and diabetic patients after nutritional intervention. Analysis of mean dietary intake of normal and diabetic patients in the beginning of the study revealed that all subjects were hyper-caloric that reduced to be 79% and 84% from RDA, respectively after treatment. After two months of supplementation with DFB, diabetic patients showed significant reduction of plasma glucose. Both diabetic patients and normal subjects showed significant improvement in plasma lipid profile and significant reduction of lipid peroxidation. Consumption of DFB in addition of dietary regimen may be helpful in preventing diabetes and its complications.

INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disorder characterised by high levels of blood glucose due to insulin resistance and impaired insulin secretion [Whitmore, 2010]. Diabetes mellitus (DM) is affecting large number of individuals worldwide. DM is a prevalent systemic disease with well documented devastating effects [Duckworth, 2001]. Hyperglycemia has been found to play a key role in reactive oxygen species (ROS) generated damage [Ugochukwu *et al*., 2003; Maritim *et al*., 2003]. ROS are formed disproportionately in diabetes by glucose oxidation, non-enzymatic protein glycation and the subsequent oxidative degradation of glycated proteins [Maritim *et al*., 2003]. Many scientific reports indicate that diabetic complications are associated with overproduction of ROS and accumulation of lipid peroxidation by-products [Palanduz *et al*., 2001]. In diabetes, major damage occurs in tissues such as kidney where the entry of glucose is not regulated by insulin [Limaye *et al*., 2003]. Free radicals generated in diabetes may lead to several kinds of diabetic complications including nephropathy, neuropathy, cardiopathy and many other diseases. Dyslipidemia has been considered as one of the complications of diabetes [Goldberg, 2001], which may enhance cardiovascular diseases. Current approaches to diabetes therapy involve mainly drugs enhancing insulin secretion or signaling as well as inhibiting endogenous glucose production [Anuradha & Selvam, 1993]. Dietary intervention, particularly the use of dietary regimen and bioactive ingredients derived from natural sources, is a mainstay in the management of diabetes.

Various dietary sources are presently receiving considerable attention across the world for the potential health benefits in relation to many diseases such as diabetes mellitus and diabetic disorders. Among them, flaxseed (*Linum usitatissimum* L., Family Linaceae) is becoming one of the traditional health food. Defatted flaxseed contains high levels of dietary fibers and phytochemicals such as lignans [Vijaimohan *et al*., 2006]. Flaxseed has been reported to possess antioxidant properties [Simopoulos, 1991] against various diseases, including atherosclerosis, diabetes, hypertension, chronic inflammation, and pre-cancerous stage [Fukuda *et al*., 1985]. In previous study we evaluated the effect of bread enriched with whole or defatted flaxseeds in hyperlipidemic rats which proved improvement of lipid profile [Mohamed *et al*., 2005]. Also Makni *et al*. [2008 & 2010] reported that flax and pumpkin seeds mixture supplemented in diets possesses free radical scavenging activity in hypercholesterolemic and diabetic rats and is helpful in preventing diabetes and its complications. The results from our previous study [Mohamed *et al*., 2005] encourage us to evaluate the beneficial effects of bread supplemented with defatted flaxseeds in normal subjects and type 2 diabetic pa-

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tients. So, the present work was established to study the protective role of flaxseed bread in normal subjects and its reducing ability of diabetic complications.

MATERIALS, SUBJECTS AND METHODS

Materials

Flaxseeds Giza 8 were obtained from Field Crops Research Institute, Agriculture Research Centre, Egypt.

Raw materials applied in the study included: flour (72% extraction), common salt, bakery shortening, and instant dry yeast.

Subjects

The subjects under study were sixteen men and women; eight normal healthy subjects and eight type 2 diabetic patients. Their age ranged from 40 to 59 years (average: 48.3±5.121, as mean ± SD).

Flaxseed preparation

Flaxseeds (Giza 8) were defatted by petroleum ether 40–60°C using a Soxhlet apparatus. Residual solvent was evaporated from defatted flaxseed using hot air oven at 40°C.

Optimization of bread formulation

Defatted flaxseeds (Giza 8) were grinded and added as 10% levels on the expense of flour, to make bread according to Mohamed *et al*. [2005].

