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Hypoglycemic and Hypolipidemic Effect of Fenugreek in Different Forms on Experimental Rats

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Abstract: Hypoglycemic and hypolipidemic effect of fenugreek was investigated on forty-two Sprague Dawley adult male rats. Seven rats kept as control (-ve) and 35 Streptozotocin induced diabetic rats. The diabetic rats were classified into control (+ve) and four treated groups which were fenugreek powder (5% in diet), aqua extract, methanolic extract (3mg/kg body weight) and oil (5% in diet). The experiment period was 60 days. Fenugreek powder, aqua extract, methanolic extract and oil groups showed improvement in nutritional status and liver and renal function of diabetic rats. Compared with control (+ve) group, the four treated groups showed significant decrease in glucose, glucosalated heamoglobin (HbA_{Ic} %) and fructoseamine (FA) values, but significant increased in insulin. Also, they produced a significant fall in various serum lipids like total cholesterol, triglycerides, low and very low density lipoprotein cholesterol (LDLc &VLDLc) and increased high density lipoprotein cholesterol (HDLc) in the diabetic rats. On the other side, they showed a significant decrease in liver cholesterol and total lipids and a significant increase in liver triglyceride and glycogen compared to control (+ve) group.

Key words: Blood lipid · Blood glucose · Diabetes · Fenugreek · Rats

INTRODUCTION

Diabetes is a chronic disease of pancreatic origin, marked by insulin deficiency, excess sugar in the blood and urine, weakness and emaciation. The diet recommended for people with diabetes is used to help control and regulate blood sugar levels and body weight. Diabetes is recognized for severe complications such as diabetic nephropathy, neuropathy and retinopathy [1, 2]. Fenugreek is an annual, leguminous plant. The plant is probably indigenous to eastern Mediterranean, West Asia and India. It has been grown as a traditional minor pulse crop in the Mediterranean area, Middle East countries, India and Africa. It is grown in small scale in Europe, North America, Latin America and China. It has tri-foliate, obviate and toothed, light green leaves. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making [3]. Fenugreek imparts a characteristic flavor and tang to food and also has several very important disease preventing characteristics. Fenugreek is one of the oldest medicinal plants. It has been used for centuries for different female conditions, brain and nervous system ailments, skin, liver and metabolic disorders. It is also considered highly beneficial for respiratory and gastrointestinal problems. It is a highly potent female herb, since it helps relaxing the uterus and relieving menstrual pains and is an excellent stimulator of milk production in nursing mothers [4, 5]. Fenugreek is usually suggested in treatments of poor digestion, gastric inflammations, enteritis, especially for convalescents. It can also be used in cases of weight loss, poor appetite and even in treatment of anorexia nervosa. Fenugreek can treated different blood conditions, such as anemia and nervous system disorders. Fenugreek is excellent in treatment of bronchitis, mucous congestions, different infections [4, 6]. The present study was designed to investigate the hypoglycemic and hypolipidemic effects of fenugreek in the form of powder, extract (aqua or methanolic) and oil.

MATERIALS AND METHODS

Materials: Streptozotocin was procured from Sigma, St. Louis, MO, USA. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt. A total of forty-two Sprague -Dawley adult male

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rats, weight 140±6 g were purchased from the National Research Center, Giza, Egypt. The standard diet was performed according to NRC [7]. Fenugreek (*Trigonella foenum-graecum*) seed and oil were purchased from Agricultural Research Center.

Methods: Fenugreek seeds were cleaned, dried and finely powdered. Fenugreek powder and oil were added in diet as 5%. Aqua extract is prepared by boiling of 100 g of fenugreek seeds in 1000 ml distilled water for 30 minutes, cooled and filtrate. Finally, the filtrate was concentrated by flash evaporation at 358°C to a thick paste. However, the methanolic extract was prepared by mixing 25 g fenugreek powder with 150 ml of 95% methanol with stirring and repeated twice. The extract was evaporated to dryness using a Rotavapor and water bath under vacuum and stored at 4°C until further analyzed. Rats administered fenugreek aqueous and methanolic extract at dose 3 mg/kg body weight by oral intragastric intubation. After adaptation period (one week), seven rats kept as normal control (-ve) fed on standard diet only. The rest of rats were injected with a single intraperitoneal dose of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection in order to prevent hypoglycemia [8]. After four days, blood samples were taken from orbital plexus for estimation of glucose. The rats having persistent hyperglycemia were considered as diabetic rats and used for the experiment. The diabetic rats were classified into control (+ve) and four treated groups that were powder, aqua extract, methanolic extract and oil of fenugreek. The food intake was calculated daily and the body weight gain was recorded weekly. Feed efficiency ratio (FER) was determined by Chapman *et al.* [9].

