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Original Article

Therapeutic Effects of *Trigonella foenum-graecum* Extract on fasting blood glucose, tissue glycogen, glycosylated haemoglobin, plasma concentrations of insulin and GLP-1 in healthy and diabetic subjects

Gulzar Ahmad Bhat[a*], Poonam Sharma[b], Rambir Singh[a]

[a]*Bundelkhand University Jhansi U.P India*
[b]*Indra Gandhi National Tribal University Amarkantak M.P India*

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ABSTRACT

Traditionally *Trigonella foenum-graecum L.* was used in gastritis, to increase milk flow, gastric ulcers, kidney problems, cervical and breast cancer prevention of diabetes etc. The aerial parts of plant are remedy for abdominal cramps associated with menstrual pain, labour pains and diarrhoea or gastroenteritis. The objective of the study is to evaluate the effect of Aqueous Extract of Trigonella foenum-graecum (AETFG) on fasting blood glucose, tissue glycogen, glycosylated hemoglobin, plasma concentrations of insulin and GLP-1 in healthy and diabetic subjects. Both normal and diabetic Male Wistar rats were treated with AETFG by gavaging (300mg/kg for 28 days). Aqueous extract of *Trigonella foenum-graecum* increases tissue glycogen, serum insulin and GLP-1 with non-significantly ($P>0.05$) in normal, but significantly ($P<0.01$) in diabetic Wistar rats. Whereas non-significantly ($P>0.05$) decrease in FBG and Glycosylated haemoglobin was found in normal, and decreases significantly ($P<0.01$) in diabetic Wistar rats. Our findings tend to provide a possible explanation for the hypoglycemic action of AETFG seed extracts as alternative nutritional therapy in the management of diabetes

Introduction

The world prevalence of diabetes among adults will be affecting 285 million in 2010, and will increase to 439 million adults by 2030. Diabetes mellitus (DM) is one of metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion or its action. Chronic hyperglycemia leads to improper regulation of carbohydrate, protein, lipid metabolism and also contributes to the progression of micro as well as macro-vascular complications in DM (Shaw et al., 2010).

Secretion of insulin from the β-cells of pancreas is a major step involved in DM. The major targets for insulin are the liver, adipose tissue and muscle where synthesis of glycogen occurs by stimulating the glycogen synthetase and inhibiting the action of glycogen phosphorylase. Recently, the incretin effect has been demonstrated to be largely impaired in patients with type 2 diabetes. Reduced incretin effect has been attributed to a small but significant reduction in postprandial secretion of GLP-1. Glucagon-like peptide (GLP-1) is secreted from the gut, and is physiologically important regulators of metabolic control which . enhances insulin release from β-cells in a glucose-dependent manner. Thus, considerable interest has emerged on the pharmacological regulation of incretin actions for type 2 diabetes. Recently GLP-1 analogs such as exenatide, metformin, liraglutide, etc have been introduced for therapeutic intervention.

A number of drugs are available for management of DM. The side effects and high cost of treatment associated with current antidiabetic drugs, it is necessitated search for alternate remedy for DM having less side effects and low cost. The plant based drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar et al., 2008). The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes.

Trigonella foenum-graecum one of the medicinal plant, have been traditionally used for the treatment of DM (Sharma, 1986). The active ingredients of this plant have been identified as 4-hydroxyisoleucine (4-HIL) has been reported with glucose-dependent insulinotropic effect by a direct effect on pancreatic

* Corresponding Author : **Gulzar Ahmad Bhat**
Gulzar Ahmad Bhat,
Dept. of Zoology Bundelkhand University Jhansi U.P India
Mobile: 7298783202, Email: bhatgulzar26@gmail.com

islets (Sauvaire et al., 1998 and Broca et al., 2000). Trigonelline has activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension, and neuron excitability. It also possesses hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation.

In this study, we investigated the effects of oral administration of Aqueous Extract of Trigonella foenum-graecum (AETFG) on FBG, tissue glycogen, glycosylated haemoglobin, serum insulin and GLP-1

MATERIALS AND METHODS:

Plant material and its extraction: The seeds of Trigonella foenum-graecum was purchased with authenticated. The seeds of plant material were pulverized into powder and extracted with double distilled water by stirring whole night with the help of mechanical stirrer. The extract was filtered and then centrifuged for further purification. The supernatant was further lyophilized for complete dryness to obtain AETFG.

Chemicals: Streptozoticin (Sigma Aldrich Germany-S0130-1G) was used to induce diabetes. All chemicals and solvents used in this study were purchased from standard companies of analytical grade.