Design of the clinical study (intervention study)

This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt. Subjects were divided into two groups. The first group comprised type 2 diabetic patients and the second included normal healthy subjects. Each group contained eight subjects. Each subject was given 200 g of DFB per day in replacement of his/her ordinary consumed bread. The study continued for 2 months. Nutritional status of all normal and diabetic patients was assessed through anthropometric measurements and nutrients' intake before and after dietary intervention. Biochemical analysis of blood was carried out at the start and end of the study.

Anthropometric measurements

Body weight and height were measured. Body mass index (BMI) was calculated according to Bray [1998]: BMI (kg/m^2) = Weight / Squared Height.

Food intake

Normal subjects and diabetic patients were subjected to questionnaire for one-day dietary recall, in addition to frequency of food items consumed to determine the daily nutrient intake. Analysis of intake of protein, fat, carbohydrates, calories, zinc, iron and vitamin E and C per day was carried out using the computer program (World Food Dietary Assessment). The adequacy of nutrient intake was evaluated as percent of RDA [FAO/WHO, 1989]. After the questionnaire, normal subjects and patients were advised to reduce calories and carbohydrates intake, substitute saturated fats, purified flour and its products and full milk and milk products by unsaturated fats, whole cereals and cereal products, and skimmed milk and low fat milk products; respectively and to take daily reasonable amount of fresh vegetables and fresh fruits (low in total sugar). At the end of the study another questionnaire for one-day dietary recall was taken from all subjects. Different nutrient intake of subjects at the end of the study was compared with that at the start.

Biochemical analysis of blood

Blood samples were obtained from fasted subjects at the start and after dietary intervention. The blood samples were mixed with heparin for separation of plasma and determination of plasma glucose [Trinder, 1969], plasma total lipids [Toro & Ackerman, 1975], total cholesterol (T-Ch) [Watson, 1960], high density lipoprotein cholesterol (HDL-Ch) [Burstein *et al*., 1970], low density lipoprotein cholesterol (LDL-Ch) [Schriewer *et al*., 1984] and triglycerides (TGs) [Megraw *et al*., 1979]. T-Ch/HDL-Ch ratio was calculated. Plasma malondialdehyde was determined [Satoh, 1978] as indicator of lipid peroxidation and oxidative stress. Plasma level of creatinine [Houot, 1985] was assessed as a kidney function test while the activity of aspartate transaminase (AST) and alanine transaminase (ALT) [Reitman & Frankel, 1957] was estimated as liver function tests. Postprandial plasma glucose [Trinder, 1969] was determined in both groups. The biochemical parameters of patients at the start of the study were compared with those of the healthy normal subjects. Also, biochemical parameters of normal subjects and patients were compared before and after dietary intervention.

Statistical analysis

Data was analysed using Student's t-test (2-tailed).

RESULTS AND DISCUSSION

Assessment of nutritional status was carried out through anthropometric parameters (Table 1) and food intake (Table 2). Body mass index (BMI) (Table 1) revealed that all normal subjects and type 2 diabetic patients were moderately obese. After two months of dietary intervention, BMI was reduced in normal and diabetic patients significantly but they were still overweight.

Mean dietary intake of normal subjects and diabetic patients (Table 2) at the beginning of the study revealed that normal subjects and diabetic patients were hyper-caloric than RDA (107% and 116%, respectively). Protein intake was noticed to be more than RDA. All micro-nutrients (vitamin E, vitamin C, iron and zinc) were lower than RDA except for zinc which was higher than RDA (103%) in healthy subjects; the values for diabetics were lower than that for the normal subjects.

Mean dietary intake of normal subjects and diabetic patients (Table 2) after two months of the study revealed that all normal subjects and diabetic patients reduced their caloric intake to be 79% and 84% from RDA, respectively. Protein intake was reduced but still higher than RDA (143% and 142 % in normal subjects and diabetic patients, respectively). It was

TABLE 1. Anthropometric parameters of normal subjects and diabetic patients before and after 2 months of dietary supplement with flaxseed bread.

