

Effect of Fenugreek (*Trigonella foenum graecum*) Seed Aqueous Extract on Blood Glucose, Lipid Profile and Some Hormonal Assay in Streptozotocin-induced Diabetic Male Albino Rats

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia over a prolonged period, since a long time, diabetes has been treated with plant medicines, one of these important plants is *Trigonella foenum-graecum* (fenugreek), thus, the investigation goals of this study to clarify the role of fenugreek seed aqueous extract in its therapeutic dose on blood glucose, triglycerides, total cholesterol, lower density lipoprotein (LDL), higher density lipoprotein (HDL), very lower density lipoprotein (VLDL), liver and kidney function by estimating alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea and serum creatinine, also to clarify the role in treatment of infertility by estimating luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone hormone level, sperm count, motility and viability in Streptozotocin induced diabetic rat for 4 weeks of treatment, 60 adult male albino rats were divided into 4 groups (15 rat for each group), control (normal rats received normal saline), diabetic rats without treatment, diabetic rats were treated with 300 mg/kg of methanolic plant extract for 4 weeks, diabetic rats treated with 650mg/kg of metformin drug. The result indicated that after 4 weeks of treatment of fenugreek seed aqueous extract, there was an improvement in blood glucose, lipid profile, liver, and kidney function. Although there was an improvement in LH, FSH, testosterone hormone, sperm count, viability and motility

Keywords: Diabetes mellitus, Blood Urea, Serum creatinine, *Trigonella foenum graecum*.

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INTRODUCTION

The DM is a complex, chronic, and very serious disease, characterized by a person's blood sugar level become too high that resulted from the pancreatic β -cells generate deficient insulin (a hormone that regulates blood glucose level) secretion.¹ It is caused by acquired or inherited a deficiency in insulin production by the pancreas or resistance of the target organ to the action of insulin. The most common complications of diabetes are ketoacidosis, blindness, cardiovascular disease, and kidney failure.² Diabetic can be classified into three main categories, type 1 due to complete destruction of β -cell, leading to absolute insulin deficiency, type 2-progressive loss of β -cell insulin secretion, and type 3 that occurred during pregnancy.³

In recent years, herbal medicines have gained importance as a hypoglycemic agent. The plants play an essential role in disease treatment because these plants are a rich source

of compounds that can be used in drug synthesis. Herbal drugs are safe, less toxic, no side effects, and low cost.⁴ *Trigonella foenum-graecum*, known as fenugreek, assumed to possess several beneficial, nutritive, restorative properties and containing numerous active medical compounds.⁵ It has been used for the treatment of many diseases, including abdominal colic, fever, antiulcer, and anti-inflammatory.⁶ Several studies have evaluated that fenugreek seeds aqueous extract can improve blood glucose and lipid parameters of diabetics patients.^{7,8}

Although the mechanism of action of fenugreek is not known accurately, various researcher assuming different route of mechanism, many researchers believe that fenugreek has the effect of increasing insulin secretion from beta-cell of the pancreas, some of them believe that fenugreek decrease glucose uptake by the intestine, others established that fenugreek has an

insulin-sensitizing effect.⁹ Hyperlipidemia is associated with DM. The antihyperlipidemic effect of fenugreek seed extract may be related to soluble fiber, which inhibits cholesterol synthesis in the liver as well as increase fecal excretion of bile salts.¹⁰

Infertility is a common problem with medical, psychosocial, and economic aspects. Infertility affects 15%–20% of couples. A one third of all cases of infertility is due to a combination of female and male problems. In another third of cases, they are because of the male problem.¹¹ Plants have been used worldwide for the screening of several human diseases,¹² and have a key role in the treatment of infertility.^{13,14} *Trigonella foenum-graecum* seed extract has beneficial effects on reproductive hormones; it has androgenic and anabolic activity in patients.¹⁵ This activity may be related to the fact that this extract contains soluble glycosides (furostanol), which are responsible for increasing testosterone level and complexation of cholesterol in the cell membrane.^{16,17} Other studies have found that *Trigonella foenum-graecum* increases sperm viability and decreases the abnormality in sperm shape.¹⁸ study aimed to evaluate the effect of *Trigonella foenum-graecum* aqueous extract on blood glucose, lipid profile, and some hormonal assay in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant extraction

