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Effect of High Protein Diet Containing Fortified Bread with Fenugreek and *Nigella sativa* Seeds on Rats Suffering from Diabetes

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Abstract: The present work was conducted to study the effect of high protein diet containing fortified bread with two levels from fenugreek seeds (FS), *Nigella sativa* seeds (NSS) and combination of them on the nutritional value, percent of liver weight/body weight, lipid parameters, kidney and liver functions, serum glucose and thyroid hormones of rats suffering from diabetes. Sixty male albino rats (150-165 g) were used in this study. After a period of adaptation the rats were divided into two main groups. The first main group (12 rats) divided into two subgroups (n = 6), these subgroups were fed on normal and high protein diets (14 and 20% protein) containing 250 g un-fortified bread, respectively, as control negative groups. While the second main group (48 rats) was injected with alloxane (150 mg/ kg body weight) to induce hyperglycemia. The second main group divided into eight subgroups (n = 6), subgroups (1 and 2) fed on normal and high protein diets containing 250 g un-fortified bread, as a positive control groups. Subgroups (3 and 4) fed on high protein diets containing 250 g bread fortified with 5 and 10% FS, respectively. Subgroups (5 and 6) fed on high protein diets containing 250 g bread fortified with 5 and 10% NSS, respectively. The last subgroups (7 and 8) fed on high protein diets containing 250 g bread fortified with (5% FS and 5% NSS) and (10% FS and 10% NSS), respectively. Addition fortified bread with FS, NSS and combination of them to high protein diets decreased feed intake and body weight gain%, these treatments decreased the percent liver weight/ body weight, serum cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein (VLDL-c), uric acid, urea nitrogen, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT), alkaline phosphatase, serum glucose, as compared to the positive control groups, while high-density lipoprotein (HDL-c), T_3 and T_4 increased. It was concluded that, treating diabetic rats with high protein diet containing fortified bread with (10% FS and 10% NSS) realized the best effect on serum glucose, lipid profile, thyroid hormones, kidney and liver functions, followed by the groups which treated with fortified bread with high levels from FS and NSS.

Key words: Fenugreek seeds, *Nigella sativa* seeds, diabetes, rats, lipid profile, kidney function

INTRODUCTION

Diabetes mellitus is one of the major metabolic disorders, afflicting a large proportion of the population all over the world (Zimmet *et al.*, 2001). Diabetes is recognized for severe complications, which include diabetic nephropathy, neuropathy and retinopathy (Gabir *et al.*, 2000).Several short-term, randomized, controlled studies have shown that the substitution of some dietary carbohydrate for protein in low-fat diets (≤30%) enhances weight loss (Baba *et al.*, 1999) and is associated with favourable changes in body composition through a preferential loss in body fat and the sparing of lean tissue (Parker *et al.*, 2002) as well as potentially exerting beneficial effects on specific risk factors for cardiovascular disease (CVD) such as insulin sensitivity (Layman *et al.*, 2003) glycemic control (Farnsworth *et al.*, 2003) and the blood lipid profile (Wolfe and Giovannetti, 1991).Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Fenugreek is a medicinal plant that uses in disease some therapy. Natural products have been a major source of new drugs (Vuorelaa *et al.*, 2000). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hashim *et al.*, 2010). Black cumin (*Nigella sativa* L. Ranunculaceae) is an annual herb commonly used in the Middle East, India and nowadays gaining worldwide acceptance. Historical and traditional uses are extensively documented in ancient texts and historical documents. Black cumin seeds and oil are commonly used as a traditional tonic and remedy for many ailments as well as in confectionery and bakery (Botnick *et al.*, 2012). Fenugreek is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. This plant use for blood lipids and sugar decreasing in diabetic and non diabetic peoples and has antioxidant and antibacterial activity (Ethan *et al.*, 2003). Fenugreek seed powder in the diet reduces blood sugar and urine sugar with concomitant improvement in glucose tolerance and diabetic symptoms in type 2 diabetic patients (Analava and Debaprasad, 2004). The hypoglycemic effects of fenugreek have been attributed to several mechanisms (Sauvaire *et al*., 1998) demonstrated *in vitro* the amino acid 4-hydroxyisolcucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells, It was observed that 4-hydroxy isoleucine extracted from fenugreek seeds has insulin tropic activity. Sauvaire *et al*. (1998) show This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells. According to Tim (1998) who reported that, the hypoglycemic effect of fenugreek is thought to be largely due to its high content of soluble fiber, which acts to decrease the rate of gastric emptying thereby delaying the absorption of glucose from the small intestine. The quality and quantity of protein in the diets have a direct effect on the levels of cholesterol. Generally plant protein appears to lower cholesterol level (James, 2004). According report (Xue *et al*., 2007), supplementation of these medicinal plants mixture (fenugreek), decreased in serum triglycerides, total cholesterol, LDL-C, VLDL-C in both raw and cooked form but increased in HDL-C with the increase in supplementation of medicinal plants. Fenugreek seeds decrease triglycerides and total cholesterol level, because fenugreek is contain fiber and fiber have effect of dietary fiber on lipoprotein cholesterol is due to its association with absorption and transport of lipids (Story and Kelley, 1982). Seeds of *N. sativa* contain a considerable amount of fixed and volatile oils (Nickavar *et al*., 2003), proteins, alkaloids and saponins (Ali and Blunden, 2003; Al-Ghamdi, 2003). Modern trials have proved that its seeds alone or in combination with other drugs are highly effective in diabetes mellitus (Al-Rowais, 2002) and improves lipid profile through increasing HDL (the healthy cholesterol) and decreasing LDL and triglycerides and It is also reported to be a potent immune modulator (Kalus *et al*., 2003). *N. sativa* has been traditionally used for treatment of diabetes and hypertension in south-eastern Morocco and Jorden (Tahraoui *et al*., 2007). A cross sectional survey of 310 diabetic patients in Jorden revealed 7.3% of them used *N. sativa* for diabetes (Otoom *et al*., 2006). Seeds of *N. sativa* have been safely consumed by human patients in many clinical trials which however were not aimed to assess its antidiabetic activity. In future clinical studies may show potential of *N. sativa*, its constituents or their synthetic analogues, in prevention and control of diabetes (Mathur *et al*., 2011). Therefore, the aim of the present study was to investigate the effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on rats suffering from diabetes