Animals: Adult male Wistar rats of age 2-3 months old and having weight about 200-250 grams were used for experimental work. The inbred male Wistar rats were purchased from animal DRDE, Gawlior (India). The animals were fed pellet diet and water ad-libitum. At the end of study all animal experiments were performed at the consent of the Institutional animal ethics committee (BU/ Pharma/ IAEC/12/031).

Induction of diabetes: Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of 50 mg/kg streptozotocin (STZ).

Treatment Schedule:

The rats were divided in 4 groups of 6 each as per treatment schedule given below.

Group I	Normal Control
Group II	Normal treated with Aqueous extract of TFG seeds of 300mg/kg bw
Group III	Diabetic control
Group IV	Diabetic treated with Aqueous extract of TFG seeds of 300mg/kg bw

Dose was given orally by gavaging for 28 days. Acute study of GLP-1 and serum insulin was done from blood serum in single dose administration of TFG. The blood was taken from tail vein at the interval of 0, 20, 30, 40, 50, and 60mins, whereas the sub-chronic study was done by same procedure after 28 days administration of plant extract.

Fasting Blood Glucose (FBG):

Fasting blood glucose (FBG) is a method for learning how much glucose (sugar) there is in a blood sample taken after an overnight fast. Blood sample was obtained through puncture tail vein and glucose was estimated on 0, 14, and 28th day by Glucometer.

Glycosilated Hemoglobin (Ghb):

Glycosylated hemoglobin was estimated by Euro diagnostic system kit based on photometric test using ion exchange resin was given by Trivelli et al., (1971); Nathan et al., (1984); Bunn (1981).

Tissue Glycogen:

This estimation indicates the distinction between free and fixed glycogen content in tissues by using the Anthrone reagent.

Tissue from liver and muscle was taken for estimation of glycogen, the tissuewas digested with 2ml of 30% boiling KOH and then cooled, after that 3ml of 95% of ethanol was added and heated until the tissue gets dissolved.Mixtures were cooled and centrifuged at 1000rpm for 5 minutes, the supernatant was discarded.The residual material was dissolved in 2ml of distilled water, and then 10ml of Anthrone reagent was added and immersed in ice bath to prevent excessive healing. The Tubes were incubated at 100oC for 4 minutes for color development and immersed in an ice bath.Theabsorbances were measured at 620 nm by using spectrophotometer (Seifter et al., 1950).

Serum Insulin:

Insulin estimation was done by using QAYEE-BIO Rat insulin autoantibodies (IAA) ELISA kit.

Glucagon-Like Peptide-1 (GLP-1):

GLP-1were determined by Ray Bio® Rat GLP-1 ELISA kit given by Toft-Neilsen et al.,(2001); and Meier et al.,(2004).

RESULTS:

The effect of Trigonella foenum-graecum on FBG level in normal and diabetic rats.

Table 1 depicts that there is non-significant (P>0.05) decrease in FBG on administration of TFG to normal rats resulted 14 days (8.05%), 28 days (11.19%) in group II as compared to 0 days, whereas, no change was found in normal control group I and in group IIIas compared to 0days in each group. Furthermore the significant (P<0.01) decrease in FBG was found (14 days 36.94%, 28days 69.85%)in group IV as compared to 0day readings. On comparing group I with group III, significant increase (P<0.01) in FBG 14 days (176.16%) and 28 days (286%) was observed in group III. However non-significant (P>0.05) decrease in FBG was found 14 days (15.60%) followed by significant (P<0.01) decrease 28 days (70.19%) in group IV as compared to group I.

Table 1. The effect of Trigonella foenum-graecum on FBG level in normal and diabetic rats.

No. of days ⟶	0	14	28
Group I	83.83±2.701	82.5±3.757	85.66±2.929
Group II	84.86±2.191	78.00±1.41 $^{\alpha*}$	75.33±2.160 $^{\alpha*}$
Group III	301.5±9.570	299.16±31.836$^{\gamma**}$	310.83±11.652$^{\gamma**}$
Group IV	306.66±9.674	193.33±12.883$^{\gamma r}$	92.43±2.883$^{\gamma r}$

The values represents as Mean ± SEM for 6 rats each. The results are expressed in mg/dl. α=(P>0.05), β=(P<0.05),γ=(P<0.01) represents comparison of 0day reading of each group with 14 and 28days. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TFG); Group-III Diabetic control; Group-IV diabetic treated (TFG).

The effect of Trigonellafoenum-graecum on level of Liver Glycogen, Muscle Glycogen and Glycosylated Haemoglobin in normal and diabetic rats.

The effect of TFG on the Liver Glycogen, Muscle Glycogen and Glycosylated Haemoglobin level ofin normal and diabetic rats is presented in Table 4.2.

