

Antihyperglycemic effects of *Trigonella Foenum graecum* seeds in diabetic Rats

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Abstract

Background: Traditionally *Trigonella foenum-graecum* (Fenugreek) has been used for treatment of diabetes mellitus however few studies are available regarding its mechanism of action as an antihyperglycemic agent. This study was designed to evaluate and compare the effect of *Trigonella foenum-graecum* seeds with Metformin and Acarbose on blood glucose, insulin, ghrelin, leptin and body weight in diabetic rats.

Method: Thirty-six rats were divided into two groups. The first group involved twelve normal rats (control). The second group involved 24 diabetic rats which were subdivided into four subgroups each group have six rats. The first subgroup served as a positive control. The second subgroup, received standard diet mixed with Fenugreek seed powder. The third and fourth subgroups received a single dose of Metformin and Acarbose respectively.

During the experimental period, body weight was individually recorded for each rat before and after treatment. Blood samples were collected from rats for determination of serum blood sugar, insulin, leptin level and ghrelin.

Results: Administration of Fenugreek seeds to non-diabetic rats did not exert any hypoglycemic or glucose lowering action and has no significant effect on the serum insulin. However, the same dose of Fenugreek seeds significantly reduced blood sugar and induced a significant rise in serum insulin of diabetic rats.

Trigonella foenum seeds powder slightly elevated serum level of leptin in normal rats. However, the same dose of the plant markedly reduced leptin in diabetic rats. Daily administration of the plant seeds powder notably decreased serum ghrelin of normal rats. Moreover, the same dose of the plant seeds could reduce the body weights of normal rats. These results support the hypothesis of the role of ghrelin in stimulating the appetite and an increase in body weight.

Conclusion: The result of this study revealed that *Trigonella foenum* has greater antihyperglycemia activities than that of Metformin and Acarbose in diabetic rats.

Key: Fenugreek, Diabetic rats, Acarbose, Metformin

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Introduction:

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels, type 2 diabetes mellitus has quickly become a global health problem due to rapidly increasing population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity¹.

A multitude of plants have been used for the treatment of diabetes throughout the world. Etuk et al² reported that many medicinal plants have been validated scientifically as potent antidiabetic plants such as: Aloe vera (L.), Burm.f. (Family: Aloaceae), Garlic *Allium sativum* L. (Family: Alliaceae), and *Trigonella Foenum*. They were found to produce beneficial effects on carbohydrate and lipid metabolism when administered as an adjunct in patients with type 2 diabetes. These plants have been reported to improve glucose tolerance in healthy human subjects and diabetic patients. They caused significant reduction in blood glucose, glycosylated haemoglobin and glycosylated plasma proteins comparable to an oral hypoglycaemic drug^{3,4}.

Trigonella foenum graecum (Fenugreek) is one of the oldest medicinal plants, is extensively cultivated in most regions of the world for its medicinal value (Petropoulos, 2002). *Trigonella foenum graecum* (Linn.) belonging to the family Papilionaceae commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated in India. Active compounds of fenugreek included soluble fiber saponins, trigonelle, diosgenin⁵⁻⁷.

Trigonella foenum-graecum is one such plant that has been extensively used as a source of antidiabetic compounds, from its seeds, leaves and extracts in different model systems⁸. Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.). *Trigonella foenum graecum* is commonly used as a condiment and seasoning in food preparations; is assumed to possess nutritive and restorative properties and has been used in folk medicine for centuries for a wide range of diseases including diabetes, fever and abdominal colic as a poultice for abscesses, boils, and carbuncles⁹.

This study was designed to evaluate and compare the effect of *Trigonella Foenum* seeds with Metformin at 100mg/kg and Acarbose at 60mg/kg on serum glucose, insulin, leptin, ghrelin and body weight in streptozotocin induced diabetic rats after three weeks of administration.

Methods

Plant material:

Seeds of *Trigonella Foenum –graecum* were used and obtained from specific local herbal market. The whole seeds were delicately powdered. The plant powder was mixed at a concentration 0.75% (w/w) with a standard diet¹⁰.