MATERIALS AND METHODS

Materials: Casein, vitamins, minerals, cellulose, choline chloride were purchased from El-Gomhoreya Company, Cairo Egypt. Sixty male albino rats (Sprague Dawley Strain) (150-165 g) were obtained from Helwan farm. Wheat flour (80% extract), yeast, salt and sugar were purchased from local market, Cairo Egypt. Fenugreek seeds (*Trigonella foenugraecum Linn)* and *Nigella sativa* were obtained from local market (Cairo-Egypt).

Methods

Preparation of fenugreek seed powder: Fenugreek seeds were soaked overnight and allowed to germinate and to eliminate the bitter taste and then the seeds were dried after removal from the water and were ground to a fine powder (Raghuram *et al*., 1993).

Preparation of bread: Normal bread consists of wheat flour (90 gm), yeast (5 gm), salt (2.5 gm), sugar (2.5 gm).

Fortification of bread: In this study, wheat flour fortified with 5 and 10 g (FS and NSS) and (5 g FS+5 NSS g and 10 FS g+10NSS g)/100 g and used in the preparation of bread.

Chemical analysis: Moisture, protein, fat, fiber and ash were determined in unfortified bread and also fortified bread with fenugreek seeds, *Nigella sativa* seeds and their combination, according to the methods outlined in AOAC (1990), while the carbohydrates content were calculated by difference.

Biological assay: Sixty male albino rats (150-165 g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet for adaptation in *ad libitum*. The basal diet in the preliminary experiment consists of 14% casein (protein>80%), corn oil 4%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the remainder is corn starch (Reeves *et al*., 1993). Vitamin mix. and minerals mix. prepared according to (AOAC, 1975).After a period of adaptation on basal diet (one week), the rats were divided into two main groups. The first main group (12 rats) fed on basal diet, while the second main group (48 rats) was injected with alloxane (150 mg/kg body weight) to induce hyperglycemia, after fasting overnight (Buko *et al*., 1996). After four days serum glucose was determined in the first and second main groups to ensure the induction. The mean value ± SD f serum glucose was (75.220±6.00 mg/dL in healthy rats fed on basal diet vs. 175.709±5.673 mg/dL in the second main group "diabetic group").The first main group (12 rats) divided into two subgroups (6 rats each). The two Subgroups were fed on normal and high protein diets (14 and 20% protein) containing 250 g un-fortified bread, as control negative groups. The second main group divided into eight subgroups, subgroups (1 and 2) fed on normal and high protein diets (14 and 20% protein) containing 250 g un-fortified bread, as a positive control groups. Subgroups (3 and 4) fed on high protein diets containing 250 g bread fortified with 5 and 10% fenugreek seed powder, respectively. Subgroups (5 and 6) fed on high protein diets containing 250 g bread fortified with 5 and 10% *Nigella sativa* seeds, respectively. The last subgroups (7 and 8) fed on high protein diets containing 250 g bread fortified with (5% fenugreek seed powder and 5% *Nigella sativa* seeds) and (10% fenugreek seed powder and 10% *Nigella sativa* seeds), respectively. During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly. At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged and the serum was separated to estimate some biochemical parameters, i.e. serum cholesterol by Allain *et al.* (1974), triglycerides by Fossati and Principe (1982), HDL-c by (Lopes-Virella *et al.*, 1977), LDL-c and VLDL-c by Friedewald *et al.* (1972), aspartate amino transferase (AST) and alanine amino transferase (ALT) by Ritman and Frankel (1957), serum alkaline phosphates (Bergmeyer and Brent, 1974), uric acid (Fossati *et al.*, 1980), urea nitrogen (Patton and Crouch, 1977), creatinine by Bohmer (1971), glucose by Trinder (1959), total thyroxin T_4 (Britton *et al.*, 1975), triiodothyronine T_3 (Ahmed *et al.*, 1974). The data obtained was analyzed statistically for standard deviation and one way ANOVA test (Steel and Torri, 1980).