The plant material of *Trigonella foenum-graecum* brought from the local market, and the park was powdered, extracted in Soxhlet apparatus with methanol, starting with hexane and chloroform, to separated lipids and terpenoid, after that methanol was used to extract the other components, then each component was dried and collected.¹⁹

Animals

Sixty adult male albino rats weighing 100-110 g were used in the current work. All rats were kept at room temperature, fed with standard rat pellet diet, and provided water ad libitum. The animal was treated with 300 mg/kg of *Trigonella* seed aqueous extract, and this dose was selected after a series of primary experiments.

Streptozotocin-induced diabetes

The rats were allowed to fast for 24 hours before experimentation and rendered diabetic by intraperitoneal injection of streptozotocin 80 mg/kg body weight dissolved in normal saline.²⁰ After 18 hours of injection, diabetes was confirmed by testing blood sugar. Then the animal was divided into four groups, each with 15 rats and treated the plant extract orally using a gavage tube.²¹

Group 1: Rats were treated with normal saline as a control group

Group 2: Diabetic rats without treatment

Group 3: Diabetic rats were treated with 300 mg/kg of methanolic plant extract

Group 4: Diabetic rats treated with 650mg/kg of metformin drug

Blood sampling:

Blood samples from rats were collected by direct heart puncture, centrifuged at 3000rpm for 15 minutes, and divided into two parts, and the first part was used for biochemical tests(glucose and lipid parameters), the second part was used for estimation of testosterone, LH and FSH. Serum glucose level was measured every 3, 6, 9 hours by using (glucose enzymatic colorimetric test kit) from Biocon Diagnostik, Germany. Triglycerides, total cholesterol, LDL, HDL, and VLDL were estimated by a commercial kit. The level of ALT was evaluated by using Elisa kit(catalog MBS 041480 from MyBiosource/ USA) , AST was estimated by using Elisa kit(catalog MBS 264975 from MyBiosource/USA), blood urea was measured using Elisa kit (catalog -MBS 752119 from MyBiosource/ USA) and serum creatinine was measured using Elisa kit (catalog-MBS 764869 from MyBiosource/USA), testosterone was assessed using rat testosterone Elisa kit(catalogMBS282195 from MyBiosource/USA) , LH level was evaluated by using Elisa kit(catalog MBS 729873 from MyBiosource/USA) and FSH level was estimated by Elisa kit (catalog MBS 2502190 obtained from MyBiosource/USA).

Sperm collection and evaluation

The right epididymis of mice in each group were removed and minced into several pieces on a Petridis with RPMI 1640 media for a few minutes to allow sperm to become motile and swim out from epididymis. The semen was then taken with 1 mL pipette and dropped on a clean slide and covered with a coverslip. The sperm concentration was determined by the conventional method using a hem cytometer chamber for the red blood cell (RBC) count, the progressive sperm motility (spm) was estimated by evaluating five fields of a sperm droplet under a coverslip on a warm glass slide (36_37c)under a light microscope (x40). The sperm vitality was assayed using a procedure of eosine _ngrosine stain (1.67 eosin 10% nigrosin and 0.1M sodium citrate)under x100 magnificent, and 100 sperm were counted, all of the sperm evaluation were estimated according to on world health organization manual sperm analysis.²²

Statistical Analysis

The data expressed as mean ± S.E. and were analyzed using SAS system²³

RESULTS

Blood glucose of Streptozotocin-induced diabetic rats showed statistically decrease after treatment by fenugreek seed aqueous extract compared with diabetic non treated groups, as shown in Table 1