Groups	Before (Mean ± SE)				After (Mean ± SE)			
	Age (years)	Weight (Kg)	Height (m)	BMI (kg/m^2)	Age (years)	Weight (Kg)	Height (m)	BMI (kg/m^2)
Normal subjects (n= 8)	46.5±1.51	81±2.56	1.62±0.016	31.0±0.896	46.5±1.51	74.3±2.17	1.62±0.016	28.3*±0.779
Diabetic patients (n= 8)	50.1±1.95	88.9±3.22	1.65±0.35	32.5±0.643	50.1±1.95	82.8±3.12	1.65±0.353	30.4*±0.651

Values significantly different compared to values before treatment: *: $p<0.05$.

TABLE 2. Mean dietary intake of different nutrients at the end of the study.

Parameters	Normal subjects Before		Normal subjects After		Diabetic patients Before		Diabetic patients After		RDA*
		% RDA		% RDA		% RDA		% RDA	
Energy (Kcal)	2517.7±42.746	107	1868.2±22.452	79	2736.2±67.002	116	1964.8±34.623	84	2350
Carbohydrate (g)	385.5±10.643	-	243.5±12.570	-	406.2±15.373	-	260.9±13.349	-	-
Protein (g)	82.6±5.101	146	80.9±9.589	143	87.5±4.519	155	80.0±6.179	142	56.5
Animal protein	35.8±6.696	-	52.9±11.465	-	42.3±5.323	-	44.1±7.449	-	-
Fat (g)	71.7±4.629	-	63.4±4.692	-	84.6±5.829	-	66.8±4.588	-	-
Saturated Fat	35.1±2.734	-	21.3±3.036	-	42.3±4.714	-	21.5±2.386	-	-
Monounsaturated Fat	22.7±1.831	-	21.5±1.782	-	27.4±2.025	-	23.0±1.815	-	-
Polyunsaturated Fat	13.9±1.201	-	20.6±1.184	-	14.9±2.818	-	22.3±1.897	-	-
Vit. E (mg α-tocopherol)	7.2±0.402	80	8.7±0.879	97	3.3±0.577	37	5.1±0.739	57	9
Vit. C (mg)	48.3±3.579	81	66.3±2.220	111	26.4±6.550	44	64.0±3.157	107	60
Iron (mg)	8.0±1.090	64	9.2±0.939	74	7.7±0.902	62	8.9±0.907	71	12.5
Zinc (mg)	13.9±0.589	103	14.0±0.585	104	11.1±0.796	82	12.2±0.551	90	13.5

* RDA according to Food and Nutrition Board, National Research Council Recommended Dietary Allowances [FAO/WHO, 1989].

noticed that all subjects in the study followed the dietary advice through increasing the intake of polyunsaturated fatty acids and reducing saturated fat. All subjects in both groups showed increase in minerals and vitamins' intake as a result of dietary advice.

Biochemical parameters of normal subjects and diabetic patients before and after supplementation with DFB together with dietary regimen are present in Table 3. As expected, plasma glucose levels in the diabetic patients were significantly higher in comparison to the normal subjects at the start of the study. Administration of DFB with dietary regimen resulted in significant decrease of glucose level in diabetic patients whereas no significant change was detected in normals. Compared to the starting level the reduction in plasma glucose level in diabetic patients was -14%. Postprandial glucose level in diabetic patients showed significant reduction (-15%) after supplementation with flaxseeds bread and dietary regimen when compared with that of the starting level.

Plasma total lipid, total cholesterol, triglycerides, LDL-Ch and T-Ch/HDL-Ch ratio showed significantly higher levels with different degrees in diabetic patients when compared with normal subjects before dietary treatment. Normal subjects and diabetic patients showed significant reduction in plasma level of plasma total lipid, total cholesterol, triglycerides, LDL-Ch and T-Ch/HDL-Ch ratio with different levels after consumption of DFB in comparison to their starting levels. Plasma level of HDL-Ch was significantly lower in diabetic patients when compared with normal subjects at the starting level. Significant elevation in plasma level of HDL-Ch was observed in normal subjects and diabetic patients after supplementation with DFB and dietary regimen when compared to their respective starting levels.

A significant higher plasma level of MDA (20%) was observed in the diabetic patients compared to those of the normal subjects at the start of the study. Supplementation with flaxseeds bread and dietary regimen induced a significant decrease of MDA levels in plasma by 25% and 16% in normal and diabetic patients, respectively.