RESULTS AND DISCUSSION

Chemical composition of non-fortified and fortified bread with two levels from fenugreek and *Nigella sativa* seeds. Bread analyzed for its content and illustrated in Table 1. The percent content of moisture, protein, fat, ash, fiber and carbohydrates of un-fortified bread were 31.00, 8.700, 1.620, 0.655, 0.570 and 57.455, respectively. Fortified bread with fenugreek seeds (FS) and *Nigella sativa* seeds (NSS) increased total protein, fat, ash and fiber, while the moisture content and carbohydrates decreased, than that of the un-fortified bread. The highest content of protein, fat, ash and fiber recorded for the bread which fortified with (10% FS 10% NSS).

Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on feed intake, body weight gain (%) and liver weight/body weight (%) of diabetic rats: Table 2 shows the effect of high protein diet containing fortified bread with 5 and 10% fenugreek seeds (FS), *Nigella sativa* seeds (NSS) and combination of them on the mean values of feed intake (g/day/rat), body weight gain% and liver

Table 1: Chemical composition of non-fortified and fortified bread with two levels from fenugreek and *Nigella sativa* seeds (w/w)

	Nutrients g/100 g					
Sample	Moisture	Protein	Fat	Ash	Fiber	Carbohydrates
Unfortified bread	31.00	8.700	1.620	0.655	0.570	57.455
Bread fortified with 5% FS	30.82	9.45	1.907	0.779	1.10	55.944
Bread fortified with 10% FS	30.600	10.142	2.077	0.910	1.550	54.721
Bread fortified with 5% NSS	30.650	9.722	2.350	0.877	0.80	55.601
Bread fortified with 10% NSS	29.800	10.700	2.960	0.950	0.962	54.628
Bread fortified with 5% FS+5% NSS	30.544	9.832	2.650	0.950	1.153	54.871
Bread fortified with 10% FS+10% NSS	30.00	10.814	3.592	1.136	1.642	52.816

Value of each parameter is the mean of two determinations

Table 2: Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on feed intake, body weight gain% and liver weight/body weight% of diabetic rats

	Parameters		
Groups	Mean values of feed intake (g/day/rat)	Body weight gain%	Liver weight/body weight%
Control (-ve) normal protein	16.675	28.470±3.516[a]	2.690±0.111[f]
Control (-ve) high protein	15.500	26.801±2.811[a]	2.642±0.064[ef]
Control (+ve) normal protein	15.368	16.320±2.691[b]	3.899±0.142[a]
Control (+ve) high protein	13.231	13.395±2.683[c]	3.699±0.115[b]
Bread fortified with 5% FS.	14.565	7.001±1.148[ef]	3.406±0.099[c]
Bread fortified with 10% FS.	13.00	5.462±1.212[f]	3.183±0.123[d]
Bread fortified with 5% NSS.	14.862	11.523±2.033[cd]	3.356±0.115[c]
Bread fortified with 10% NSS.	14.500	8.271±1.351[e]	3.360±0.124[c]
Bread fortified with 5% FS+5%NSS.	14.00	9.663±1.733[de]	3.071±0.092[d]
Bread fortified with 10% FS+10% NSS.	13.775	8.082±1.377[ef]	2.810±0.101[e]

Values are expressed as mean±SD. Significant at $p<0.05$ using one way ANOVA test. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant weight/body weight% of rats suffering from diabetes. The mean value of feed intake in the negative control group fed on normal protein diet containing 250 g un-fortified bread was 16.675 g, while it was 15.500 g in the negative control group fed on high protein diet containing the same type and amount of bread, in this respect (Thomas and Frank, 2004) reported that, higher protein intake increases thermogenesis and satiety compared to diets of lower protein content. Injected rats with alloxan decreased the mean value of feed intake in the normal and high protein diets groups containing 250 g un-fortified bread, than that of the control negative groups, especially with the high protein diet. Feeding diabetic rats on high protein diets containing bread fortified with (5 and 10% FS), (5 and 10% NS) and (5% FS and 5% NSS and 10% FS and 10% NSS) decreased the mean value of feed intake, than that of the positive control groups.