Administration of aqueous extract of TFG in normal rats the liver glycogen level was non-significantly (P<0.05) increased (5.18%) in group II. Whereas the significant (P<0.01) decrease in liver glycogen was observed (65.16%) in group III on comparison with group I. The significant (P<0.01) increase in level of liver glycogen was reported in Group IV (160.6%) as compared with group III.

However, non-significant (P<0.05) increase in muscle glycogen was found (4.96%) in group II. On the contrary, significant (P<0.01) decrease in muscle glycogen was reported in group III(58.48%)when compared with group I. Muscle glycogen in TFG treated groups increases significantly (P<0.01) in group IV (100.3%) as compared to group III.

HbA1C percentage non-significantly (P>0.05) decreased (9.06%) in group II, whereas in diabetic control the HbA1C significantly (P<0.01) increased in group III(305.33%)as compared with group I. Diabetic treated with TG, the HbA1C percentage significantly (P<0.01) decreased in group IV (66.6%) when compared with group III.

Table 2. The effect of single dose of Trigonella foenum-graecum on Serum insulin level in normal and diabetic rats.

Parameter	Group I	Group II	Group III	Group IV
Liver Glycogen(mg/g)	23.91±1.74	25.15±1.472*	8.33±0.741***	21.7±1.868 ^{r}
Muscle Glycogen (mg/g)	19.75±1.109	20.73±1.089*	8.2±0.958***	16.43±0.604 ^{r}
Glycosylated Hemoglobin (%)	5.06±0.100	4.60±0.119*	20.51±5.154***	6.845±0.183^{r}

The values represents as Mean ± SEM for 6 rats each. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-II, III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TGF); Group-III Diabetic control; Group-IV diabetic treated (TGF).

The effect of single dose of Trigonella foenum-graecum on Serum insulin level in normal and diabetic rats.

The effects of TFG on the serum insulin level in normal and diabetic rats are presented in Table 3.

Serum insulin level increased non-significantly (P>0.05) in acute administration of TFG to normal rats from 30mins (6.13%), 40mins (6.66%) and tries to compensate towards normal at 60mins (1.41%) in comparison to 0min in group II. The non-significant increase (P>0.05) in serum insulin was observed 30mins (75%) followed by significant (P<0.05) increase (83.3%) at 40mins, then non-significantly falls towards normal at 60mins (8.3%) in group IV as compared to its 0min. Furthermore, whereas significant (P<0.05) change in serum insulin was observed from (20mins 0.71%, 30mins 47.79%, 40mins 64.17%, 50mins 7.69%, and 60mins 4.0%) in group IV on comparison with group III.

The effect of sub-chronic administration of Trigonella foenum-graecum on Serum insulin level in normal and diabetic rats.

On sub-chronic administration of aqueous extract of TFG in normal rats, the serum insulin level increased non-significantly (P>0.05) at 30mins (12.85%), 40mins (14.95%), and then drops towards normal at 60mins (2.10%) in comparison to 0min in group II. The table also depicted a significant (P<0.01) increase in insulin at 30mins (86.14%), 40mins (86.82%) and then non-significantly (P>0.05) concentrates towards normal at 60mins (17.56%) in group IV. There was significant (P<0.01) increase in serum insulin level was observed (20mins 115.1%, 30mins 305.14%, 40mins 312%, 50mins 223%, and 60mins 178.4%) in group IV, on comparison with group III (Table 4).

The effect of single dose of Trigonella foenum-graecum on GLP-1 level in normal and diabetic rats.

In the present investigations, GLP-1 level increased significantly (P<0.01) at 30mins (54.06%), 40mins (67.88%) and non significantly (P>0.05) at 60 min (4.13%) as compare to 0 min, during short term administration of TFG in normal rats in group II. A significant (P<0.01) increase in GLP-1 level was observed in group IV from 20mins (29.12%), 30mins (131.26%), 40mins (154.36%) and at 60mins (31.45%) when compared with its individual 0min readings.

Table 5-shows that the GLP-1 level decreases significantly (P<0.01) in group IIIas compared with group I. Significant (P<0.05) increase in GLP-1 was observed (20mins 32.55%, 30mins 33.6%, 40mins 81.6%, 50mins 72.91%), followed by non-significant (P>0.05) increase (60mins 16.32%) in group IV on comparison with group III.

The effect of long term exposure of Trigonella foenum-graecum on GLP-1 level in normal and diabetic rats.