At the end of experiment (60 days), rats were sacrificed then blood and liver of each rat were collected. Blood hemoglobin (HB), packed cell volume (PCV), fructoseamine (FA) and glucose were estimated according to Drabkin [10], Mc Inory [11], Delpierre and Schaftingen [12] and Sasaki *et al.*[13]. Serum insulin and glucosalated heamoglobin (Hb A1c %) were estimated according to Wilson and Miles [14] and Abraham *et al.* [15], respectively. Serum alanine and aspartate aminotransferase (ALT&AST) and alkaline (ALP) and gama glutamyle transferase (γGT) activity enzymes, creatinine, urea and uric acid were estimated according to Reitman and Frankel [16], Kind and King [17], Henry [18], Bonsens and Taussky [19], Patton and Crouch[20] and Fossati *et al.* [21], respectively. Serum cholesterol, triglycerides(TG), high density lipoprotein cholesterol (HDLc), low and very low density lipoprotein cholesterol (LDLc & VLDLc) were determined according to Trinder and Ann [22], Young and Pestaner [23], Richmond [24], Fruchart [25] and Friedwald *et al.* [26], respectively. Atherogenic index (cholesterol /HDL-c) was calculated according to Castelli and Levitar [27]. Livers samples were analyzed for estimation of cholesterol, total lipids, glycogen and triglyceride according to Abell *et al.* [28], Folch *et al.* [29], Rerup and Lundquist [30] and Scheletter and Nussel [31], respectively.

Statistical Analysis: Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry [32].

RESULTS

Table 1 illustrated that weight gain was significantly decreased in control (+ve) group at $p<0.001$ and in both powder and aqua extract fenugreek rat groups at $p<0.01$compared to control (-ve) group. Food intake was insignificantly difference among all experimental groups while FER was significantly decreased at $p<0.001$ in control (+ve) group and at $p<0.01$ in diabetic groups that treated with powder, aqua extract, methanolic extract and oil of fenugreek compared to control (-ve) group. The four treated groups showed improvement in nutritional status due to increase of weight gain and FER compared to control (+ve) group.

Table 2 showed that PCV value was significantly decreased at $p<0.01$ but HbA_{IC} % was significantly increased at $p<0.001$in control (+ve) group compared to control (-ve) group. The diabetic rat groups which treated with powder, aqua extract, methanolic extract and oil of fenugreek showed non significant difference in HB, PCV and HbA_{IC} % compared to control (-ve) group. HB value was insignificantly difference but HbA_{IC} % was significantly decreased in treated groups compared to control (+ve) group. PCV value was significantly increased in aqua extract, methanolic extract and oil of fenugreek compared to control (+ve) group.

Table 3 recorded that glucose and fructose amine (FA) values were significantly increased at $p<0.001$ but insulin was significantly decreased at $p<0.001$in control (+ve) group compared to control (-ve) group. The diabetic rat group which treated with fenugreek powder showed significant increased in glucose and FA at $p<0.01$and 0.05 but significant decreased in insulin at $p<0.05$ compared to control (-ve) group. The diabetic rat groups which treated with fenugreek aqua extract and methanolic extract showed non significant difference in glucose, insulin and FA at $p>0.05$ but diabetic rat group which treated with fenugreek oil showed insignificant difference in