The mean values of BWG% decreased significantly $p<0.05$ in the positive control groups, compared to the negative control groups (16.320±2.691 and 13.395±2.683 vs., 28.470±3.516 and 26.801±2.811, respectively). Feeding diabetic groups with high protein diets containing bread fortified with 5 and 10% (FS, NSS and their combination) decreased BWG% significantly ($p<0.05$), as compared to the positive control groups. The highest decrease in BWG% recorded for the group treated with bread fortified with 10% fenugreek seeds. Fortified bread with the low levels of FS or the combination of FS and NSS showed non-significant changes in BWG% of diabetic rats, as compared to the high levels from them. On the other hand BWG% of the group which treated with bread fortified with 10% NSS recorded significant decrease $p<0.05$, as compared to the group which treated with 5% NSS. In this respect, some researchers indicated that fenugreek seed extract supplementation in reducing the body and adipose tissue weight (Handa *et al*., 2005). The probable mechanism of fenugreek decreasing the total body and adipose tissue weight may be that (1) fenugreek flushes out the carbohydrates from the body before they enter the blood stream resulting in weight loss (2) Fenugreek seeds contain a high proportion (40%) of soluble fiber. This fiber forms a gelatinous structure (similar to gaur gum) which may have effects on slowing the digestion and absorption of food from the intestine and create a sense of fullness in the abdomen, thus suppresses appetite and promotes weight loss (Geetha *et al*., 2011). Mathur *et al*. (2011) reported that, *N. sativa* may be beneficial in diabetic individuals and those with glucose intolerance as it reduces appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, cholesterol, triglycerides, body weight and simulates glucose induced secretion of insulin from beta-cells in pancreas; yet it has not shown significant adverse effects and has very low toxicity. Data in the same table showed that, the mean value of liver weight/body weight% of healthy group fed of normal protein diet containing 250 g un-fortified bread/ kg diet recorded non-significant difference, as compared to the healthy group which fed on high protein diet containing the same type and amount of bread. On the other hand, feeding diabetic rats on high protein diet containing un-fortified bread showed significant decrease in liver weight/body weight%, as compared to diabetic rats fed on diet containing normal protein diet. Injected rats with alloxan showed significant increase in liver weight/body weight% of the control positive groups, as compared to the negative control groups. All treated diabetic groups with high protein diets containing fortified bread with 5% and 10% (FS, NSS and their combination) showed significant decrease in the mean values of liver weight/body weight%, as compared to the positive control groups, especially the group of rats which treated with bread fortified with (10% FS and 10% NSS), followed by the groups which treated with fortified bread with (5% FS and 5% NSS) and (10% FS), respectively.

Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on lipid profile of diabetic rats: Table 3 shows the effect of high protein diet containing fortified bread with 5 and 10% FS, NSS and combination of them on the mean values of serum cholesterol, triglycerides, high density lipoprotein-cholesterol HDL-c, low and very low density lipoprotein-cholesterol (LDL-c and VLDL-c) of rats suffering from diabetes. Feeding healthy rats on high protein diet containing 250 g unfortified bread/kg diet led to significant decrease in serum cholesterol, triglycerides, LDL-c and VLDL-c, as compared to healthy rats fed on normal protein diet containing the same amount and type of bread. While feeding diabetic rats on high protein diet containing 250 g un-fortified bread induced significant decrease $p<0.05$ in serum cholesterol and LDL-c only, as compared to diabetic rats fed on normal protein diet containing the same amount and type of bread. In this respect, Layman *et al*. (2003) demonstrates that increasing the proportion of protein to carbohydrate in the diet of adult women has positive effects on body composition, blood lipids, glucose homeostasis and satiety during weight loss. Injected rats with 150 mg alloxan/kg body weight (control positive groups) led to significant increase $p<0.05$ in serum cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c decreased, as compared to non-injected rats (control negative groups).Feeding diabetic groups with high protein diets containing 250 g fortified bread with (5 and 10% FS), (5 and 10% NSS) and (5% FS+5%NSS and 10% FS+10% NSS) led to significant improvement in all lipid profile, as compared to the positive control groups fed on normal and high protein diets. The mean value of serum cholesterol, triglycerides, LDL-c and

Table 3: Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on lipid profile of diabetic rats

	Parameters mg/dL				
Groups	Ch	Tg	HDL-c	LDL-c	VLDL-c
Control (-ve) normal protein	90.281±2.370^{h}	47.038±2.170^{f}	45.923±1.998^{a}	34.950±0.792^{h}	9.407±0.434^{f}
Control (-ve) high protein	84.768±2.506^{i}	42.240±1.758^{g}	44.551±2.259^{a}	31.769±0.875^{i}	8.448±0.351^{g}
Control (+ve) normal protein	183.608±4.921^{a}	84.772±4.910^{a}	23.363±3.609^{f}	143.290±1.368^{a}	16.954±0.982^{a}
Control (+ve) high protein	176.413±5.107^{b}	82.776±4.269^{a}	28.212±3.153^{e}	131.650±2.057^{b}	16.555±0.853^{a}
Bread fortified with 5% FS.	154.606±4.258^{d}	72.378±3.304^{c}	35.197±3.246^{cd}	104.933±2.746^{d}	14.475±0.661^{c}
Bread fortified with 10% FS.	131.496±4.906^{f}	60.209±3.053^{e}	38.060±2.006^{bc}	81.393±5.048^{f}	12.041±0.611^{e}
Bread fortified with 5% NSS.	160.450±4.057^{c}	76.883±2.448^{b}	28.750±2.285^{e}	116.334±1.526^{c}	15.365±0.489^{b}
Bread fortified with 10% NSS.	142.153±3.929^{e}	65.172±4.059^{d}	34.557±1.405^{d}	94.550±2.004^{e}	13.034±0.811^{d}
Bread fortified with 5% FS+5%NSS.	130.866±6.524^{f}	60.988±3.776^{e}	37.826±3.916^{bcd}	80.844±2.705^{f}	12.196±0.752^{e}
Bread fortified with 10% FS+10% NSS.	118.322±3.278^{g}	46.679±1.508^{f}	40.806±2.385^{b}	68.179±2.010^{g}	9.335±0.301^{f}