Significant (P<0.01) increase in GLP-1 in sub-chronic experiment was observed hailing from 30mins (55.96%) to 40mins (59.9%) and tends to be normalize at 60mins (7.3%) in comparison to 0min in group II. However significant increase in GLP-1 level was depicted at 30mins (270.14%) 40mins (280.36%) and then falls towards normal at 60mins (71.47%) in comparison with 0min in group IV. Long term administration of TFG in diabetic rats results significant (P<0.05) increase in GLP-1 level from (20mins 14.11%, 30mins 270%, 40mins 282.55%, 50mins 159.21%, and 60mins 71.47%) in group V, on comparison with group IV Table 6.

Table 3.The effect of single dose of Trigonella foenum-graecum on Serum insulin level in normal and diabetic Wistar rats.

Time in Second	0 min	20 min	30 min	40 min	50 min	60 min
Group I	4.10±0.217	4.20±0.206	4.17±0.210	4.16±0.211	4.14±0.211	4.12±0.212
Group II	4.24±0.289	4.29±0.286$^{\alpha*}$	4.50±0.296 $^{\alpha*}$	4.52±0.299 $^{\alpha*}$	4.34±0.271 $^{\alpha*}$	4.30±0.285 $^{\alpha*}$
Group III	1.25±0.239	1.39±0.194 $^{\alpha***}$	1.36±0.205 $^{\alpha***}$	1.34±0.207 $^{\alpha***}$	1.30±0.219 $^{\alpha***}$	1.25±0.234 $^{\alpha**}$
Group IV	1.20±0.213	1.40±0.196$^{\alpha r}$	2.01±0.319$^{\alpha r}$	2.2±0.300$^{\beta r}$	1.40±0.310$^{\alpha r}$	1.30±0.252$^{\alpha r}$

The values represents as Mean ± SEM for 6 rats each. The results are expressed in ng/ml. α=(P>0.05), β=(P<0.05),γ=(P<0.01) represents comparison of 0 min reading of each group with 20,30,40,50 and 60 min. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TGF); Group-III Diabetic control; Group-IV diabetic treated (TGF).

Table 4.The effect of sub-chronic administration of Trigonella foenum-graecum on Serum insulin level in normal and diabetic Wistar rats.

Time in Second	0 min	20 min	30 min	40 min	50 min	60 min
Group I	4.10±0.217	4.20±0.206	4.17±0.210	4.16±0.211	4.14±0.211	4.12±0.212
Group II	4.28±0.241	4.35±0.242$^{\alpha*}$	5.83±0.376$^{\alpha*}$	5.94±0.411 $^{\alpha*}$	4.16±0.330 $^{\alpha*}$	4.37±0.270$^{\alpha*}$
Group III	1.25±0.239	1.39±0.194 $^{\alpha***}$	1.36±0.205 $^{\alpha***}$	1.34±0.207 $^{\alpha***}$	1.30±0.219 $^{\alpha***}$	1.25±0.234 $^{\alpha**}$
Group IV	2.96±0.125	2.99±0.089$^{\alpha r}$	5.51±0.386$^{\gamma r}$	5.53±0.399$^{\gamma r}$	4.20±0.229$^{\beta r}$	3.48±0.275$^{\alpha r}$

The values represents as Mean ± SEM for 6 rats each. The results are expressed in ng/ml. α=(P>0.05), β=(P<0.05),γ=(P<0.01) represents comparison of 0 min reading of each group with 20,30,40,50 and 60 min. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-II,III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TGF); Group-III Diabetic control; Group-IV diabetic treated (TGF).

Table 5.The effect of single dose of Trigonella foenum-graecum on GLP-1 level in normal and diabetic Wistar rats.

Time in Second	0 min	20 min	30 min	40 min	50 min	60 min
Group I	12.00±0.288	15.20±0.970	14.81±0.788	13.81±0.484	12.30±0.463	12.08±0.422
Group II	11.81±0.914	13.30±0.616$^{\alpha*}$	18.16±0.792$^{\gamma**}$	19.81±0.760$^{\gamma**}$	15.51±0.622$^{\gamma*}$	12.58±0.508$^{\alpha*}$
Group III	5.10±0.289	9.86±0.603$^{\gamma***}$	8.91±0.534$^{\gamma***}$	7.21±0.391 $^{\beta***}$	6.35±0.396$^{\alpha***}$	5.82±0.311$^{\alpha***}$
Group IV	5.15±0.489	6.65±1.08$^{\alpha q}$	11.91±0.891$^{\gamma q}$	13.10±0.924$^{\gamma r}$	6.77±0.562$^{\gamma r}$	6.46±0.820$^{\gamma p}$

The values represents as Mean ± SEM for 6 rats each. The results are expressed in pg/ml. α=(P>0.05), β=(P<0.05),γ=(P<0.01) represents comparison of 0 min reading of each group with 20,30,40,50 and 60 min. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TGF); Group-III Diabetic control; Group-IV diabetic treated (TGF).