Table 2 illustrated the significant decrease in triglyceride, cholesterol, HDL, and VLDL while LDL shows increasing in its concentration compared to the diabetic nontreated group

Table 1: Effect of *Trigonella* seed aqueous extract on blood glucose after four weeks of treatment

Groups	Control	Diabetic	Diabetic + <i>Trigonella</i>	Diabetic + metformin
Glucose mg\dl	80.54 ± 3.21	300 ± 6.11	170 ± 5.3 9	192 ± 4.29

Table 2: Effect of *Trigonella* seed aqueous extract on lipid profile after four weeks of treatment

Groups	Control	Diabetic	Diabetic + <i>Trigonella</i>	Diabetic + metformin
Triglycerides mg/dL	77.65 ± 2.29	96.45 ± 4.34	83.38 ± 3.44	80.44 ± 0.23
Cholesterol mg/dL	90.32 ± 4.34	119.34 ± 4.32	105.22 ± 5.32	102.39 ± 2.54
HDL mg/d	45.23 ± 1.24	32.27 ± 1.29	40.26 ± 1.43	35.19 ± 0.45
LDL mg/d	30.22 ± 2.32	68.29 ± 2.54	49.52 ± 3.25	L 53.42 ± 1.46
VLDL mg/dL	15.62 ± 1.04	18.32 ± 4.25	16.63 ± 0.96	L 16.00 ± 0.46

Table 3: Effect of *Trigonella* seed aqueous extract on some liver and kidney function

Groups	Control	Diabetic	Diabetic+ <i>Trigonella</i>	<i>Trigonella</i>	Diabetic+metformin
AST IU/L	40.22 ± 2.13	94.20 ± 3.44	57.322 ± 3.76	42.45 ± 2.34	51.54±2.72
ALT IU/L	35.34 ± 1.43	72.53 ± 2.18	43.33 ± 3.22	37.42 ± 2.54	39.09±2.82
Creatinine mg/dL	0.70 ± 0.09	1.81 ± 0.07	0.89 ± 0.021	0.63 ± 0.01	0.78±0.09
Urea mg/dL	25.22 ± 1.55	46.12 ± 2.33	28.18 ± 1.23	24.17 ± 1.23	22.20±1.92

Table 4: Effect of *Trigonella* on hormone concentration in male rats

Groups	LH mIU/L	FSH mIU/L	Testosterone ng/mL
control	1.4 ± 0.29	2.9 ± 0.44	3.5 ± 0.28
Diabetic	1.2 ± 0.23	1.4 ± 0.35	2.86 ± 1.4
<i>Trigonella</i>	2.9 ± 0.22	3.7 ± 0.11	5.45 ± 2.9
<i>Trigonella</i> + diabetic	2.00 ± 0.19	2.19 ± 0.15	3.95 ± 2.96
Diabetic+ metformin	1.43 ± 0.06	2.52 ± 0.11	2.76 ± 0.8

Table 5: Effect of *Trigonella* seed aqueous extract on sperm count and motility of male rats

Groups	Sperm count(Sperm x 10 ⁶)	Motility %	Viability %
Control	40.14 ± 3.91	90.68 ± 3.66	89.6 ± 2.42
Diabetic	33.22 ± 2.61	67.29 ± 2.89	75.2 ± 2.70
<i>Trigonella</i>	50.25 ± 2.77	92.14 ± 2.67	93.3 ± 2.38
<i>Trigonella</i> +Diabetic	43.24 ± 3.52	84.11 ± 3.65	82.5 ± 3.16
<i>Trigonella</i> + metformin	40.22 ± 2.37	82.34 ± 3.61	80.21 ± 2.34

Table 3 shows a decrease in, ALT, AST, urea and creatinine after four weeks of treatment by fenugreek seed aqueous extract

Table 4 shows a significant increase in LH, FSH, and Testosterone level after 4 weeks of treatment by fenugreek seed extract

