M.F. ELSADEK[1],
MONA A. ABD EL-BASSET[2],
A. ALMAJWAL[1]

Antihyperglycemic and antihyperlipidemic effects of olive and fenugreek leaves in alloxan-induced diabetic rats

PROGRESS IN NUTRITION
VOL. 15, N. 3, 175-183, 2013

TITLE
Effetto ipoglicemizzante e ipolipidemico di foglie di ulivo e di fieno greco in ratti affetti da diabete indotto da allossano

KEY WORDS
Olive leaves, fenugreek leaves, alloxan, diabetic rats, lipid profile

PAROLE CHIAVE
Foglie di ulivo, foglie di fieno greco, allossano, ratti affetti da diabete, profilo lipidico

[1]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Saudi Arabia.
[2]Home Economic Dept., Faculty of Specific Education, Fayoum University, Egypt.

Address for correspondence:
Dr. Mohamed Farouk El-Sadek,
Assistant Professor
Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University
P.O.Box 10219, Riyadh, 11433
Saudi Arabia
Office Tel: +966-01-4697433
E-mail: mfelsadek@gmail.com

Summary
This study was performed to evaluate the glucose and lipid - lowering effects of Olive leaves (OL) and Fenugreek leaves (FL) on diabetic rats. Forty-eight healthy albino rats were injected with alloxan inducing hyperglycemia except normal control group then diabetic rats randomly divided into eight groups. Two groups are normal control and diabetic control. Six diabetic groups were fed on basal diet containing different levels 2.5%, 5% and 7.5% OL and FL respectively. Results revealed that consuming diet containing 7.5% OL and 7.5% FL lowers serum glucose level by 28.3% and 21.68% respectively and restoring all biochemical parameters to near normal levels, from another hand with elevating serum Ca & P intracellular storing levels by (17.3% and 20.1%) and (22.7% and 23.8%) for 5% FL and 5% OL respectively as compared to (+) control group.

Riassunto
Questo studio è stato condotto per valutare gli effetti sull'abbassamento dei livelli di glucosio e di lipidi delle foglie di ulivo (OL) e di fieno greco (FL) in ratti affetti da diabete. A 48 ratti albini sani è stato iniettato allossano per indurre iperglicemia tranne nel gruppo di controllo normale, e poi i ratti che hanno sviluppato diabete sono stati divisi casualmente in 8 gruppi. Due gruppi sono il controllo normale e quello diabetico. I sei gruppi diabetici sono stati alimentati con una dieta di base che conteneva diversi livelli: 2,5%, 5% e 7,5% rispettivamente di OL e FL. I risultati hanno rivelato che consumando una dieta contenente il 7,5% di OL e il 7,5% di FL si abbassavano i livelli di glucosio sierici rispettivamente del 28,3% e del 21,68%, e si ripristinavano tutti i parametri biochimici a livelli quasi normali, dall'altro lato si innalzavano i livelli di Ca e P sierici, immagazzinati a livello intracellulare, da 17,3% e 20,1% e da 22,7% e 23,8% per rispettivamente il 5% di FL e il 5% di OL se paragonati al gruppo di controllo positivo (+).

Introduction

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. Despite the efficiency of insulin treatment and other chemical therapies to control many features of diabetes, there are common incidents of diabetic complications such as vascular dysfunctions, nephropathy, neuropathy and retinopathy (1).

