

COMPARATIVE EFFECT OF AQUEOUS EXTRACT OF *TRIGONELLA FOENUM-GRÆCUM* AND *MOMORDICA* *CHARANTIA* ON DIABETIC WISTAR RATS

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ABSTRACT

Diabetes is one of biggest healthcare challenges faced by the NHS, and is one of the most common endocrine diseases affecting all the age groups. There are so many drugs which are now available to fight against the deadly disease diabetes, but the common side effects of these drugs like fluid retention, weight gain, headache, upper respiratory tract infection and urinary tract infection. In recent years to overcome these side effects, emphasis has been placed on development of plant-based therapeutics which is basically perceived as safe.

The objective of the study is to evaluate the effect of Aqueous Extract of *Trigonella foenum-graecum* (AETFG) and *Momordica Charantia* (AEMC) on fasting blood glucose, tissue glycogen, glycosylated haemoglobin, plasma concentrations of insulin and GLP-1 in diabetic subjects. Both experimental groups of Male Wistar rats were treated with AETFG and AEMC by gavaging (300mg/kg for 28 days). Aqueous extract of both increases tissue glycogen, serum insulin with significantly ($P < 0.01$) in diabetic Wistar rats. Whereas significantly decrease in FBG and Glycosylated haemoglobin in diabetic Wistar rats. Our findings tend to provide a possible explanation for the hypoglycemic action of AETFG seed extracts and AEMC fruit extract as alternative nutritional therapy in the management of diabetes.

Keywords: Tissue glycogen, Serum insulin, Diabetic and Seed extracts.

INTRODUCTION

Diabetes is one of biggest healthcare challenges faced by the NHS, and is one of the most common endocrine diseases affecting all the age groups. Diabetes mellitus is a universal health problem which has a global prevalence of 1.3% (Dhaar and Robbani, 2006). More than 220 million people worldwide have diabetes. Globally, 3.2 million deaths are attributed to diabetes every year. At least one in 10 deaths among the adults who are between 35 and 64 years of age is attributed to diabetes (Diabetes Action Now Booklet, 2011). The WHO has projected that the diabetes deaths would double between 2005 and 2030. Diabetes is not only a disease of the affluent countries, it is prevalent in the

developing countries too (Dhaar and Robbani, 2006). India is known as the "Diabetes capital of the world". In India, the prevalence of diabetes in the urban areas increased tenfold from 1.2-12.1% during the years 1971- 2000 (Ramachandran, 2005), while in the rural areas, it increased three times from 2.2% to 6.4% during the years, 1989-2003 (Ramachandran *et al.*, 2004).

Diabetes mellitus is caused by lack of insulin secretion by the pancreas (type I diabetes) or by insufficient insulin secretion to compensate for decreased sensitivity to the effects of insulin (type II diabetes) (Guyton and Hall, 2006). Patients with diabetes experience significant morbidity and mortality from microvascular (retinopathy, neuropathy,

nephropathy etc) and macrovascular complications viz. heart attack, stroke and peripheral vascular disease (Halder et al., 2003 and Merlin et al., 2005)

There is no cure for diabetes, but the progression of the disease may be slowed down considerably through proper diet and regular physical activity. Present treatment is aimed at maintaining strict glycemic control and while some patients may be managed by diet and exercise, more typically, one or a combination of oral hypoglycemic agents are required for effective glycemic control. However, even with current pharmacological treatment the disease progressively worsens with time. For these reasons, the development of new drugs is actively being pursued. According to recommendations by the WHO expert committee on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become important (Viturro et al., 1999). Medicinal plant extracts may have adjuvant effects to existing antidiabetic agents. Many compounds isolated from these plants are used in combinational therapy for diabetes (Srivastava et al., 2012).

MATERIALS AND METHODS

Procurement and maintenance of Animals

The inbred male Wistar rats were purchased from animal DRDE, Gawlior (India). The animals were kept for acclimatization in the animal house of Institute Of Basic Sciences, Department of Zoology at ambient temperature of 25°C and 45-55% relative humidity, with 12h each of dark and light cycles. The different groups of animals were maintained in polypropylene cages measuring 45×30×30 with husk bedding. Principles of Laboratory Animal Care (NH publication no, 85-23, revised 1985) will be followed for animal care during the course of experimentation and all the principles of laboratory animal care were followed.