Table 5 shows an elevation in sperm count, motility and viability after 4 weeks of treatment by fenugreek seed aqueous extract in Streptozotocin-induced diabetic rats

DISCUSSION

The objective of this paper is to evaluate the role of fenugreek aqueous extract on blood glucose, lipid profile, and some hormonal parameters in streptozotocin-induced diabetic rats. In the present study, there was a marked decrease in the level of blood glucose in diabetic groups treated with *Trigonella foenum-graecum* aqueous extract. The hypoglycemic effects of this extract have been attributed to several mechanisms, one of these mechanisms mentioned that 4-hydroxy isoleucine amino acid extracted and purified from fenugreek seeds increased insulin secretion in rat and human pancreatic cells,²⁴ 4-hydroxy isoleucine is also displayed an insulin tropic property *in vitro*, activated insulin production *in vivo* and enhanced tolerance of glucose in normal rats and dogs and in rat model of type 2 DM. Also, this amino acid inhibits hepatic glucose production and accelerates the use of glucose and possibly a decrease in the

plasma glucose value,²⁵⁻²⁷ as well as the hypoglycemic role of this extract maybe related to the effect of pectin which delays glucose absorption from the intestine.²⁸ Another highly active hypoglycemic material is trigonelline, which improves glucose tolerant after treatment in alloxan-induced diabetic rats.²⁹

Hypercholesterolemia and hypertriglyceridemia are associated with diabetes.^{30,31} Hypertriglyceridemia is also related to metabolic consequences of hyper-insulinemia, glucose intolerance, insulin resistance, and hypercoagulability.³² In this study, treatment of diabetic induced rats by *Trigonella foenum-graecum* improved lipid profiles, including decreases in total cholesterol, LDL, and triglyceride, as well as increases in HDL level, the best explanation of this result, may be related to decreased synthesis of cholesterol and fatty acid,³³ this effect due to saponins agent, which lower serum cholesterol level by increasing biliary cholesterol excretion.^{34,35} Saponins formed a large particle with bile salts and reduce cholesterol by inhibiting cholesterol absorption and increasing excretion of bile salts.³⁶ The hypolipidemic effect, could also be the result of fat absorption and carbohydrate retardation due to the existence of soluble fiber in the extract,³⁷ as well as Fenugreek seeds, contain a large amount of mannose which leads to reduce cholesterol synthesis.³⁸ Studies indicated treatment of diabetic rats with fenugreek extract significantly reduced the damage in liver and kidney and improve their normal function.³⁹ This study supports the important effects

of fenugreek seeds aqueous extract on hyperglycemia and hyperlipidemia in diabetic induced animals, further studies would be done in mammals.

Our finding clarified that the level of testosterone, LH, FSH hormone as well as the sperm count, viability and motility increased in diabetic animals after treatment with *Trigonella foenum-graecum* aqueous extract. Potential mechanisms by which *Trigonella foenum-graecum* may increase serum testosterone level include stimulation of pulsatile gonadotropin-releasing hormone (GnRH)/LH, increased testicular sensitivity to LH, and increased testosterone synthesis. An animal study found that after administration of fenugreek steroids to diabetic rats over 60 days induced a considerable improvement in testosterone level in the serum of the rats, another independent study demonstrated that, treatment of diabetic rats with *Trigonella foenum-graecum* extract, there was a significant decrease of sperm shape abnormality and improvement of the sperm count. Furthermore, the potential effect of *Trigonella foenum-graecum* extract was observed on reproductive systems, as reported by histological studies on epididymis and testis in rats.⁴⁰ In addition, *Trigonella foenum-graecum* seed contain diosgenin (an important material for the synthesis of testosterone) and saponins (especially protodioscin-like compounds). Therefore, a glycoside-rich fraction of *Trigonella foenum-graecum* seed is worth investigating for possible androgenic and anabolic activity.^{41,42} The present study results may reflect the effect of fenugreek seeds aqueous extract on fertility in male rats by increasing the level of testosterone, LH, and FSH hormone.

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