Olive (*Olea europaea L.*) leaves have been used as a medical herb to treat diabetic hyperglycemia, hypertension and infectious diseases (2). It has also been reported that some components in these leaves are useful in preventing oxidative stress (3), olive leaves present a unique opportunity to study the effects of *O. europaea* - derived polyphenols since the leaf contains polyphenols and only a small amount of oleic acid. Methanolic extracts of olive leaves contain secoiridoids, flavonoids (apigenin, kaempferol, luteolin), as well as phenolic compounds (caffeic acid, tyrosol, hydroxytyrosol) (4). It showed that the *O. europaea*-derived components present in a polyphenol-enriched dry olive leaf extract (DOLE) with oleuropein as major component, can ameliorate disease progression (5). *Trigonella foenum-graecum* (Fenugreek) is traditionally used in India, especially in the Ayurveda and Unani systems (6, 7). Preliminary animal and human trials suggest possible hypoglycaemic and anti-hyperlipedemic properties of fenugreek seed powder taken orally. Fenugreek is one such plant that has been extensively used as a source of antidiabetic compounds, from its seeds, leaves and extracts in different model systems (7-9).

The present study was carried out to investigate the comparison of olive leave vs fenugreek leave and fix the effective dose in diabetic rats.

Materials and methods

Chemicals

Alloxan, casein, vitamins, minerals, cellulose and choline chloride were purchased from El-Nasr Pharm. and Chem. Ind. Comp. Cairo, Egypt. Corn oil and corn starch were obtained from local market. Kits used to determine serum biochemical parameters were purchased from Alkan Pharm. Ind. Comp. Cairo, Egypt.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection with alloxan (100 mg/Kg body weight) (10). Rats were fast overnight before injection with freshly prepared aqueous solution of alloxan monohydrate. Blood was extracted from the tail vein for glucose analysis and rats with fasting glucose ranging from 210-220 mg/dl, showing clear signs of polyuria, polyphagia and polydipsia were considered diabetic and were analyzed 48 hours after alloxan treatment. Animals with fasting blood glucose less than 200 mg/dl were rejected.

Animals and experimental design

A total of forty eight male healthy albino rats, weighing between (100-120 gm) were obtained from Helwan Farm and housed in polypropylene cages at laboratory temperature (26 ± 2°C), water was available *ad libitum* and fed standard basal diet for one week as adaptation period, rats were divided into eight groups each group containing 6 rats. All rats were intraperitoneally injected by alloxan, except rats of negative control. Control groups (1&2) negative and positive were fed on basal diet without supplementation. All treated diabetic groups (3-5) were fed on basal diet containing (2.5%, 5% and 7.5% FL respectively), groups (6-8) were fed on basal diet containing (2.5%, 5% and 7.5% OL respectively). Fenugreek leaves and Olive leaves were supplemented in the diet daily to diabetic rats for 6 weeks, and feed intake was recorded daily.

Preparation of diet

The basal diet consisted of protein (14%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), choline (0.2%), cellulose (5%) and the remainder was starch (11).

Preparation of plant materials

Fresh Olive leaves (OL) and Fenugreek leaves (FL) were obtained from local market in Cairo, Egypt, and washed with fresh water and left to dry under shade and ground by using a grinder then stored until use.

Sample collections and analytical methods of blood serum

Blood samples were taken from aorta under diethyl ether anesthesia, centerfuged and serum were collected for analytical parameters. Serum cholesterol was determined according to the enzymatic method described by Allain et al. (12), serum triglycerides were colorimetrically determined according to Wahlefeld (13), the HDL-c was determined according to Albers et al. (14), while concentration of VLDL-c was estimated according to the Fridewald's equation (15).
According to Fridewald et al. (15) low density lipoprotein cholesterol can be calculated as follows:
LDL-c = Total cholesterol – (HDL-c) – (VLDL-c).

Serum activities of aspartate amino transferase AST, alanine amino transferase ALT (16), Alkaline Phosphatase (ALP) activities was measured according to the method described by Bergmeyer and Brent (17). From another hand serum urea nitrogen, uric acid, creatinine were determined according to the methods described by Patton and Crouch (18); Fossati et al. (19); Henry (20) respectively. Finally serum calcium and phosphorus were estimated according to method described by Baginski et al. (21); Yee (22) respectively. Burrin and Price (23) described the analytical method of serum blood glucose.