Animal:

Adult rats weighting between 140-500 g (75-90 days old) were used in the study. All animals kept in the animal house at the college of medicine under controlled condition of 12 hours light and 12 hours dark cycle in a room temperature of 27 c←

Experimental design

Thirty six rats were divided into two groups. The first group involved twelve normal rats (control). The second group involved 24 diabetic rats. Hyperglycemia was induced by using single dose intraperitoneal administration of streptozotocin in a dose of 40mg/kg¹¹ and dissolve 18 gm of sugar in 1000-liter water gives to rats for 48 hours in order to overcome sudden decrease in blood glucose level (hypoglycemia) induced by streptozotocin¹¹. The hyperglycemic rats subdivided into four subgroups each group have six rats. The first subgroup served as a positive control (hyperglycemic rats). The second subgroup, received standard diet mixed with powder *Trigonella foenum* for about 3 weeks. The third and fourth subgroups received a single dose of Metformin in a dose of 100 mg/kg¹² and Acarbose in a dose of 60 mg/kg¹³ orally respectively. The solution of two drugs Metformin (100mg/kg) and Acarbose (60mg/kg) were freshly prepared in tap water and given to animals by oral gavages every day.

At the end of treatment period (3 weeks), rats were fasted overnight and the following day blood samples were taken. The procedure started by anaesthetizing the rats by giving them combination of ketamine in a dose of 35mg/kg with xylazine in a dose 5mg/kg¹⁴ which was followed by a cardiac puncture by a sterile disposable plastic syringe which was then put into a specified numerically labeled blood tubes.

During the experimental period, body weight was individually recorded for each rat before and after treatment.

Blood samples were collected from rats for determination serum blood sugar, insulin, leptin level and ghrelin.

Statistical analysis

All data are expressed as mean \pm standard error of means (M \pm SEM) and statistical analysis was carried out using statistically available software (SPSS Version 21). Data analysis was made using one-way analysis of variables (ANOVA). Comparisons between groups were done using Duncan test and unpaired student t-test. $P \leq 0.05$ was considered as statistically significant.

Results

Effects of *Trigonella foenum graecum* seeds on serum glucose and insulin of normal rats

Oral administration of *Trigonella Foenum Graecum* seeds powder at a concentration of (0.75% w/w), non significantly ($P=0.22$) and slightly increased the level of blood sugar in normal rats as shown in Table (1). Daily ingestion of *Trigonella foenum* seeds powder at a concentration of (0.75% w/w) had no significant effect on insulin level in normal rats ($P=0.116$) Table (1).

Table (1). Effects of *Trigonella foenum graecum* seeds 0.75% (w/w) on blood glucose and insulin of normal rats (n=12).

Parameter	Control	<i>Trigonella normal</i>	P value
Blood glucose (mg/dl)	110.92 \pm 10.59	127.33 \pm 7.16	0.22
Insulin (uU/ml)	1.26 \pm 0.3439	0.898 \pm 0.21227	0.116

Effects of *Trigonella foenum graecum* seeds, Metformin and Acarbose on serum glucose

Intraperitoneal injection 40mg/kg of streptozotocin induced a significant and a highly increase and rise in blood sugar of rats. Daily oral administration of *Trigonella foenum* significantly reduced blood sugar of diabetic rats. While oral administration of Metformin and Acarbose for 3 weeks non significantly reduced blood sugar of diabetic rats Table (2).

Table (2). Effects of *Trigonella foenum graecum* seeds on blood glucose and insulin of diabetics rats (n=18).

Parameter	Control	Positive control diabetics rat	<i>Trigonella diabetics</i>
Blood glucose (mg/dl)	110.92 \pm 10.59 a	373.72 \pm 52.43 b	206.55 \pm 44.88 c
Insulin (uU/ml)	1.2683 \pm 0.0343 a	0.5605 \pm 0.2261 b	1.2567 \pm 0.0310 a

Effect of *Trigonella foenum graecum* seeds on serum insulin

Intraperitoneal injection of streptozotocin on normal rats caused a significant reduction in the level of insulin and the basal value was 1.268 ± 0.0343 and it decreased to 0.5605 ± 0.226 . Comparing to the diabetic rats, the insulin level of rats treated with *Trigonella foenum*, have significantly increased

Effects of *Trigonella Foenum graecum* seeds on body weights of normal rats

The body weight of normal rats in pre-treatment (basal value) was 427.50 gram, after 3 weeks of treatment with *Trigonella* mean value of the body weight decreased to 403.33 gram figure. (1).