The Animals were fed pellet diet and water *ad-libitum*. The cages were cleaned regularly in order to avoid any type of infection and abnoxious odour. Adult male Wistar rats of age 2-3 months old and having weight about 200-250 grams were used for experimental work. At the end of study rats will be sacrificed

and all animal experiments were performed at the consent of the animal Ethical committee (BU/ Pharma/ IAEC/12/031).

CHEMICALS

Streptozotocin (Sigma Aldrich Germany-S0130-1G) was used to induce diabetes. All chemicals and solvents used in this study were purchased from different companies like Merck, Himedia, Rankem, Qualikems, Loba, Sigma and other chemical companies of analytical grade.

PLANT MATERIAL

The seeds of *Trigonella foenum-graecum* and the fresh fruits of *Momordica Charantia* were purchased from the market of Jhansi.

Extraction of Plant Material

Preparation of different solvent extracts

The seeds of *Trigonella foenum-graecum* were pounded into powder in grinder. The seed powder of plant material was extracted with aqueous by stirring whole night with the help of homogenizer. The supernatant was further lyophilized in Martin christ alpha 1-2 LD plus lyophilizer for complete dryness before use in this way the aqueous extract is obtained through serial extraction.

The fruits of *Momordica Charantia* were crushed in grinder. The crushed fruit of plant material was extracted with aqueous by stirring one hour with the help of homogenizer then was lyophilized.

Experimental Design

Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of 50 mg/kg streptozotocin (STZ) in citrate-buffered saline (pH 4.5, 0.1 M). 30 days after STZ induction, the development of diabetes was confirmed by tail vein blood glucose levels. Animals with blood glucose levels more than 200 mg/dl were included in the study.

Treatment Schedule

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

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

Dose was given orally by gavaging for 28 days. Sub-chronic study of serum insulin was done from blood serum in daily dose administration of TFG and MC. The blood was taken from tail vein at the interval of 0, 20, 30, 40, 50, and 60mins. At the end of study rats was sacrificed as per the guideline of Institutional Animal Ethics Committee (IAEC), and then the liver and muscle were removed and used for estimation of free and fixed Glycogen in tissues. Intestine was also removed for the estimation of α -glucosidase.

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.

Glycosylated 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 tissue was 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 heating. The Tubes were incubated at 100°C for 4 minutes for color development and immersed in an ice bath. The absorbances 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.

RESULTS AND DISCUSSION

The effect of *Trigonella foenum-graecum* and *Momordica charantia* on FBG level in normal and diabetic rats

Significant ($P < 0.01$) decrease in FBG was found (14 days 36.94%, 28 days 69.85%) in group II and (14 days 41.42%, 28 days 69.57) in group III was observed as compared to 0 day readings whereas, no change was found in normal control group I. On comparing group I with group II, III FBG decrease significantly ($P > 0.05$) was found 14 days (15.60%) followed by significant ($P < 0.01$) decrease 28 days (70.19%) in group V. whereas significantly ($P > 0.05$) decrease in FBG level was depicted 14 days (23.35%), followed by significant ($P < 0.01$) decrease at 28 days (70.23%) on comparison with group IV. The antidiabetic effect of TFG and MC 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 emptying, thereby preventing the rise in blood sugar levels following a meal (Gupta *et al.*, 2001). Experiment of Wehash *et al.*, 2012 and Rejarajeswari *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.

MC has also been reported to inhibit absorption of glucose by inhibiting α -glucosidase and suppressing the activity of disaccharidases in the intestine (Chaturvedi, 2012). Whereas Lal *et al.*, 2011, experiment on diabetic rats, reported the hypoglycemic activity of MC.