Statistical analysis

The obtained data were statistically analysed using SPSS statistical software version 11. The results were expressed as mean ± SD. Data analysed by one way analysis of variance (ANOVA).

Results and discussion

Data presented in table (1) showed the mean value of food intake of diabetic groups decreased than that of negative group (healthy rats). While, the mean value of food intake of all diabetic groups administrated with different levels of Olive Leaves (OL) and Fenugreek Leaves (FL) with levels (2.5%, 5% and 7.5%) increased compared with (+) control group. Treating diabetic rats with the three levels from each leaves increased the mean value of body weight gain %, as compared to the (+) control group. On other hand organs weight/body weight % for liver and kidneys revealed that diabetes mellitus raised the weight of all internal body organs indicating inflammative changes.
There were significant decrease $p<0.05$ in kidney and liver weight/body weight % between all treatments specially for 7.5% OL feeding groups, as compared to the (+) control group. Results are in agreement with those of Siddiqui et al. (24) which demonstrated that FL showed a significant increase in body weights as compared with the diabetic rats. Also liver weight of the diabetic rats decreased in comparison with the controls' from another hand there was an increase in kidney and liver weights of diabetic groups as compared with the controls
The effects of different levels of FL and OL on serum glucose and lipid profile of diabetic rats are presented in tables 2. It could be indicated that alloxan injection in rats caused a significant increase in serum glucose level in comparison with control rats. Moreover, all diabetic groups administrated

Table 1 - Effects of FL and OL leaves on feed intake, body weight % and some organs weight / body weight % of diabetic rats

Parameters Groups		Feed Intake (g/day/rat)	Body Weight Gain %	Organs weight / body weight % Liver	Kidney
Healthy rats (-)control group		14.98	39.016 a ± 2.666	2.786 e ± 0.119	0.566 e ± 0.053
Diabetic rats (+) control group		12.84	11.285 c ± 1.662	3.582 a ± 0.090	0.779 a ± 0.071
Diabetic rats fed on diet containing	2.5% fenugreek leaves	13.22	12.858 bc ± 1.143	3.314 b ± 0.087	0.693 b ± 0.059
	5% fenugreek leaves	13.77	14.294 b ± 1.088	3.112 c ± 0.066	0.648 b c d ± 0.048
	7.5% fenugreek leaves	13.98	13.956 b ± 1.203	3.111 c ± 0.068	0.640 b c d ± 0.039
	2.5% olive leaves	13.76	13.874 b ± 0.733	3.266 b ± 0.076	0.665 b c ± 0.036
	5% olive leaves	13.99	14.548 b ± 0.860	2.927 d ± 0.059	0.623 c d e ± 0.039
	7.5% olive leaves	13.71	14.092 b ± 0.882	2.799 e ± 0.035	0.595 d e ± 0.014

** Values with the same letters indicate non significant difference (P<0.05) and vice versa.*

with different levels of OL and FL with levels (2.5%, 5% and 7.5%) for each had significant decrease in serum glucose.

The results indicated significant elevation p<0.05 in serum glucose level in diabetic (+) control group as compared to (-) control group. Feeding diabetic rats on diet containing 2.5%, 5% and 7.5% OL and FL induced significant reduction (P<0.05) in serum glucose level with increasing the level of two leaves. It was clearly observed that groups fed on diet containing 7.5% OL and FL had the lowest values of serum glucose with 28.4% and 21.7% respectively compared with (+) control group. Feeding diabetic rats on diet containing 2.5%, 5% and 7.5% OL and FL induced significant reduction (P<0.05) in serum glucose level with increasing the level of two leaves. It was clearly observed that groups fed on diet containing 7.5% OL and FL had the lowest values of serum glucose with 28.4% and 21.7%, compared with (+) control group. Olive leaves significantly decreased the serum glucose level (25) and FL extract administrated to STZ-diabetic rats reduced blood glucose level by 58% (26, 27).