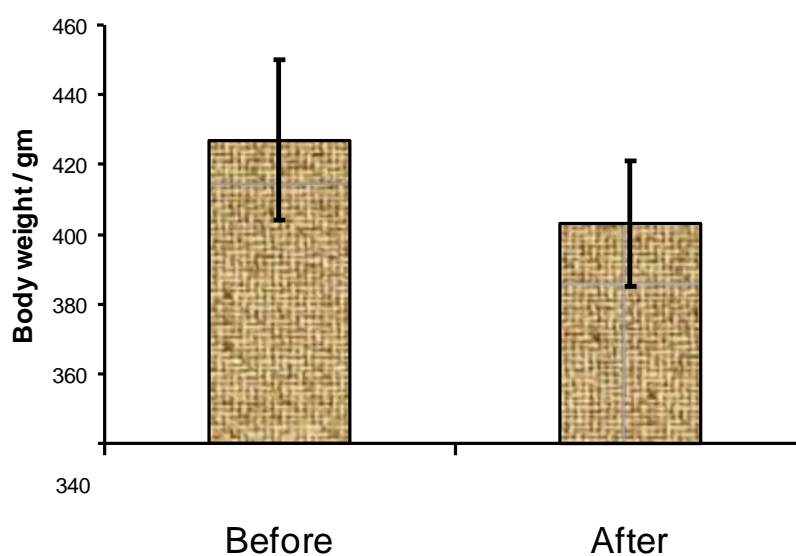


Fig. (1). Effects of *Trigonella foenum graecum* seeds (0.75% w/w) on the body weight of normal rats (n=6)

Effect of *Trigonella foenum graecum* seeds, Metformin and Acarbose on body weight of diabetic rats

Three weeks after intraperitoneal injection of streptozotocin the body weight of diabetic rat significantly reduced from 214 gram to 170 gram. After daily ingestion of *Trigonella foenum* (0.75% w/w) for 3 weeks, the body weight of diabetic rats was significantly improved from 170-204 gm. Both Metformin and Acarbose slightly increased the body weight of diabetic rats Figure (2).

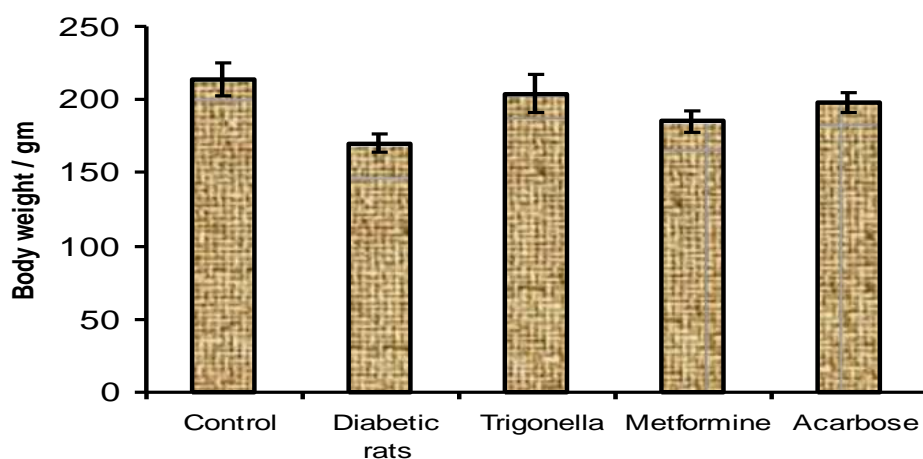


Fig. (2). Effect of Trigonella foenum graecum seeds (0.75%w/w), Metformin (100mg/kg), Acarbose (60mg/kg) on the body weight of diabetics rats (n=30).

Effects of Trigonella foenum graecum seeds on serum leptin and ghrelin in normal rats

Serum leptin in normal rats treated with Trigonella Foenum seeds was non significantly increased in comparison with the control Table (3). Oral administration of Trigonella Foenum for 3 weeks induced a marked decrease in the ghrelin level however statistical analysis turned out to be non significant (P=0.099) Table (3).

Table (3). Effect of Trigonella foenum graecum seeds 0.75% (w/w) on serum leptin and ghrelin of normal rats (n=12).

Parameter	Control	Trigonella	P value
Leptin(ng/ml)	0.3637±0.0869	0.432±0.15	0.16
Ghrelin(ng/ml)	1.5267±0.13120	0.57±1.6631	0.099

Effects of Trigonella foenum graecum seeds, Metformin and Acarbose on serum leptin and ghrelin of diabetics rats

Serum leptin was significantly increased in diabetic rats when it compared with control rats. Administration of Trigonella foenum in diabetic rats for 3 weeks induced a non significant but a marked reduction in the serum leptin. Whereas significant changes in the serum leptin were observed in diabetic rats treated by both metformin and acarbose Table (4).

Comparing to the normal rats the serum level of ghrelin non significantly decreased in diabetic rats. Trigonellafoenum has no detectable effects on the serum level of ghrelin when it was given to diabetic rats for 3 weeks, whereas both Metformin and Acarbose caused a significant increase in serum level of ghrelin in diabetic rats Table (4).