Table 1: Mean values ± SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	Powder	Aqua extract	Methanolic extract	Oil
Weight Gain (g)	80.14±7.81^{a}	32.66±4.31^{d***}	61.41±7.51^{c**}	69.45±7.71^{bc**}	73.20±8.11^{ab}	81.61±8.24^{a}
Food Intake(g/d)	15.21±1.31^{a}	11.31±1.22^{a}	14.35±1.41^{a}	15.12±1.39^{a}	15.67±1.25^{a}	16.24±1.33^{a}
FER	0.087±0.005^{a}	0.048±0.004^{e***}	0.071±0.003^{cd**}	0.076±0.001^{c**}	0.077±0.002^{c**}	0.083±0.001^{b**}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 2: Mean values ± SD of HB, PCV and HbA_{IC} of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	Powder	Aqua extract	Methanolic extract	Oil
HB (g/dl)	12.96±1.51^{a}	11.01±1.67^{ab}	12.71±1.35^{a}	12.44±1.51^{a}	12.66±1.33^{a}	13.01±1.24^{a}
PCV %	35.16±3.17^{a}	30.61±2.66^{b**}	31.99±2.01^{ab}	34.21±3.61^{a}	33.41±4.20^{a}	32.31±3.61^{a}
HbA_{IC} %	3.99±0.38^{b}	6.80±1.10^{a***}	4.40±0.55^{b}	4.01±0.65^{b}	4.31±0.87^{b}	4.12±0.88^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 3: Mean values ± SD of glucose, insulin and FA of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	Powder	Aqua extract	Methanolic extract	Oil
Glucose (mg/dl)	99.61±8.96^{c}	275.11±21.20^{a***}	137.40±13.27^{b**}	119.41±13.14^{bc}	120.36±14.13^{bc}	117.99±12.16^{bc}
Insulin (µ/l)	16.33±2.10^{a}	8.51±1.61^{c***}	12.11±1.49^{b*}	13.21±1.25^{ab}	14.31±1.41^{a}	14.27±1.33^{a}
FA (µmol/l)	155.71±10.15^{c}	230.14±22.04^{a***}	182.22±17.11^{b*}	170.50±15.41^{bc}	165.14±13.61^{bc}	172.22±14.66^{b*}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 4: Mean values ± SD of serum ALT, AST, ALP and γGT enzymes of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	powder	Aqua extract	Methanolic extract	Oil
ALT (µ/ml)	22.66±2.11^{c}	45.67±5.41^{a***}	29.61±2.60^{b*}	30.41±3.20^{b*}	31.41±3.07^{b*}	29.61±2.88^{b*}
AST (µ/ml)	37.40±4.41^{c}	71.30±8.61^{a***}	45.51±5.31^{b*}	47.71±5.61^{b*}	46.42±4.90^{b*}	43.61±5.01^{bc}
ALP (i/ml)	29.66±2.55^{b}	55.67±5.41^{a***}	28.96±2.95^{bc}	35.61±4.21^{b}	31.61±3.51^{b}	33.61±3.61^{b}
γGT(i/ml)	5.81±1.35^{b}	9.11±1.96^{a***}	6.67±1.41^{b}	6.53±1.31^{b}	5.84±1.25^{b}	5.71±1.34^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table 5: Mean values ± SD creatinine, urea and uric acid of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	Powder	Aqua extract	Methanolic extract	Oil
Creatinine (mg/dl)	0.68±0.05^{d}	1.24±0.29^{a***}	0.75±0.04^{bc*}	0.78±0.03^{b*}	0.72±0.01^{c*}	0.85±0.03^{b**}
Urea (mg/dl)	32.41±3.11^{bc}	65.33±5.17^{a***}	38.41±4.25^{b}	36.61±4.11^{b}	37.21±3.28^{b}	35.61±3.19^{bc}
Uric acid	3.44±0.22^{bc}	6.16±1.01^{a***}	4.80±0.77^{b}	4.30±0.56^{b}	4.16±0.70^{b}	4.24±0.84^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 6: Mean values ± SD of serum lipid patterns of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	Powder	Aqua extract	Methanolic extract	Oil
Cholesterol (mg/dl)	160.11±19.12^{bc}	225.11±30.61^{a***}	175.17±20.40^{b}	180.61±21.22^{b}	170.54±19.66^{b}	178.61±18.81^{b}
TG (mg/dl)	131.61±12.13^{b}	185.33±15.21^{a***}	135.41±13.71^{b}	141.25±14.96^{b}	140.31±14.19^{b}	135.41±13.22^{b}
HDLc (mg/dl)	35.40±2.47^{a}	20.66±1.98^{c***}	28.96±2.14^{b*}	29.91±2.55^{b*}	31.21±3.31^{ab}	30.31±3.61^{ab}
LDLc (mg/dl)	98.39±8.91^{c}	167.39±14.19^{a***}	119.13±13.17^{b*}	122.45±12.14^{b*}	11.27±12.10^{c}	121.22±11.61^{b*}
VLDLc (mg/dl)	26.32±3.11^{b}	37.06±3.14^{a***}	27.09±2.61^{b}	28.25±3.01^{b}	28.06±2.19^{b}	27.08±2.61^{b}
Cholesterol /HDLc	4.52±0.55^{bc}	10.89±1.41^{a**}	6.04±1.22^{b}	6.03±1.03^{b}	5.46±0.69^{b}	5.89±0.78^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 7: Mean values ± SD of liver cholesterol, total lipid, triglyceride (TG) and glycogen of the experimental rat groups.