From table 2 it was clearly observed that 7.5% OL and 7.5% FL feeding groups had the lowest values of TC and TG (37.1% and 31.7%) for 7.5% OL and (32.8% and 28.7%) for 7.5% FL respectively compared to (+) control group.

Also table 2 illustrated that consuming the supplemented diet with 2.5%, 5% and 7.5% from both OL and FL to diabetic rats were significantly reversed all bad changes to near normal levels. This is in agreement with the facts that: (i) the level of glycaemic control is the major determinant of total cholesterol, VLDL-c and triglyceride levels; (28) and (ii) improved glycemic control following sulphonylurea therapy decreases the levels of serum VLDL-c and total triglycerides (29). It was clearly observed that 7.5% OL and 7.5% FL feeding groups had the lowest values of LDL-c and VLDL-c vice versa for HDL-c as compared to (+) control group.

Data presented are in agreement with Annida and Stanely (30) who's revealed that Fenugreek leaf

Table 2 - Effects of FL and OL leaves on serum glucose and lipid profile of diabetic rats

Parameters Groups		Glucose	Cholesterol	Triglycerides	HDL-c	LDL-c	VLDL-c
			mg/dl				
Healthy rats (-) control group		77.600 g ± 4.159	82.336 f ± 3.820	43.760 f ± 2.789	45.322 a ± 2.838	28.262 g ± 1.361	8.752 f ± 0.557
Diabetic rats (+) control group		185.800 a ± 5.263	165.721 a ± 5.697	73.110 a ± 4.383	26.276 e ± 1.669	124.822 a ± 3.369	14.622 a ± 0.876
Diabetic rats fed on diet containing	2.5% fenugreek leaves	173.00 b ± 4.00	152.861 b ± 3.605	62.850 b ± 2.080	31.620 d ± 2.437	108.677 b ± 1.226	12.569 b ± 0.416
	5% fenugreek leaves	154.600 d ± 1.516	126.843 c ± 4.526	56.082 c d ± 2.145	36.548 c ± 1.845	79.079 d ± 3.849	11.216 c d ± 0.429
	7.5% fenugreek leaves	145.519 e ± 3.386	111.344 d ± 5.192	52.102 e ± 1.323	39.470 b ± 2.405	61.454 e ± 2.655	10.420 e ± 0.264
	2.5% olive leaves	167.600 c ± 3.911	148.520 b ± 2.892	58.675 c ± 1.792	33.972 c d ± 2.157	102.812 c ± 1.847	11.734 c ± 0.358
	5% olive leaves ± 2.338	143.592 e ± 3.940	113.388 d ± 2.283	53.007 d e ± 2.159	40.322 b ± 2.055	62.525 e ± 0.443	10.541 d e
	7.5% olive leaves	133.168 f ± 3.524	104.282 e ± 4.184	49.951 e ± 3.805	42.005 b ± 1.447	52.337 f ± 2.290	9.990 e ± 0.761

** Values with the same letters indicate non significant difference (P<0.05) and vice versa.*

powder supplementation significantly lowered lipid peroxidation and significantly increased the antioxidant system in diabetic rats. Fenugreek leaf powder reduces oxidative stress in experimental diabetes rats. The hypoglycemic and hypolipidemic effects of OL may due to its total phenolic content (31) that ranged between 11.7-40.1 g/kg dried leaves which associated with the prevention of several diseases such as atherosclerosis and heart disease (32). FL has hypoglycemic and hypolipidemic effects may be due to its total dietary fibers nearly 24.6 g /100g (33), soluble fiber of fenugreek can improve glucose homeostasis by delaying carbohydrate digestion and absorption, and enhancing insulin action (34).

Table 2 showed that feeding diabetic rats on diet containing 2.5%, 5% and 7.5% from both OL and FL had recorded significant noticeable improvement in blood urea nitrogen (BUN), creatinine and uric acid parameters when compared with the (+) control group, especially for 7.5% groups followed by 5% then the 2.5% for both OL and FL respectively.