Table (4). Effects of Trigonella foenum graecum seeds, Metformin and Acarbose on serum leptin and ghrelin of diabetics rats (n=30).

Parameter	Control	Positive control Diabetics rat	Trigonella	Metformin	Acarbose
Leptin ng/ml	0.3637±0.087 a	1.8873±0.999 b	0.6120±0.218 b	0.3233±0.0621 a	0.2143±0.06 a
Ghrelin ng/ml	1.52±0.1312 a	0.5375±0.2109 a	0.8515±0.1865 a	4.4300±1.4554 b	3.8563±1.6382 b

Discussion:

Trigonella foenum graecum seeds have been historically used for the treatment of various chronic human diseases and studies concerned with application of fenugreek seeds in diabetes and dyslipidemia support this hypothesis.

In this study, intraperitoneal injection 40mg/kg of streptozotocin induced a significant and a highly increase and rise in blood sugar of rats as compared to the control group. This result is in agreement with other studies^{15,16}, which suggested that the elevated blood glucose levels in diabetes mellitus are caused by a defect in production and/or secretion of the hormone insulin. In this study the elevation of blood glucose indicates that this effect is caused by the direct influence of streptozotocin on pancreatic β cells.

The result of the current study, showed that administration of Trigonella foenum graecum seeds (0.75%) to non diabetic rats did not exert any hypoglycemic or glucose lowering action and has no significant effect on the serum insulin. However, the same dose of Trigonella foenum graecum seeds (0.75%) significantly reduced serum glucose of diabetic rats and induced a significant rise in serum insulin. This result was similar to the finding of Wehash *et al*,¹⁷ who reported that giving daily doses of fenugreek ethanolic extract (50 mg/kg) to diabetic rats for four weeks produced a significant reduction in serum glucose. In another study Ali *et al*,¹⁸ observed that oral administration of Fenugreek seeds extract (0.5gm/500 ml and 1.0gm/500 water) for 40 days to diabetic rats induced by streptozotocin showed a significant decrease in plasma glucose concentration by 10% on 25th day and 32% on 40th day of therapy with the plant extract. The hypoglycemic effect of fenugreek could be attributed to dietary fibers present in the fenugreek seeds, which help in the management of metabolic abnormalities associated with diabetes as peripheral insulin resistance and lipid abnormalities¹⁹. Whereas another study showed that the fenugreek seeds delayed gastric emptying and caused the inhibition of glucose transport as the seed contains around 50% pectin that forms a colloid suspension when hydrated can decrease the rate of gastric emptying and slow carbohydrate absorption²⁰.

In this study, Trigonella foenum seeds (0.75% w/w) increased serum insulin level in diabetic rats. This result was in agreement with the result of (El-Soud *et al*)²¹ who observed that treatment of diabetic rats with 60mg/kg alkaloidal extract for 21 days resulted in a significant reduction of blood glucose and increase in serum insulin. Gaddam *et al*²² revealed that consuming of 5 g of fenugreek powder twice a day before meals for 3 months caused a significant elevation in insulin level of patient with diabetes mellitus type 2. They suggested that the powder has acted as insulin secretor as they observed the rise in insulin secretion in animal. The result of this study revealed that Trigonella foenum has greater antihyperglycemia activities than that of Metformin and

Acarbose. These results indicate that the mechanism of antihyperglycemic effect of the plant is not due to the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption through inhibiting of α -Glucosidase²³.

The most probable mechanism of the antihyperglycemic effect of the plant could be due to the presence of the bioactive compounds present in it, including 4-hydroxyisoleucine, a novel amino acid known to facilitate insulin secretion²⁴. In addition, the soluble dietary fibers present in aqueous extraction of *Trigonella foenum* could decrease the absorption of glucose in the gastrointestinal tract.

In the present study, serum leptin is significantly increased in diabetic rats when it compared with the control. This effect is similar to the finding of Rafique and Latif, 25 who observed that serum leptin level was significantly higher in diabetic rats than the normal rats. This effect could be explained on the basis of insulin resistance, chronically elevated plasma insulin levels, and diabetic nephropathy, Type 2 diabetes is characterized by insulin resistance, which is positively associated with hyperleptinemia²⁶. Insulin induces leptin synthesis in a dose-dependent fashion²⁷. Hence, in the presence of insulin resistance and chronic hyperinsulinemia, type 2 diabetics are expected to exhibit hyperleptinemia. The other possibility of increased serum leptin levels could be diabetic renal damage. It was found that type 2 diabetic patients with impaired renal functions had higher serum leptin concentrations than control subjects matched for age, sex, and body fat²⁸. It remains to be determined whether the increased serum leptin concentrations in patients with diabetic nephropathy is due to increased production, reduced clearance, or increased leptin resistance.