Table 6.The effect of long term exposure of Trigonella foenum-graecum on GLP-1 level in normal and diabetic rats.

Time in Second	0 min	20 min	30 min	40 min	50 min	60 min
Group I	12.00±0.288	15.20±0.970	14.81±0.788	13.81±0.484	12.30±0.463	12.08±0.422
Group II	16.26±1.104	16.80±1.066$^{\alpha*}$	25.36±1.491$^{\gamma***}$	26.00±1.786$^{\gamma***}$	20.01±1.238$^{\alpha***}$	17.45±1.022$^{\alpha***}$
Group III	5.10±0.289	9.86±0.603$^{\gamma***}$	8.91±0.534$^{\gamma***}$	7.21±0.391$^{\beta***}$	6.35±0.396$^{\alpha***}$	5.82±0.311$^{\alpha***}$
Group IV	9.78±0.627	11.16±0.406$^{\alpha p}$	36.20±1.751$^{\gamma r}$	37.46±1.462$^{\gamma r}$	25.35±1.846$^{\gamma r}$	16.77±1.358$^{\gamma r}$

The values represents as Mean ± SEM for 6 rats each. The results are expressed in pg/ml. α=(P>0.05), β=(P<0.05),γ=(P<0.01) represents comparison of 0 min reading of each group with 20,30,40,50 and 60 min. *=(P>0.05), **=(P<0.05), ***=(P<0.01) represents comparison of Group-III with group-I. p=P>0.05), q=(P<0.05), r=(P<0.01) compared group IV with group III. Group-I Normal Control; Group-II Normal treated (TGF);; Group-III Diabetic control; Group-IV diabetic treated (TGF).

Discussion:

STZ are widely used to induce experimental diabetes in animals to decrease insulin level. STZ is a drug that selectively destroys the β-cells, by entering into the β-cell via a glucose transporter (GLUT2) and causes alkylation of DNA, enhanced ATP dephosphorylation to form superoxide radicals. These β-cells are insulin producing pancreatic endocrine cells, and thus induce experimental diabetes mellitus (Brenna et al., 2003).

The hypoglycemic effect of TFG seeds is evaluated in present study. Fasting blood glucose levels in the normal control group remained unchanged throughout the experimental work. Administration of aqueous extracts of TFG to normal rats showed reduction in blood sugar levels are from day 1 to day 28. But in case of diabetic treated groups the blood sugar level reaches near to normal control within 28 days of experiment. The antidiabetic effect of TFG may be due to increase the utilization of glucose, or decrease of glucose absorption from GI tract or controls on the insulin secretion or inhibits the α-glucosidase activity.

The seed fibers of TFG reduces the rate of glucose absorption, enhancing its utilization and may also delay gastric empting, thereby preventing the rise in blood sugar levels following a meal (Gupta et al., 2001). Experiment of Wehash et al., 2012 and Rajarajes wari et al., 2012 was also reported that the administration seed extract TFG in both normal and diabetic rats restored the FBG level near to normal control. Results of other scientists on TFG treatment (Ahmad et al., 2011; Puri et al., 2012; Radhika et al., 2013; Bera et al., 2013), are also in conformity with the present work.

The Glycosylated hemoglobin (HbA1c) assay has become the most commonly used measure of chronic glycaemia in epidemiological studies, clinical trials and the management of diabetes. In the present study, the diabetic rats have shown significant increase in HbA1c level as compared to normal control which indicates that diabetic rats have poor glycemic control. The long term administration of aqueous extracts TFG in diabetic rats results a significant decrease in the HbA1c levels in the diabetic rats, but no significant difference in HbA1c percentage found in normal treated groups. HbA1c levels which were noted in consistent with other reports may be due to low plasma level of insulin or high glucose utilization in the peripheral tissues as reported in the present work. The various experimental reports obtained from recent papers on TFG (Xue et al., 2007; Preet et al., 2007; Kulkarni et al., 2012 and Bera et al., 2013) concords with our results.

It is well known that in diabetes mellitus there will be marked depletion in glycogen storage in hepatic cells and muscle cells in diabetic rats. This observation is in accordance with the finding of Bhuvaneshwari 2012; Radhka et al., 2013; Beraet al., 2013. Liver glycogen and muscle glycogen is drastically reduced in diabetic group and on administration of aqueous extract of TFG for 28 days in the normal and diabetic rats corrects the glycogen level, but not equivalent to normal control group. The decrease in tissue glycogen may be due to enhanced catabolic process such as glycogenolysis, lipolysis and proteolysis, which are the outcomes of lack of insulin or oxidative stress by diabetes may inactive the oxygen synthase or decrease in GLUT4 transporter protein of muscles and cellular glucose in liver cells.