Table 3 showed that feeding diet containing 2.5%, 5% and 7.5% from both OL and FL to diabetic rats resulted in sufficient amelioration in both AST and ALT thus may be due to olive leaves and fenugreek leaves flavonoids contents. Reactive oxygen species and other free radicals cause hepatocellular damage, the treatment with phenolic compounds was found to be hepatoprotective

Table 3 - Effects of FL and OL leaves on kidney functions of diabetic rats

Parameters Groups		Uric acid	Urea nitrogen	Creatinine
			mg/dl	
Healthy rats (-) control group		1.310 f ± 0.055	27.386 f ± 1.969	0.518 f ± 0.069
Diabetic rats (+) control group		2.409 a ± 0.101	56.952 a ± 2.027	1.199 a ± 0.087
Diabetic rats fed on diet containing	2.5% fenugreek leaves	2.226 b ± 0.055	51.736 b ± 2.687	1.031 b ± 0.081
	5% fenugreek leaves	2.036 c ± 0.091	44.626 d ± 2.438	0.904 c ± 0.057
	7.5% fenugreek leaves	1.807 d ± 0.085	38.900 e ± 1.792	0.777 d ± 0.054
	2.5% olive leaves	2.072 c ± 0.079	48.366 c ± 1.778	0.932 c ± 0.041
	5% olive leaves	1.855 d ± 0.046	37.832 e ± 2.099	0.785 d ± 0.051
	7.5% olive leaves	1.685 e ± 0.060	33.561 f ± 1.731	0.653 e ± 0.039

** Values with the same letters indicate non significant difference (P<0.05) and vice versa.*

Table 4 - Effects of FL and OL leaves on kidney functions of diabetic rats

Parameters Groups		AST	ALT	ALP
			u/l	
Healthy rats (-) control group		60.086 f ± 3.367	24.230 f ± 1.819	87.012 f ± 4.176
Diabetic rats (+) control group		104.794 a ± 5.594	57.356 a ± 2.577	197.900 a ± 5.949
Diabetic rats fed on diet containing	2.5% fenugreek leaves	97.714 b ± 4.254	52.364 b ± 2.809	189.180 b ± 7.057
	5% fenugreek leaves	88.165 c ± 4.198	46.478 c ± 2.649	170.684 c ± 6.688
	7.5% fenugreek leaves	81.759 d ± 3.206	41.071 d ± 2.009	161.353 d ± 4.108
	2.5% olive leaves	92.238 c ± 3.648	46.679 c ± 2.744	183.800 b ± 7.259
	5% olive leaves	79.396 d ± 4.673	40.867 d ± 2.613	155.163 d ± 3.709
	7.5% olive leaves	71.468 e ± 2.855	35.237 e ± 2.922	145.916 e ± 2.908

** Values with the same letters indicate non significant difference (P<0.05) and vice versa.*

agents, because it acts as free radicals scavenging (28).

The activities of ALT and AST in rat groups fed on diet containing 7.5% from OL and FL recorded the best results, respectively as shown in table 4. The results are in agreement with those of Baynes (35) who reported that olive extracts scavengers of oxygen radicals are effective in preventing diabetes in experimental animal models, thus my led to improve liver functions.

Data in table 5 showed significant differences between the diabetic groups and non-diabetic group. Results illustrated that groups of

Table 5 - Effects of FL and OL leaves on serum calcium and phosphorus of diabetic rats