			Fenugreek			
Variables Groups	Control (-ve)	Control (+ve)	powder	Aqua extract	Methanolic extract	Oil
Cholesterol (mg/100g)	4.11±0.66^{b}	7.21±1.31^{a***}	4.81±0.65^{b}	4.55±0.60^{b}	4.33±0.56^{b}	4.21±0.55^{b}
Total lipid (mg/100g)	36.11±3.51^{bc}	48.14±4.41^{a***}	40.31±3.61^{b}	41.21±4.10^{b}	38.31±5.11^{bc}	39.35±4.11^{b}
TG (mg/100g)	3.11±0.77^{a}	1.88±0.11^{b***}	2.75±0.29^{a}	2.99±0.35^{a}	2.87±0.31^{a}	2.96±0.44^{a}
Glycogen (mg/100g)	8.22±1.75^{ab}	3.20±0.56^{c***}	5.99±1.14^{b}	6.80±1.30^{b}	6.70±1.25^{b}	6.88±1.41^{b}

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

glucose and insulin at p>0.05 and significant increased in FA at p<0.05 compared to control (-ve) group. Fenugreek powder, aqua extract, methanolic extract and oil groups showed significant decrease in glucose and (FA) values but significant increased in insulin compared to control (+ve) group.

Concerning the results in Table 4, the control (+ve) rat group showed a significant increase in serum ALT, AST, γGT and ALP (P<0.001) however powder, aqua extract and methanolic extract fenugreek rat groups showed a significant increase in serum ALT and AST at p<0.05 and non significant difference in ALP and γGT compared to control (-ve) group. Fenugreek oil group showed a significant increase in serum ALT at p<0.05 and insignificant difference in AST, ALP and γGT compared to control (-ve) group. Powder, aqua extract, methanolic extract and oil of fenugreek rat groups showed a significant decrease in serum ALT, AST, γGT and ALP compared to control (+ve) group.

Concerning the results in Table 5, the control (+ve) rat group showed a significant increase in serum creatinine, urea and uric acid (P<0.001), however four treated rat groups showed a significant increase in serum creatinine at p<0.05 and 0.01 and insignificant difference in urea and uric acid (P>0.05) compared to control (-ve) group. Powder, aqua extract, methanolic extract and oil of fenugreek rat groups showed a significant decrease in serum creatinine, urea and uric acid compared to control (+ve) group.

Table 6 illustrated that the control (+ve) rat group showed a significant increase in serum cholesterol, TG, LDLc, VLDLc and cholesterol/HDLc at P<0.001 and significant decrease in serum HDLc compared to control (-ve) group. Powder, aqua extract, methanolic extract and oil of fenugreek rat groups showed a non significant difference in cholesterol, TG, VLDLc and cholesterol/HDLc compared to control (-ve) group. Powder and aqua extract groups showed a significant decrease in HDLc and a significant increase in LDLc, while fenugreek oil group showed a significant decrease in LDLc compared to control (-ve) group. On the other side, four treated rat groups showed a significant decrease in serum cholesterol, TG, LDLc, VLDLc and cholesterol/HDLc but a significant increase in serum HDLc compared to control (+ve) group.