Table 1: The effect of *Trigonella foenum-graecum* and *Momordica charantia* on FBG level in diabetic rats

No. of days	0	14	28
Group I	301.5 \pm 9.570	299.16 \pm 31.836 ^v	310.83 \pm 11.652 ^v
Group II	306.66 \pm 9.674	193.33 \pm 12.883 ^{vf}	92.43 \pm 2.883 ^{vf}
Group III	304.00 \pm 9.292	177.93 \pm 7.923 ^{vf}	92.50 \pm 13.200 ^{vf}

The values represents as Mean \pm 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 0 day reading of each group with 14 and 28days. ^p= $P > 0.05$), ^q=($P < 0.05$), ^r=($P < 0.01$) compared group II,III with group I. Group-I Diabetic control; Group-II diabetic treated (TFG); Group-III diabetic treated (MC).

The effect of *Trigonella foenum-graecum* and *Momordica charantia* on level of Liver Glycogen, Muscle Glycogen and Glycosylated Haemoglobin diabetic rats

The effect of MC and TFG on the Liver Glycogen, Muscle Glycogen and Glycosylated Haemoglobin level of diabetic rats is presented in Table 2.

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; Bera *et al.*, 2013; Choudhary *et al.*, 2012,. Administration of aqueous extract of TFG and MC in normal rats the liver glycogen level was significant ($P<0.01$) increase in level of liver glycogen was reported in Group II (160.6%) and in group III (171.42%) as compared with group I. However, Muscle glycogen in TFG and MC treated groups increases significantly ($P<0.01$) in group II (100.3%) and group III (110.36%) as

compared to group I. 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.

HbA1C percentage Diabetic treated with TG and MC, the HbA1C percentage significantly ($P<0.01$) decreased in group II (66.6%) and in group III (65.7%) when compared with group I. 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 report obtained from recent papers on TFG (Preet *et al.*, 2006; Kulkarni *et al.*, 2012 and Bera *et al.*, 2013) and on MC (Fernandes *et al.*, 2007; Mohammady *et al.*, 2012) and in *Elaeodendron glaucum* (Lanjhiyana *et al.*, 2011) concurs with our results.

Table 2: The effect of *Trigonella foenum-graecum* and *Momordica charantia* on Liver Glycogen, Muscle Glycogen and Glycosylated Hemoglobin in diabetic rats

Parameter	Group I	Group II	Group III
Liver Glycogen (mg/ml)	8.33±0.741	21.7±1.868 [†]	22.61±1.795 [†]
Muscle Glycogen (mg/ml)	8.2±0.958	16.43±0.604 [†]	17.25±0.909 [†]
Glycosylated Hemoglobin (%)	20.51±5.154	6.845±0.183 [‡]	7.021±0.187 [‡]

The values represents as Mean ± SEM for 6 rats each. [‡]= $P>0.05$, [‡]=($P<0.05$), [†]=($P<0.01$) compared group V,VI with group IV.

Group-I diabetic Control; Group-II diabetic treated (TGF); Group-III diabetic treated (MC).

The effect of sub-chronic administration of *Trigonella foenum-graecum* and *Momordica charantia* on Serum insulin level in diabetic rats

On sub-chronic administration of aqueous extract of TFG in normal rats, significant ($P<0.01$) increase in insulin at 30mins (86.14%), 40mins (86.82%) and then concentrates towards normal at 60mins (17.56%) in group II. However, in MC treated rats, the insulin level was found significantly ($P<0.01$) higher at 20mins (85.1%) and then reaches non-significantly ($P>0.05$) towards normal at 60mins (8.15%) in group III (Fig. 1).

The Fig. 1-also clarifies that 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 II, and (20mins 275.5%, 30mins 229.4%, 40mins 202.2%, 50mins 158.4% and 60mins 144%) in group III on comparison with group I.