Liver and kidney function tests showed non-significant change in diabetic patients at the start of the study when compared with normal subject and also at the end of the study when compared with the starting level.

Diabetes mellitus is an increasing world health problem; particularly the prevalence of type 2 diabetes has assumed epidemic dimensions in Western industrialized societies. It is mainly the environmental, dietary and lifestyle behavioral factors that are the control keys in the progress of this disease [Pegklidou *et al*., 2010]. Several epidemiological studies have linked over nutrition and lack of physical activity with type 2 diabetes. Indeed, the excessive consumption of energy dense foods as source of carbohydrates and fats along with ineffective medical management has negative impact on controlling blood glucose levels and on insulin response. This usually leads to a hyperglycemic state, which is associated with the development of the devastating secondary complications [Pegklidou *et al*., 2010].

TABLE 3. Biochemical changes of normal subjects and diabetic patients before and after 2 months of dietary supplement with flaxseed bread.

Parameters	Normal subjects (n= 8) (Mean ± SE)		Diabetic patients (n= 8) (Mean ± SE)	
	Before	After	Before	After
Fasting glucose (mg/dL)	92.9 ± 2.41	90.1 ± 2.534	137.6*** ± 3.39	117.9[a] ± 2.29
% Change		-3		-14
Postprandial glucose (mg/dL)	110.2 ± 2.56	108.7 ± 2.74	202.2*** ± 3.53	170.9[a] ± 4.39
% Change				-15
Total lipids (mg/dL)	596.8 ± 8.12	499.5*** ± 10.76	824.1*** ± 10.53	723.9[a] ± 9.20
% Change		-16		-12
Total cholesterol (mg/dL)	165.4 ± 6.96	142.9* ± 6.01	206.1*** ± 5.57	167.2[a] ± 4.90
% Change		-14		-19
HDL-cholesterol (mg/dL)	48.6 ± 0.924	53** ± 0.981	43*** ± 0.597	46.4[b] ± 0.777
% Change		9		8
LDL-cholesterol (mg/dL)	98.9 ± 5.94	76.5* ± 5.959	134.1*** ± 5.42	99.7[a] ± 4.13
% Change		-23		-26
Total cholesterol/ HDL-cholesterol	3.3 ± 0.202	2.7* ± 0.107	4.8*** ± 0.143	3.61[a] ± 0.124
% Change		-18		-25
Triglycerides (mg/dL)	101.5 ± 4.67	82.2** ± 4.14	137.5*** ± 2.71	114.8[a] ± 3.27
% Change		-19		-17
MDA (mmol/L)	16.6 ± 0.939	12.5** ± 0.779	19.9*** ± 0.359	16.8 [a] ± 0.465
% Change		-25		-16
AST (IU/L)	9.8 ± 1.03	7.3 ± 0.745	10 ± 0.756	9 ± 0.654
% Change		-26		-10
ALT (IU/L)	5.5 ± 0.732	5 ± 0.654	7.5 ±1.12	7.5 ± 0.499
% Change		-9		0
Creatinine (mg/dL)	0.831 ± 0.015	0.791 ± 0.019	0.875 ± 0.016	0.86 ± 0.015
% Change		-5		-2

Values significantly differ from normal initial values: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Values significantly differ from before values of patients: a: $p<0.001$, b: $p<0.005$.

Dietary guidelines are very important for people with diabetes mellitus to improve health quality and to maintain blood glucose levels at normal range so as to reduce the risk for diabetes complications. In this research, supplementation of DFB together with nutrition advices was applied for the management of diabetes type 2 and the prevention of its complications and also for protection of normal subjects from occurrence of hyperlipidemia and hyperglycemia.

The present results showed that DFB along with dietary regimen exhibited significant reduction in fasting and postprandial plasma glucose and improvement of plasma lipid profile in type 2 diabetic patients and significant improvement of lipid profile in normal subjects.