Table 7 illustrated that the control (+ve) rat group showed a significant increase in liver cholesterol and total lipid at P<0.001 and a significant decrease in liver triglyceride (TG) and glycogen however powder, aqua extract, methanolic extract and oil fenugreek rat groups showed a non significant difference in liver cholesterol, total lipid, TG and glycogen compared to control (-ve)

group. On the other side, four treated rat groups showed a significant decrease in liver cholesterol and total lipid and a significant increase in liver TG and glycogen compared to control (+ve) group.

DISSCUSION

The improvement of the obtained nutritional status results were related to the constituents of the fenugreek seeds that are a rich source of vitamins, minerals and antioxidants, which help protect the body's cells from damage caused by unstable molecules known as free radicals. Fenugreek traditionally used to promote digestion, improve appetite and to support respiratory health. The high-fiber seeds also support for healthy bowel function and its lecithin content promotes fat metabolism [33]. The obtained biochemical results showed hypoglycemic effect of fenugreek. These results were agreed with the dietary intervention studies that have found improvements in glycaemic control after increasing the dietary intake of fenugreek. The benefit has been attributed to slow release carbohydrate and increase in soluble fiber, which helps lower blood sugar by slowing down digestion and absorption of carbohydrates. Fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble) and the gum is composed of galactose and mannose. The latter compounds are associated with reduced glycemia and cholesterolemia. Fenugreek seeds contain alkaloid trigonelline and protein high in lysine and L-tryptophan. Fenugreek has steroidal saponines, diosgenin, yamogenin, tigogenin and neotigogenin [34, 35]. Fructoseamine may undergo further spontaneous reactions resulting in advanced glycation end product. Fructoseamine in plasma is an alternative to measure glycosalated hemoglobin for evaluation of diabetic control. Fenugreek showed the most consistency in lowering fasting blood sugar or glycated hemoglobin (HbA1c) levels in diabetic patients [3,12]. Daily oral administration of Soluble dietary fiber fraction (SDF)of T. foenum-graecum seeds to type 2 diabetic rats for 28 day decreased serum glucose increased liver glycogen content and enhanced total antioxidant status. SDF exerts antidiabetic effects mediated through inhibition of carbohydrate digestion and absorption and enhancement of peripheral insulin action [4]. *In vitro*, the amino acid 4-hydroxyisoleucine in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. In human studies, fenugreek reduced the area under the plasma glucose curve and increased the number of insulin receptors. Fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism [36].

There is accumulating evidence to support the hypothesis that fenugreek consumption is associated with an improvement of liver and kidney function in diabetic individuals by increasing activities of antioxidants and inhibiting accumulation of oxidized DNA in the kidney, suggesting a potential drug for the prevention and therapy of diabetic nephropathy. Serum creatinine, blood urea nitrogen, 24-hour content of urinary protein and creatinine clearance were significantly decreased in fenugreek seed aqueous extract compared with non treated diabetic nephropathy rats [37]. Fenugreek seeds reduced serum total cholesterol, LDL and VLDL cholesterol and triglycerides and unchanged HDL cholesterol fraction. These results indicate the usefulness of fenugreek seeds in the management of diabetes in human [38]. HDL cholesterol increased significantly in group who received 1 gm/day hydroalcoholic extract of fenugreek seeds as compared to diabetic group patients. Fenugreek extract had no effect on calorie intake or body weight and opposed the development of experimental high fat diet in diabetes in mice and had an anti-diabetic effect in mice with established diabetes [5, 39]. Improved activities of antioxidants that protect the organs such as liver and pancreas against the oxidative damage induced by diabetes and a significant decline in the levels of thiobarbituric acid reactive substances (TBARS) were observed in fenugreek treated diabetic rats [40]. Rats treated with Trigonella foenum-graecum extract had lower blood glucose, glycated hemoglobin, triglycerides, total cholesterol and higher higher-density-lipoprotein-cholesterol in a dose-dependent manner. *Trigonella foenum-graecum* extract can lower kidney/body weight ratio, blood glucose, blood lipid levels and improve hemorheological properties in experimental diabetic rats following repeated treatment for 6 weeks [37, 41]. It may be concluded that fenugreek in the form of the powder, extract and oil can reduce hyperglycemia and hyperlidemia with improvement of nutritional status, liver and kidney function in experimental diabetic rats. It is recommended to make dietary supplements with fenugreek supplement in any form either powder or extract or oil to control blood glucose and making functional food products at home for prevention of diabetes.

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