In present investigation diabetic group showed low insulin than control group which indicates the β-cell failure in diabetic rats. The insulin level increases non-significantly 20 to 60mins in short term administration of TFG, but in diabetic TFG treated serum insulin increase significantly. The possible mechanism behind the increase in insulin secretion in acute administration TFG may increases the stimulation of β-cell by binding to its receptors or shows insulin like activity, or decreases blood glucose concentrations by acting on peripheral tissues.

In long term exposure of TFG the serum insulin level remains same as in short term exposure of TFG rats. The serum insulin level was elevated from 20 to 60mins in TFG diabetic group. However, the serum insulin level was increased at 0min on sub-chronic administration of TFG in diabetic groups as compared with 0min readings of acute diabetic TFG treated group. Serum insulin was also increased in the normal TFG treated rats. This significant change in serum insulin level in long term exposure of TFG might be due to increasing the number of insulin receptors or β-cell regeneration. The results suggests that both TFG increases the renewal and number of β-cells in the pancreas as compared to untreated diabetic rats or may permit the recovery of STZ destroyed β-cells and stimulates pancreatic insulin secretion.

In support with the present study the elevation of insulin levels was observed with the administration of TFG by (Ahmad et al., 2012; Rhadika et al., 2013; Gad et al., 2006 and Wehash et al., 2012).

The progressive loss of β-cell function and mass is an early feature of type 2 diabetes, eventually leading to insulin dependence in many patients. Intervening early in the course of diabetes or perhaps in the prediabetic state to stimulate β-cell differentiation and/or reduce

apoptosis could theoretically halt the progression of the disease. GLP-1 has shown promise in this respect. Increase in GLP-1 level, form 0 to 60 min was observed in normal and diabetic rats on short term treatment with TFG. In diabetic rats highest increase in GLP-1 level was after 40mins of TFG administration, compared to 0min. The results also suggest that induction of GLP-1 on administration of TFG was more pronounced in diabetic rats as compared to normal. It is possible that in normal rats high increase in GLP-1 is prohibited due to normal physiology and normal level of glucose regulating hormones including GLP-1. Since there is decrease in insulin as well as GLP-1 in diabetic rats, GLP-1 induction is more pounced to decrease enhanced glucose level.

In our study the long term administration of TFG, increase in GLP-1 level was observed in normal from 20 to 60mins as compared to 0min of each group. In diabetic treated significant change GLP-1 level in TFG treated group, but no significant change found in normal control. This result also suggests that the increase in GLP-1 level was higher at 0min in long term administration of TFG when compared with 0min acute TFG diabetic group. These findings indicate that the GLP-1 level is highly significant in sub-chronic administration of TFG compared to acute administration of TFG. The Mechanism of elevation of GLP-1 on sub-chronic administration of TFG in normal and diabetic rats may be due to increase the L-cell receptors or L-cell regeneration.

The secretion of GLP-1 by aqueous extract TFG may be due to polar molecules, such as sugars, amino acids, small peptides, water soluble alkaloids and plant secondary metabolites, are known to stimulate GLP-1 secretion (Ramos et al., 2012). The nutrient and tastents sensing in enetroendocrine cells are mainly mediated by G-protein coupled receptors (Reimann et al., 2012; Iwatsuki et al., 2012). When tastent binds to taste receptors like bitter taste receptors, a conformational change occurs at the receptor level resulting in the activation of a series of signal transducers such as G protein α-gustducin, phospholipase C beta 2 (PLCβ2),inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), and transient receptor potential (TRP) channels that eventually depolarize the cell through elevation of intracellular Ca2+ concentration (Iwatsuki and Uneyama, 2012) which releases GLP-1 from GI L-cells and this GLP-1 in turn binds with GLP-1R receptor of pancreatic beta-cells to increase serum insulin level in blood Fig. no.1.

In support with the present study Cicero and Tartagni, 2012 reported that administration of Beriberis plant at the dose of 500mg/kg bw, shows that Berberine is an alkaloid present in Beriberis plant that affects glucose metabolism, increasing insulin secretion, stimulating glycolysis, Berberine also increases glucose transporter-4 (GLUT-4) and GLP-1 levels, other results in support of increase in GLP-1 was observed in Smallanthus sonchifolius (Habib et al., 2011), Agave tequilana Gto. & Dasylirion spp (Silvas et al., 2008), Ilex paraguariensis (Hussein et al., 2011), Cinnamomum zeylanicum (Hlebowicz et al., 2009), Pinus koraiensis (Pasman et al., 2008), Glycine max (Park et al., 2010) and Berberis (Cicero and Tartagni, 2012).