Type 2 diabetes mellitus is one of the most common human metabolic diseases, and derangements in lipid metabolism in diabetic patients are often important determinants of the course and status of the disease [Stolar, 2011]. The increase in plasma lipids levels in diabetic patients are associated with increase in the risk factor for coronary heart diseases [Al-Shamaony *et al*., 1994]. A decrease of serum lipid concentration through drug therapy or dietary measures seems to decrease the risk of vascular diseases [Rhoads *et al*., 1976]. High levels of total lipids, TG, T-Ch, LDL-Ch and T-Ch/HDL-Ch in diabetic patients were observed in the present study when compared to normal healthy subjects before dietary treatment. Abnormalities in insulin action and not hyperglycemia *per se* are associated with dyslipidemia in type 2 diabetes. Several factors are likely to be responsible for diabetic dyslipidemia; insulin effects on liver apoprotein production, regulation of lipoprotein lipase, actions of cholesteryl ester transfer protein and peripheral actions of insulin on adipose and muscle [Goldberg, 2001]. Administration of DFB with dietary regimen for two months improved lipid profile in diabetic patients and normal healthy subjects. Dietary intervention not only lowered the T-Ch, TG and LDL-Ch but also elevated the HDL-cholesterol which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed ‘‘reverse cholesterol transport”, and is considered to be a cardio protective lipid and anti-atherogenic agent [Shah *et* al., 2001; Young, 2005]. It has been reported that the anti-atherogenic effect of HDL-Ch is due to its capacity to inhibit LDL oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL [Assmann & Nofer, 2003]. The increase in HDL-Ch levels, observed in the present study, might be due to the stimula-

tion of pre-β HDL-Ch and reverse cholesterol transport, as demonstrated by Gupta *et al*. [1993].

In the present study, the elevation of lipid peroxidation in the plasma of diabetic patients (represented by high MDA) compared with normal subjects before dietary treatment suggested exposure of tissue and membranes to damage and the failure of antioxidant defense mechanisms to combat free radicals [Amresh *et al*., 2007]. The reduction in intake of the antioxidant (vitamins E and C) in all subjects and zinc in diabetic patients at the beginning of the study may participate in the increased oxidative stress that may lead to complications. Supplementation of DFB under dietary regimen significantly reduced MDA thereby may reduce tissue damage.

The role of DFB in reducing plasma glucose and MDA and improvement of lipid profile in the present study might be due to dietary fibers (gum), proteins and lignans contents of flaxseed. Flaxseed have been reported to possess antioxidant activity and the ability to lower blood glucose, serum lipids, serum total cholesterol, LDL-Ch and triacylglycerol while increasing serum HDL-Ch [Prasad, 2008]. Flaxseed gum has an important role in the management of hyperglycemia and hypercholesterolemia in humans [Oomah & Mazza, 2000] which might be due to its ability to reduce absorption of glucose and lipids from the gastrointestinal tract.

The hypocholesterolemic effect of plant protein has been proved previously [Atwal *et al*., 1997]. The major protein isolated from flaxseed has high contents of the amino acids arginine, glutamate/glutamine, and aspartate/asparagines [Chung, 2001]. Food sources rich in arginine have been reported to have potential preventative functions against heart disease [Pszczola, 2000].

Flaxseed is a rich source of lignans, which are converted by gut bacteria into the bioactive mammalian lignans enterolactone and enterodiol with a potent antioxidant activity [Prasad, 2005] that may result in reduction of MDA in the present study. Lignans may block androgen or progesterone receptors, thereby may alter cardiovascular disease risk by changing HDL-cholesterol metabolism [Thompson *et al*., 1989]. Flaxseed intake caused a reduction in LDL oxidation in obese adults with insulin resistance [Jenkins *et al*., 1999]. Lignan significantly prevented or delayed the onset of diabetes and improved glycemic control in rats with type 1 [Prasad, 2000] and type 2 diabetes [Prasad, 2001]. Reduction in body mass index and improvement of different biochemical parameters after dietary intervention may also be ascribed to reduction in caloric intake, saturated fat and increase in polyunsaturated fatty acids intake, in addition of the fibers content of flaxseed.

CONCLUSION

Supplementation of normal subjects and diabetic patients with DFB together with dietary advice improved plasma lipid profile, reduced plasma glucose and oxidative stress in diabetic patients and has potential protective effect on normal subjects against occurrence of hyperlipidemia, hyperglycemia and oxidative stress. The dietary treatment also produced reduction in BMI.

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