Values are expressed as mean±SD. Significant at $p<0.05$ using one way ANOVA test. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant

Ch: Cholesterol

Tg : Triglyceride

HDL-c: High Density Lipoprotein-cholesterol

LDL-C: Low Density Lipoprotein-cholesterol

VLDL-c: Very Low Density Lipoprotein-cholesterol

VLDL-c decreased gradually with increasing the level of FS, NSS and their combination. The highest improvement in lipid fractions recorded for the group which treated with high protein diet containing fortified bread with (10% FS and 10% NSS). These treatment decreased serum cholesterol, triglycerides, LDL-c and VLDL-c by about 32.93, 43.61, 48.21 and 43.61%, than that of the positive control group fed on high protein diet, respectively, while HDL-c increased by about 44.64%.In this respect, Chattopadhay and Bandyopadhyay (2005) reported that, premature and extensive arteriosclerosis involving renal, peripheral and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. Also, Krentz (2003) reported that, diabetes mellitus (DM) is the most common metabolic disorder worldwide. Patients with type 2 DM have a complex alteration in plasma lipids characterized by elevated level of triglycerides, decreased level of (HDL-c) and a preponderance of (LDL-c). Fenugreek seeds lowered serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) (Stark and Madar, 1993; Valette *et al*., 1984; Al-Habori *et al*., 1998; Al-Habori and Raman, 1998). These effects may be due to sapogenins, which increase biliary cholesterol excretion, in turn leading to lowered serum cholesterol levels (Sauvaire *et al*., 1991). The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T_4.

Diosgenin decreased the elevated cholesterol in serum LDL and HDL fractions in cholesterol-fed rats and had no effect on serum cholesterol in normo cholesterolemic rats. In addition, diosgenin inhibited cholesterol absorption suppressed its uptake in serum and liver and its accumulation in the liver (Cayen and Dvornik, 1979). A study on the extent of degradation of the saponin and/or diosgenin another steroid saponins in the alimentary tract of alloxan diabetic dogs suggested that steroid saponin and sapogenin might have a role in lowering cholesterol (Sauvaire *et al*., 1991). Srichamroen *et al*. (2008) revealed that, feeding Galactomannan from Canadian-grown fenugreek seeds has the potential to alter glycemic and lipidemic status and reduce abdominal fat in normal rats. Belguith-Hadriche *et al*. (2010) reported that, significant hypocholesterolemic effects and antioxidant activity in fenugreek seed, which may be partly due to the presence of flavonoids, especially naringenin. On the other hand, Vijayakumar *et al*. (2010) reported that, thermostable extract of fenugreek seeds TEFS may have potential application in the management of dyslipidemia and its associated metabolic disorders. Narender *et al*. (2006) reported that, amino acid, 4-hydroxy isoleucine 5, has been isolated, from the seeds of *Trigonella foenum-graecum* which significantly decreased the plasma triglyceride levels by 33% ($p<0.002$), total cholesterol (TC) by 22% ($p<0.02$) and free fatty acids by 14%, accompanied by an increase in HDL-C/TC ratio by 39% in the dyslipidemic hamster model. Treatment of rats with the seed extract of *N. sativa* decrease in plasma concentrations of cholesterol, triglycerides and glucose (Zaoui *et al*., 2002; Mathur *et al*., 2011). N. sativa shows significant decrease in serum LDL-c level and increase in serum HDL-c level (Dahri *et al*., 2005).N. sativa supplementation at a dose of 2 g/day for 12 weeks may improve the dyslipidemia associated with type 2 diabetic patients. Therefore, NS is a potential protective natural agent against atherosclerosis and cardiovascular complications in these patients (Huda *et al*., 2012). N. sativa has a favorable effect on TG and lipoprotein pattern in normal rats Le *et al*. (2004). Similar findings were encountered by the administration of thymoquinone, the active ingredient of NS, to rabbits fed on cholesterol-enriched diet (Nader *et al*., 2010). Oxidative stress and reactive oxygen species are now accepted as a likely causative factor in the development of insulin resistance (Soskic *et al*., 2011). Thymoquinone, the active constituent of NS has been demonstrated to attenuate oxidative stress in streptozotocin-induced diabetic rats (Hamdy and Taha, 2009) and in hypercholesterolemic rats (Ismail *et al*., 2010).

Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on liver enzymes of diabetic rats: The effect of high protein diets containing two levels from FS, NSS and their combination on liver enzymes aminotransferases (AST and ALT) and alkaline phosphatase (ALP) activity presented in Table 4. Feeding normal rats on high protein diet containing 250 g un-fortified bread/kg diet led to non-significant differences in AST, ALT and ALP, as compared to healthy rats fed on normal protein diet containing 250 g un-fortified bread. On the other hand, injected rats with alloxan and fed on high protein diet decreased the mean value of serum AST and ALP, while serum ALT showed non-significant changes, as compared to diabetic group fed on normal protein diet.
Feeding diabetic groups on high protein diets containing 250 g fortified bread with (5 and 10% FS), (5 and 10% NSS) and (5% FS+5% NSS and 10% FS+10% NSS) decreased the mean values of AST, ALT and ALP, as compared to the positive control groups fed normal and high protein diets. Treating diabetic groups with fortified bread with the two levels (5 and 10% NSS) improved the liver enzymes, as compared to the groups which treated with the same levels from FS. Diabetic rats which treated with fortified bread with (10% FS and 10% NSS) recorded the best results in AST, ALT and ALP, followed by the group treated with 10% NSS. Barbora *et al*. (2002) reported that, high ALT is a marker of risk for type 2 diabetes and suggest a potential role of the liver in the pathogenesis of type 2 diabetes. Kaviarasan *et al*. (2006, 2007) suggested that the polyphenolic compounds of fenugreek seeds can be considered cytoprotective during ethanol (EtOH) induced liver damage. Fenugreek seed polyphenol extract (FPEt) administration had a positive influence on both lipid profile and on the quantitative and qualitative properties of collagen in alcoholic liver disease. The protective effect is presumably due to the bioactive phytochemicals in fenugreek seeds. Saber and Samah (2012) concluded that the aqueous extract fenugreek seeds has a beneficial impact on anticancer drug adriamycin ADR-induced hepatotoxicity due to its antioxidant effect in albino rats. Kaviarasan and Anuradha (2007) demonstrated that fenugreek seed polyphenolic extract (FPEt) acts as a protective agent against ethanol-induced abnormalities in the liver. The effects of FPEt are comparable with those of a known hepatoprotective agent, silymarin. Meera *et al*. (2009) reported that significant hepatoprotective effects were obtained by ethanolic extract of leaves of *Trigonella foenum-graecum Linn* against liver damage induced by H_2O_2 and CCl_4 as evidenced by decreased levels of antioxidant enzymes (enzymatic and non-enzymatic). The extract also showed significant ant lipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging, indicating their potent antioxidant effects. Hassanin and Hassan (1996) reported that, significant decrease in AST and ALT enzymes due to treatment by *Nigella sativa* for 9 days. Also Mady (2000) found that, gave *Nigella sativa* seeds orally at two doses (250 and 500 mg/kg) for five days, led to protective against hepatotoxicity of liver. Mazentaip (2004) observed that, N. Sativa seeds powdered had the lower values of AST and ALT enzymes in diabetic rats, than that of control.

Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on kidney functions of diabetic rats: The mean values of serum uric acid, urea nitrogen and creatinine of diabetic rats fed on high protein diet containing fortified bread with 5 and 10% (FS and NSS) and (5% FS+5% NSS and 10% FS+10% NSS) are illustrated in Table 5. The mean value of serum uric acid and urea nitrogen increased significantly p<0.05 in normal

Table 4: Effect of fortified bread with fenugreek and *Nigella sativa* seeds on liver enzymes of diabetic rats

	Parameters U/L		
Groups	AST	ALT	ALP
Control (-ve) normal protein	69.492±2.680[h]	29.008±2.231[f]	81.811±3.095[h]
Control (-ve) high protein	67.382±2.789[h]	27.023±0.968[f]	78.997±2.768[h]
Control (+ve) normal protein	109.421±4.981[a]	67.827±4.527[a]	159.503±7.309[a]
Control (+ve) high protein	101.272±4.038[b]	63.944±4.176[a]	153.060±5.994[b]
Bread fortified with 5% FS.	94.126±2.198[c]	56.124±3.982[b]	140.258±6.279[c]
Bread fortified with 10% FS.	85.365±2.685[de]	44.937±3.688[d]	124.498±4.810[e]
Bread fortified with 5% NSS.	88.270±3.406[d]	50.068±3.781[c]	133.682±7.877[d]
Bread fortified with 10% NSS.	79.277±2.759[f]	39.051±3.919[e]	115.531±3.861[f]
Bread fortified with 5% FS+5%NSS.	83.704±3.796[e]	45.134±2.944[d]	126.030±4.450[e]
Bread fortified with 10% FS+10% NSS.	74.700±3.520[g]	38.446±3.518[e]	108.717±4.650[g]

Values are expressed as mean±SD. Significant at p<0.05 using one way ANOVA test. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant

Table 5: Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on kidney functions of diabetic rats