CONCLUSION:

Results of the present study supported the traditional use of Aqueous Extract of Trigonella foenum graecum shows the significant hypoglycemic action in management of diabetes.

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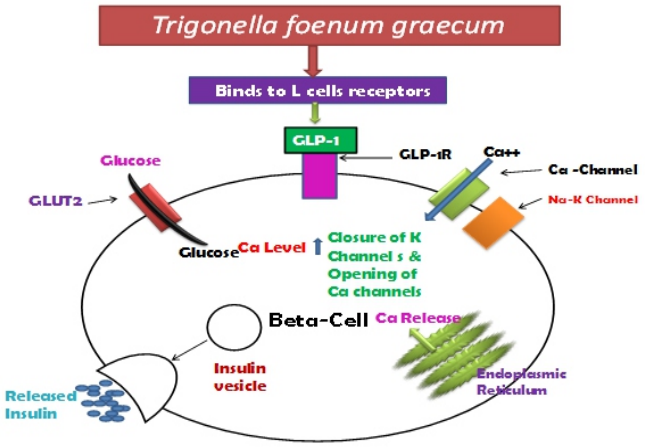
Figure No. 1 Mechanism of Action of AETFG



References

1. Ahmed MA, Abd-El M, Husam EH, (2011). Osman Elicitation of Trigonelline and 4-Hydroxyisoleucine with Hypoglycemic Activity in Cell Suspension Cultures of Trigonella foenum graecum L. The Open Conference Proceedings Journal; 2:80-87.
2. Bera TK, Ali KM, Jana K, Ghosh A, Ghosh D (2013). Protective effect of aqueous extract of seed of Psoralea corylifolia (Somraji) and seed of Trigonella foenum-graecum L. (Methi) in streptozotocin-induced diabetic rat: A comparative evaluation. Pharmacognosy Res.; 5(4):277-285.
3. Bhuvaneswari P, Krishnakumari S (2012). Antihyperglycemic potential of sesamumindicum (linn) seeds in streptozotocin induced diabetic rats. Inter J Pharmacy and Pharmaceutical Sci.; 2(2):8-13.
4. Brenna O, Qvigstad G, Brenna E, Waldum HL, (2003). Cytotoxicity of streptozotocin on neuroendocrine cells of the pancreas and the gut. J Dig Dis Sci.; 48:906-910.
5. Broca C, Manteghetti M, Gross R, Baissac Y, Jacob M, Petit P, Sauvaire Y, Ribes G. (2000). 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion. Eur J Pharmacol.; 390:339–345.
6. Bunn HF (1981). Lab Manag.; 16.
7. Cicero AF, Tartagni E. (2012). Antidiabetic properties of berberine: from cellular pharmacology to clinical effects. Hosp Pract (Minneap); 40(2):56-63.
8. Gad MZ, Sawalhi MME, Ismail MF, El-Tanbouly ND (2006). Biochemical study of the anti-diabetic action of the Egyptian plants Fenugreek and Balanites. Molecular and Cellular Biochem.; 28:73-183.
9. Gupta A, Gupta R, Lal B (2001). Effect of Trigonella foenum-graecum seeds on glucaemic control and insulin resistance in type 2 diabetes mellitus. J Assoc Physicians India; 49:1057-1061.

10. Habib NC, Honoré SM, Genta SB, Sánchez SS (2011). Hypolipidemic effect of Smallanthus sonchifolius (yacon) roots on diabetic rats. Chem Biol Interact.; 194(1):31-9.

11. Hlebowicz J, Hlebowicz A, Lindstedt S, Björgell O, Höglund P, Holst JJ, Darwiche G, Almér LO (2009). Effects of 1 and 3 g cinnamon on gastric emptying, satiety, and postprandial blood glucose, insulin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and ghrelin concentrations in healthy subjects. Am J Clin Nutr.;89(3):815-21.

12. Hussein GM, Matsuda H, Nakamura S, Hamao M, Akiyama T, Tamura K (2011). Yoshikawa mate tea (Ilex paraguariensis) promotes satiety and body weightlowering in mice: involvement of glucagon-like peptide-1. Biol Pharm Bull.; 34(12):1849-55.

13. Iwatsuki K, Torii K, (2012). Peripheral chemosensing system for tastants and nutrients. Current Opinion in Endocrinology, Diabetes, and Obesity; 19(1):19-25.