Parameters Groups		Calcium	Phosphorus
			Mmol/L
Healthy rats (-) control group		3.262 a ± 0.070	2.737 a ± 0.119
Diabetic rats (+) control group		2.250 f ± 0.071	1.843 f ± 0.107
Diabetic rats fed on diet containing	2.5% fenugreek leaves	2.407 e ± 0.092	1.992 e ± 0.111
	5% fenugreek leaves	2.639 c d ± 0.082	2.213 b c ± 0.059
	7.5% fenugreek leaves	2.527 d ± 0.077	2.076 d e ± 0.080
	2.5% olive leaves	2.532 d ± 0.081	2.137 c d ± 0.094
	5% olive leaves	2.761 b ± 0.097	2.282 b ± 0.058
	7.5% olive leaves	2.705 b c ± 0.089	2.239 b c ± 0.047

** Values with the same letters indicate non significant difference (P<0.05) and vice versa.*

rats fed on diet containing 7.5% OL and group of 5% FL had a significant increase in serum calcium and phosphorus as compared with (+) control group, this results in agreement with Anastassios et al. (36) who reported that diabetics often have low blood concentrations of calcium and low intake of dietary calcium. Calcium also is critical to the intracellular communication between the hormone insulin and the tissues in the body that work with insulin. Our results are in agreement with those of Abbas and Ebenezer (37) how reported that Severe derangement of water and electrolyte balance occurs in Diabetic ketoacidosis (DKA), hyperglycemia, and hyperketonemia associated with fluid and electrolyte abnormalities. Also our findings inline of those of DeFronzo et al. (38) whom revealed that osmotic diuresis resulting from hyperglycemia promotes net loss of multiple minerals and electrolytes, including calcium, sodium, potassium, magnesium, chloride, and phosphate. And also with Joseph et al. (39) who revealed that data from animal models of diabetes and from studying patients with diabetes reveal that intracellular calcium levels are increased in most tissues. The activities of the membrane, adenosine triphosphatase (ATPase) associated cation pumps, which determine intracellular calcium level.

Conclusion

The present study concluded that 7.5% dietary supplement of Olive leave (OL) having more potential than Fenugreek leave (FL) at same doses. Our results suggest that olive leave (OL) and fenugreek leave (FL) reduced the risk of diabetes and hyperlipidemia may due to the rich dietary fibers and phenolic compounds are contained in both leaves. However, further isolation and characterization of antidiabetic active principle(s) from Olive leave should be carried out.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saudi University for funding the work through the research group project no RGP-VPP-193).