	Parameters mg/dL		
Groups	Uric acid	Urea nitrogen	Creatinine
Control (-ve) normal protein	1.521±0.082[g]	29.116±1.115[g]	0.490±0.027[f]
Control (-ve) high protein	1.644±0.061[f]	32.616±1.013[f]	0.580±0.028[f]
Control (+ve) normal protein	2.271±0.094[b]	58.475±3.053[a]	1.150±0.154[c]
Control (+ve) high protein	2.544±0.103[a]	60.955±3.339[a]	1.637±0.194[a]
Bread fortified with 5% FS.	2.019±0.114[c]	46.784±2.750[c]	1.165±0.119[c]
Bread fortified with 10% FS.	1.879±0.075[de]	40.675±2.611[d]	0.901±0.120[d]
Bread fortified with 5% NSS.	2.246±0.106[b]	51.924±2.170[b]	1.330±0.134[b]
Bread fortified with 10% NSS.	2.013±0.111[c]	44.794±1.902[c]	1.022±0.112[cd]
Bread fortified with 5% FS+5%NSS.	1.896±0.105[d]	40.437±2.898[d]	0.977±0.102[d]
Bread fortified with 10% FS+10% NSS.	1.775±0.065[e]	36.661±2.251[e]	0.727±0.101[e]

Values are expressed as Mean±SD. Significant at $p<0.05$ using one way ANOVA test. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant

rats fed on high protein diet containing 250 g un-fortified bread, while the mean value of serum creatinine did not changed, as compared to normal rats fed on normal protein diet containing the same amount of bread.

Injected rats with alloxan increased the mean values of serum uric acid, urea nitrogen and creatinine significantly ($p<0.05$), as compared to non-injected rats. Our data showed that uric acid levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triglycerides and cholesterol (Madinov *et al*., 2000). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Meki, 2003). Treating diabetic groups with high protein diet containing bread fortified with two levels of FS, NSS and (FS+NSS) decreased the mean values of serum uric acid, urea nitrogen and creatinine, as compared to the positive control groups. All parameters decreased gradually with increasing the level of FS, NSS and their combination which used in preparing the bread. The best results in serum uric acid, urea nitrogen and creatinine recorded for the group which treated with high protein diet containing fortified bread with 10% FS+10% NSS, followed by the groups treated with (5% FS+5% NSS) and 10% FS, respectively. In this respect, (Suresh *et al*., 2005) reported that, In Southeast Asia, the water extract of fenugreek seeds is used in the management of diabetes and is known to improve kidney function during diabetes. Laroubi *et al*. (2007) showed that the amount of calcification in the kidneys and the total calcium amount of the renal tissue in rats treated with *Trigonella foenum graecum* (Tfg) was significantly reduced compared with the untreated group. Consequently, Tfg may be a useful agent in the treatment of patients with calcic urolithiasis. Mazentaip (2004) observed that, N. Sativa seeds powdered had the lower values of urea and creatinine in diabetic rats, than that of control. Al-Okbi *et al*. (1997) found that, oral administration of *Nigella sativa* seeds and its extracts reveled significant reduction in blood urea nitrogen and serum creatinine.

Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on serum glucose and thyroid hormones of diabetic rats: The effect of high protein diet containing fortified bread with two levels from FS, NSS and mixture from FS and NSS on serum glucose, triiodothyronine T_3 and thyroxin T_4 presented in Table 6. Feeding healthy rats on high protein diet containing 250 g un-fortified bread decreased serum glucose significantly and increased T_3 and T_4, as compared to healthy rats fed on normal protein diet containing the same amount of bread. On the other hand, injected rats with alloxan led to significant increase $p<0.05$ in serum glucose and decreased T_3 and T_4, as compared to non-injected rats. Serum glucose decreased in all diabetic groups which were treated with high protein diet containing fortified bread with 5 and 10% (FS and NSS) and (5% FS+5% NSS and 10% FS+10% NSS). Serum glucose decreased gradually with increasing the levels of FS, NSS and the combination from them. The data in this Table revealed that, treating diabetic groups with fortified bread with FS improved the mean value of serum glucose, as compared to diabetic groups which treated with NSS. The best results in serum glucose recorded for the group which treated with fortified bread with (10% FS+10% NSS), this treatment decreased serum glucose by about 37.40%, than that of the positive group fed on high protein diet containing unfortified bread.

Annida *et al*. (2004) showed that blood glucose and serum and tissue lipids were elevated in diabetic rats. On the other hand, Mondal *et al*. (2004) reported that fenugreek decreases the fasting blood glucose (FBG) level considerably by improving diabetes mellitus. Gad *et al*. (2006) suggested that the hypoglycemic effect of fenugreek and balanites is mediated through insulinomimetic effect as well as inhibition of intestinal

Table 6: Effect of high protein diet containing fortified bread with fenugreek and *Nigella sativa* seeds on serum glucose and thyroid hormones of diabetic rats

	Parameters		
Groups	Serum glucose (mg/dL)	T_3 (ng/dL)	T_4 (µg/dL)
Control (-ve) normal protein	88.286±3.729[h]	101.525±6.316[a]	8.305±0.554[a]
Control (-ve) high protein	82.179±4.220[i]	105.384±7.972[a]	8.626±0.546[a]
Control (+ve) normal protein	190.204±4.306[a]	47.577±2.980[f]	2.694±0.216[f]
Control (+ve) high protein	175.458±3.599[b]	50.560±5.068[ef]	2.865±0.317[f]
Bread fortified with 5% FS.	152.810±4.095[d]	52.841±6.601[e]	4.123±0.235[d]
Bread fortified with 10% FS.	125.918±3.185[f]	54.335±4.399[e]	5.813±0.346[b]
Bread fortified with 5% NS.	160.408±4.160[c]	67.323±6.376[d]	3.614±0.125[e]
Bread fortified with 10% NS.	134.449±4.710[e]	73.089±5.765[bc]	4.825±0.104[c]
Bread fortified with 5% FS+5%NS.	125.692±4.165[f]	68.990±3.848[cd]	3.925±0.242[de]
Bread fortified with 10% FS+10% NS.	109.834±5.347[g]	77.880±3.997[b]	5.480±0.254[b]