Serum insulin was increased 1.6%, 37.25%, 38.78%, 20.56%, 2.10% and 34.44%, 31.25%,

5.0%, 2.08%, 0.40% on treatment with TFG and MC as compared to 0min level of the normal rats. This significant change in serum insulin level in long term exposure of TFG and MC might be due to increasing the number of insulin receptors or β -cell regeneration. The results suggests that both TFG and MC 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. Furthermore, MC displays insulin-like properties, remarkably stimulates glycogen storage by the liver and improves peripheral glucose uptake (Reyes *et al.*, 2006). 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 at al., 2006 and Wehash *et al.*, 2012) and MC by (Nagy, 2012; Mohammady *et al.*, 2012 and Saha *et al.*, 2012).

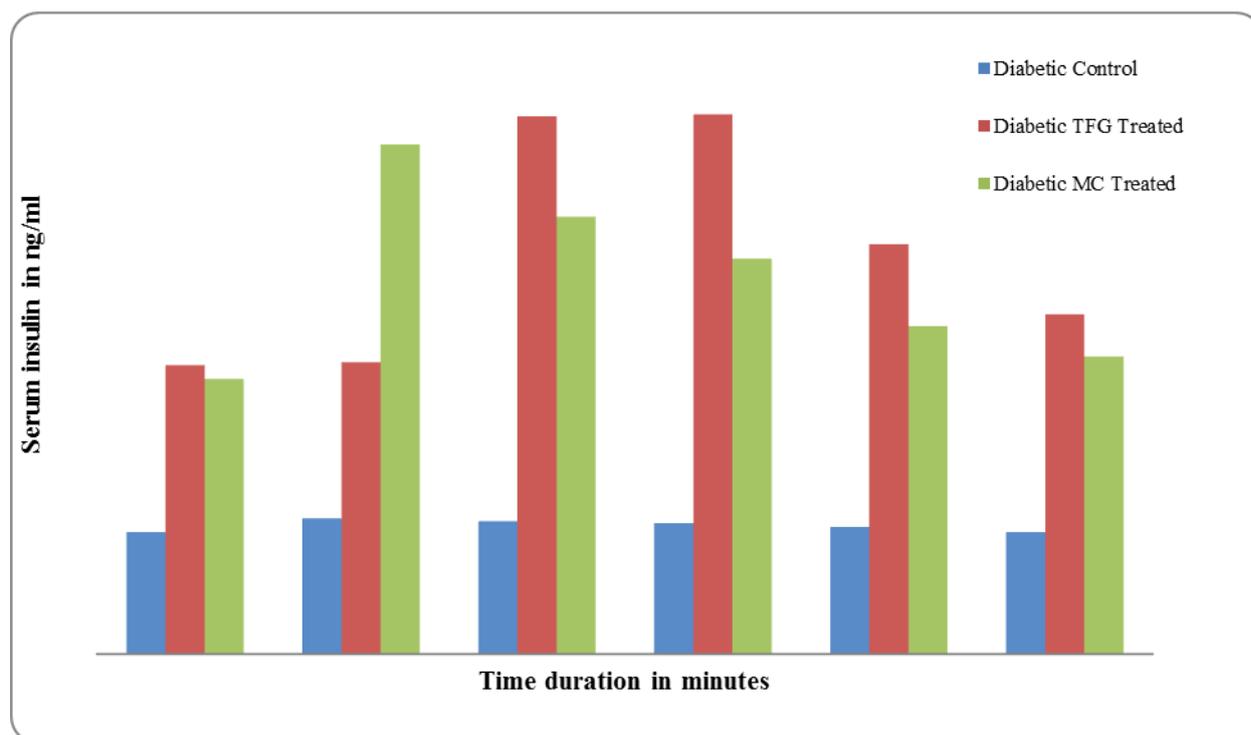


Fig. 1: The effect of sub-chronic administration of *Trigonella foenum-graecum* and *Momordica charantia* on Serum insulin level in diabetic Wistar rats

CONCLUSION

This study showed that the ingestion of TG and MC increases the serum insulin concentrations, tissue glycogen and decreases blood glucose, HbA1c concentrations in diabetic subjects. In conclusion, this study provides evidences that TGF and MC can exert an antidiabetic effect which might contribute, at least in part, to a better glucose homeostasis control. The TFG shows more antidiabetic effect than MC plant.

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