In another study²⁹ reported that fasting serum leptin of the diabetic patients is significantly higher than healthy people with normal weight. An increase in leptin level has been witnessed in some other studies on obese or diabetic people³⁰. The result of this study is also in accordance with the finding of Kanaley *et al*, (2001)³¹ who found that the plasma leptin concentration is higher in diabetic patients even with the same fat mass than in healthy people. Apart from the body fat levels, high level of serum leptin can be a sign of an increase in leptin resistance in obese and people with related diseases. The plasma leptin level has a direct relation with the fat tissue and a negative relation with the body fitness or physical activity³².

In this study administration of *Trigonella foenum* seeds powder slightly elevated serum level of leptin in normal rats. However, the same dose of the plant reduced leptin in diabetic rats. This result is similar to the study of Kumar *et al*³³ who observed that the administration of aqueous extract of *Trigonella foenumgraecum* to rats on high fat diet showed the reduction of leptin levels. Their finding was further supported by the aqueous extract of *Trigonella foenum- graecum*

mediated reductions in white adipose tissue, weights and adiposity index. This reduction in the serum leptin most probably is due to the improvement of diabetic rats induced by the plant

Comparing to the normal rats the serum level of ghrelin non significantly decreased in diabetic rats. This is in accordance with the study of Farajallah *et al*³⁴, who concluded that ghrelin concentrations of patients with type 2 diabetes was significantly lower than those of control group. The results of blood glucose, serum insulin and ghrelin of diabetic rats in this study can be confirmed by the report of ³⁵who proved that low ghrelin levels are independently associated with insulinresistance in type 2 diabetes mellitus. Daily administration of *Trigonella foenum* seeds powder at a concentration (0.78%w/w) notably decreased serum ghrelin of normal rats. Moreover, the same dose of the plant seeds could reduce the body weights of rats from 427-403. These results support the hypothesis of the role of ghrelin in stimulating the appetite and an increase in body weight.

In the present study, fenugreek did not change the ghrelin level of diabetic rats. However, the plant seeds could significantly increase the body weight of the diabetic rats. This effect on the body weight most probably due to beneficial effect of *Trigonella* on blood sugar and insulin. The reduced growth rate and weight loss seen in the diabetic group are probably due to insulin depletion provoking loss of adipose tissue, and/or a decrease of food intake due to appetite loss resulted from a marked reduction in ghrelin level³⁶.

In the present study, significant change in leptin level was seen in rats received Metformin. This result is agreement with result of Kadhim *et al.*,³⁷ who observed that Metformin significantly decreased leptin level in female with diabetes mellitus.

The present study, showed that acarbose significantly reduced serum leptin level in diabetic rats which was similar to the result of Rosenbaum *et al* ³⁸ who observed that acarbose has decreased leptin level in hypertensive diabetic patients.

In the present study, Metformin significantly increased the level of serum ghrelin of streptozotocin induced diabetic rats. This result is similar to result of Doogue *et al*³⁹ who found that treatment with metformin for 6 weeks resulted in a significant increase in plasma ghrelin in patients with type 2 diabetes. It is also in accordance with the report of Shaker *et al*, (2010) who noticed that treatment with metformin significantly increased ghrelin serum level in women with polycystic ovary syndrome (PCOS).

In the present study, metformin slightly increased the body weight of diabetic rats this is in contrast with the finding of Pavo *et al*⁴⁰ who recorded that Metformin decreased body weight in patient with diabetes. This slightly rise in body weight caused by the uses of metformin could be related to

a minor improvement of the glucose level of diabetic rats.

Daily administration of acarbose for 3 weeks slightly increased serum ghrelin of diabetic rats. This effect is dissimilar with the finding of⁴¹ who reported that four weeks of treatment with acarbose significantly enhanced postprandial total ghrelin suppression at 120 min in patients with type 2 diabetes mellitus.

Conclusions:

Fenugreek significantly reduced blood sugar and induced a significant rise in serum insulin of diabetic rats. It has greater antihyperglycemia activities than that of Metformin and Acarbose in streptozotocin induced diabetic rats. The body weight of diabetic rats was significantly improved by daily ingestion of fenugreek seeds powder to diabetic rats for three weeks.

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