References

1. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005; 59:365–373.
2. Jacotot B. L'huile d'olive - De la gastronomie à la santé. Artulen, Paris; 1993.
3. Tutour LB, Guedon D. Antioxidative activities of Olea europaea leaves and related phenolic compounds. Phytochemistry 1992;31: 1173–1178.
4. El SN, S Karakaya. Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. Nutr. Rev 2009; 67: 632-638.
5. Cvjetićanin T, Miljković D, Stojanović I., Dekanski D, S Stosić-Grujicić. Dried leaf extract of Olea europaea ameliorates islet-directed autoimmunity in mice. Br J Nutr 2010; 103: 1413-1424.
6. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2002; 81: 81–100
7. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. Food Rev Int 2006; 22: 203–224
8. Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ. TSP foenum graecum (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol Cell. Biochem 2001; 224: 45–51
9. Khalki L, M'hamed SB, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from *Trigonella foenum-graecum* (L.) in mice. J Ethnopharmacol 2010; 15: 321–325
10. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216-226.
11. Reeves PG, Nielsen FH, Fahmy GG, AIN-93. Purified diets for laboratory rodents: Final report of the American Institute of Nutrition adhoc writing committee on the reformulation of the AIN-76 A Rodent diet . J Nutrition 1993;123: 1939-151.
12. Allain CC, Poon LS, Chan CS. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-475
13. A.W. Wahlefeld, Enzymatic Determination of Triglycerides. Methods of Enzymatic Analysis. Vol. 5. Bergmeyer, Ed: New York, 1974.
14. Albers N, Benderson V, Warnick G. Enzymatic determination of high density lipoprotein cholesterol : Selected Methods .Clin Chem 1983; 10: 91-99.
15. Fridewald WT, Leve RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein. Clin Chem 1972; 18:499-502.
16. Reitman S, Frankel S. A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28: 56.
17. H.U. Bergmeyer, and E. Brent, Methods of enzymatic analysis, Vol.2. Bergmeyer, Ed: New York, 1974.
18. Patton C, Grouch SR. Enzymatic determination of urea. Anal Chem 1977; 49: 464-468.
19. Fossati P, Prencipe L, Berti G. Uric acid measurements with enzymatic colorimetric method. Medicinal Clin Chem 1980; 26: 227-273.
20. R.J. Henry, Creatinine measurements with colorimetric method, principles and techniques, Second Edition. Harper& Row: New York, 1974.
21. Baginski ES. Method of calcium determination. Clin Chem Acta 1973; 46: 49.
22. Yee HY. Dietary Calcium: adequacy a vegetarian diet. Am J Clin Nutr 1968; 59: (suppl). 12385.
23. Burrin JM, Price CP. Measurement of blood glucose. Ann Clin Biochem 1985; 22: (Pt 4):327-42.
24. Siddiqui MR, Moorthy K, Taha A, Hussain EM., Baquer NZ. Low doses of vanadate and Trigonella synergistically regulate Na+/K+ ATPase activity and GLUT-4 translocation in alloxan diabetic rats. Mol Cell Biochem 2006; 285: 17–27.
25. Hedya J, Abdelfattah E, Sami S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J Agric Food Chem 2009; 19:, pp 8798–8804.
26. Gad MZ, El-Sawalhi MM, Ismail MF, El-Tanbouly ND. Biochemical study of the anti-diabetic action of the Egyptian plants fenugreek and balanites. Mol Cell Biochem 2006; 281(1-2):173-83.
27. Annida B, Stanely M, Prince P. Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. J Med Food 2004; 2:153-6.
28. Laakso M. Epidemiology of diabetic dyslipidemia. Diabetes Rev 1995;3: 408–22.
29. Taskinen MR, Beltz WF, Harper I. Effects of NIDDM on verylow- density lipoprotein, triglyceride and apolipoprotein B metabolism. Studies before and after sulfonylurea therapy. Diabetes 1986; 35: 1268– 77.
30. Annida B, Stanely PM. Supplementation of Fenugreek Leaves Reduces Oxidative Stress in Streptozotocin-Induced Diabetic Rats. J of Med Food 2005; 3:382-385.
31. Romani A., Mulinacci N, Pinelli P, Vincieri F, Cimato A. Polyphenolic content in five Tuscany cultivars of Olea europaea L. JAFC 1999; 47: 964–967.
32. Silva S, Gomes L, Leitão F, Coelho AV, Vilas Boas L. Phenolic Compounds and Antioxidant activity of olea europaea l. fruits and leaves. Food Sci Tech Int 2006; 12(5):385–396.
33. USDA National Nutrient Data Base. [Internet]. c2002. Available from:.

http://www.nutrition-and-you.com/fenugreek-seeds.html
34. Hannan JM, Ali L, Rokeya B, et al. Soluble dietary fibre fraction of Trigonella foenum-graecum (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. Br J Nutr 2007; 97(3):514-21.
35. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405–412.
36. Anastassios G, Pittes MD, Josephalu Frank HU, Bess D. The role of vitamin d and calcium in type 2 diabetes. a systematic review and meta-analysis. J Clin Endocrinol Metab 2007; 6: 2017–2029.
37. Abbas EK, Ebenezer AN. Hyperglycemic crises in diabetes mellitus: diabetic ketoacidosis and hyperglycemic hyperosmolar state. Endocrinol Metab Clin N Am 2006; 35:725–751.
38. DeFronzo RA., Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. J Clin Invest 1975; 55: 845–55.
39. Joseph L, James RG, James RS. Diabetes mellitus: A disease of abnormal cellular calcium metabolism. Am J of Med 1994; 260-273.