14. Kulkarni CP, Bodhankar SL, Ghule AE, Mohan V, Thakurdesai PA (2012). Antidiabetic activity of Trigonella foenum graecun L. seeds extract (INDO1) in neonatal streptozotocin-induced (N-STZ) rats. Daibetloggia Croatica.:41-1.

15. Meier J, Weyhe D, Michaely M, Senkal M, Zumtobel V, Nauck M, Holst J, Schmidt W, Gallwitz B (2004). Intervenous glucagon-l-like peptide 1 normalizes blood glucose after major surgery in patients with type 2 diabetes. Crit Care Med.; 32(3):848-51.

16. Nathan DM et al., (1986). New Eng.J.Med.; 310:341-346.

17. Park S, Ahn IS, Kim JH, Lee MR, Kim JS, Kim HJ (2010). Glyceollins, one of the phytoalexins derived from soybeans under fungal stress, enhance insulin sensitivity and exert insulinotropic actions. J Agric Food Chem.; 58(3):1551-7.

18. Pasman WJ, Heimerikx J, Rubingh CM, Berg R, O'Shea M, Gambelli L, Hendriks HF, Einerhand AW, Scott C, Keizer HG, Mennen LI (2008). The effect of Korean pine nut oil on in vitro CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. Lipids Health Dis.; 20(7):10.

19. Preet A, Siddiqui MR, Taha A, Badhai J, Hussain ME, Yadava PK, Baquer NZ, (2007). Long-term effect of Trigonella foenum graecum and its combination with sodium orthovanadate in preventing histopathological and biochemical abnormalities in diabetic rat ocular tissues. Asia Pac J ClinNutr; 16(1):422-426.

20. Puri D, Prabhu KM, Murthy PS, (2012). Antidiabetic Effect of GII Compound Purified from Fenugreek (Trigonella foenum graecumL.) seeds in Diabetic Rabbits. Ind J Clin Biochem.; 27(1):21-27.

21. Radhika J, Jothi G, Haliya KR (2013). Prophylactic role of a poly herbal formulation on alloxan induced diabetes in experimental models. E. J. Pharmacol. Therapy; 6:7-11.

22. Rajarajeswari A, Vijayalakshmi P, Sadiq A M (2012). Influence of Trigonella foenum graecum (fenugreek) in alloxan induced diabetic rats. The bioscan; 7(3):395-400.

23. Ramos SM, Tovar AR, Torres N, (2012). Diet: friend or foe of enteroendocrine cells–how it interacts with enteroendocrine cells. Adv in Nutr.;3(1):8-20.

24. Reimann F, Tolhurst G, Gribble FM, (2012). G-protein-coupled receptors in intestinal chemosensation. Cell Metabol.;15(4):421-431.

25. Sauvaire Y, Petit P, Broca C, Manteghetti M, Baissac Y, Fernandez AJ, Gross R, Roye M, Leconte A, Gomis R, Ribes G (1998). 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion. Diabetes; 47:206–210.

26. SeifterS, Dayton S, Novic B and Muntwyler E (1950). The estimation of glycogen with the anthrone reagent. Arch. Biochem. Biophys., 50: 191–200.

27. Sharma RD. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses in human subjects. Nutr Res 1986; 6:1353–64.

28. Shaw JE, Sicree RA, Zimmet PZ (2009). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract.; 87(1):4-14.

29. Silvas UJE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM (2008). Physiological effects of dietary fructans extracted from Agave tequilana Gto. And Dasylirion spp. Unidad de Biotecnología e Ingeniería Genética de Plantas, México. Br J Nutr.; 99(2):254-61.

30. Toft-Nielsen M, Madsbad S, Holst J (2001). Determints of the effectiveness of Glucagon-Like peptide-1 in type 2 diabetes. J Clin Endocrinol Metab.; 86(8):3853-60.

31. Trivelli LA, Ranney HM, Lai HT (1971). New Eng.J.Med.; 284:357.

32. Wadkar KA, Magdum CS, Patil SS, Naikwade NS (2008). Antidiabetic potential and Indian medicinal plants. J Herbal Med and Toxicol.; 2:45-50.

33. Wehash FE, Ismail IAG, Saleh RM, (2012). Some Physiological Effects of Momordica charantia and Trigonella foenum-graecum Extracts in Diabetic Rats as Compared with Cidophage. World Academy of Science, Engineering and Technology; 2012:64.

34. Xue WL, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ (2007). Effect of Trigonella foenum-graecum (fenugreek) extracts on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr.; 16(S1):422–426.