Values are expressed as Mean±SD. Significant at $p<0.05$ using one way ANOVA test. Values which have different letters in each column differ significantly, while those with have similar or partially are not significant

T_3 : Triiodothyronine

T_4 : Total thyroxin

alpha-amylase activity. Hannan *et al.* (2007) investigated that the soluble dietary fibre (SDF) fraction of *T. foenum-graecum* seeds exerts antidiabetic effects mediated through inhibition of carbohydrate digestion and absorption and enhancement of peripheral insulin action. Fenugreek (Trigonella foenum-graceum) is a spice possessing amazing therapeutic and medicinal properties. The seeds are used for the treatment of diabetics (Suchandra *et al.*, 2010). The hypoglycemic effects of fenugreek have been attributed to several mechanisms. Sauvaire *et al.* (1998) demonstrated in vitro the amino acid 4-hydroxy isoleucine in fenugreek seeds increased lucose-induced insulin release in human and rat pancreatic islet cells. (Raghuram *et al.*, 1994) reported that, this amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. In human studies, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors. In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells (Ajabnoor and Tilmisany, 1988), as well as by inhibiting the activities of alpha-amylase and sucrase, (Amin *et al.*, 1987) two intestinal enzymes involved in carbohydrate metabolism. Fenugreek seed is an excellent source of fiber that is mainly comprised of galactomannans (Madar and Stark, 2002). Fenugreek seeds contain high amount of fiber (60% from them soluble fiber and 40% insoluble fiber) that can slow the rate of postprandial glucose absorption. Galactomannan in fenugreek blocks intestinal absorption of glucose. Water soluble fiber increases the viscosity inside the intestine and then inhibits absorption of glucose (Ethan *et al.*, 2003). Hedaya (1995) reported that the decrease in serum glucose may be related to the increase of insulin secretion by α-cells of pancreas due to dietary *Nigella sativa* seeds supplementation. Zaoui *et al.* (2002) reported that oral treatment of rats with fixed oils of *Nigella sativa* (1 mL/kg/12 weeks) decreased serum cholesterol and glucose levels. Recently demonstrated an improvement in glycemic control and insulin resistance in type 2 diabetic patients induced by *Nigella sativa* treatment (Bamosa *et al.*, 2010).Injected rats with alloxan showed non-significant changes in T_3 and T_4 in the group fed on normal and high protein diets (control+groups). The mean value of serum T_3 and T_4 increased significantly $p<0.05$ in all diabetic groups which treated with high protein diet containing fortified bread with NSS (5 and 10%), as compared to the positive control groups. The highest increase in serum T_3 and T_4 recorded for the group which treated with 10% NSS. On the other hand treated diabetic rats with high protein diet containing fortified bread with 5 and 10% FS increased serum T_4, while T_3 did not changed significantly, as compared to the positive control group fed on high protein diet containing un-fortified bread. Treating diabetic rats with high protein diet containing fortified beard with (5% FS+5% NSS) and (10% FS+10% NSS) improved T_3 and T_4, as compared to the positive control groups. In this respect, Tanyeri *et al.* (1993) reported that, the function of the thyroid gland may be altered in diabetes mellitus. It is known that serum triiodothyronine (T3) levels decrease in diabetic patients, possibly due to inhibition of peripheral 5-deiodinase enzyme activity (Sluszkiewicz, 1986). Ismail *et al.* (2003) suggested that oral *Nigella sativa* treatment might decrease the diabetes-induced disturbances of thyroid hormone metabolism. Nigella sativa oil (NSO) given orally significantly increases the concentration of T4 and T3 and decreases the TSH in hypothyroid rats as compared to untreated rats. It is apparent that recovery of thyroid parenchyma is related to protection offered by NSO against hyperplasic changes well known to be associated with hypothyroid status (Stelios *et al.*, 2007). Sharif *et al.* (2012) reported that *Nigella sativa* ethanolic extract increase T_3 serum concentration in diabetic rat.

N.S. may also repair the thyroid gland and resynthesize the thyroid hormone. There is evidence that *Nigella sativa* L. crushed extract returns the decreased T3 in alloxan-induced diabetic rabbit to the normal level. (Meral *et al.*, 2003). Administration of fenugreek seed extract to both mice and rats revealed its effect on thyroid hormone that fenugreek inhibits the synthesis of triiodothyronine concentration estimated by decrease in serum triiodothyronine concentration and T3/T4 ratio and consequently increased thyroxine levels which can be mediated through fenugreek-induced hypoglycaemia (Panda *et al.*, 1999).

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