

# **TRIGONELLA FOENUM GRACUM SEED EXTRACT INHIBIT UPTAKE OF GLUCOSE ACROSS RAT EVERTED GUT SACS *IN VITRO***

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**ABSTRACT:** To investigate the absorption and the inhibition of glucose by the aqueous seed extracts of *Trigonella foenum gracum*. The small intestine of all animals were removed, washed with 0.9% (w/v) normal saline and intestinal segments were everted the empty sac was filled with 0.5 ml of the KHB buffer and placed in a stoppered conical flask with 5 ml of the buffer with varying concentration of glucose and 3.62 ml of *Trigonella foenum gracum* seed extract. The conical flask was placed in metabolic shaker at a frequency of 100-110 shakes/min. The incubation medium was bubbled for 1 min with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. After 30 min of incubation medium the sacs were removed and the serosal fluid was drained into a test tube. Glucose in the mucosal and the serosal compartment was measured. Incubation of the *Trigonella foenum gracum* seed extracts with rat everted gut sacs resulted in the inhibition of the transport of glucose across the membrane. Based on the data obtained in this study, we can propose that aqueous seed extract of *Trigonella foenum gracum* may possess hypoglycemic properties that inhibit the glucose transport at the site of intestinal brush border membrane.

**KEYWORDS:** Hypoglycemic, *Trigonella foenum gracum*, Aqueous extracts.

## **INTRODUCTION**

Diabetes mellitus is a debilitating and often threatening disease with increasing incidence throughout the world. It was postulated that DM is the most common chronic disorder affected more than 176 million people worldwide, and this global figure has been set to double by the year 2030<sup>1</sup>. The treatment of DM relied heavily on dietary measures, which included the use of traditional plant therapies. Several reviews on plants with known antidiabetic activity or with traditional use as antidiabetic remedies have been reported<sup>2,3,4</sup>. Traditional antidiabetic plants might provide a useful source for developing new oral hypoglycemic compounds as pharmaceutical entities or simple dietary adjuncts to the existing therapies. Studying such traditional medicine might offer an alternative and natural key to unlock diabetologists pharmacy. On the other hand, suggested mechanism

describing therapeutic effects of several traditional medicinal plant systems are holistic<sup>5</sup>. Among various forms of treatments for DM, diet is of vital importance. Foods of medicinal value have been proved effective and thus are widely used as they combine two basic central factors: food and medication<sup>6</sup>.

*Trigonella foenum-graecum* (Family: Fabaceae) is commonly known as 'fenugreek'. It is native to many Asian, Middle Eastern and European countries. The seeds and leaves of fenugreek are edible and are used as condiments and as Ayurvedic medicine in the Indian subcontinent for their carminative, toxic and aphrodisiac effects and to treat diabetes, high cholesterol, wounds, inflammation, and gastro intestinal ailments. The potential of fenugreek seeds to modulate several enzymes, including those associated with glucose and lipid metabolism, has been described earlier.

Compounds extracted from the plant have shown cardiotoxic, hypoglycaemic, diuretic, antiphlogistic and hypotensive activity<sup>7</sup>. One of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. The seed contains the saponin diosgenin, an important substance in the synthesis of oral contraceptives and sex hormones<sup>8</sup>. Various reports have demonstrated that *Trigonella foenum-graecum* seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals<sup>9,10,11,12</sup>. The present study was therefore undertaken to assess the possible biological property of *Trigonella foenum-graecum* seed extract on glucose transport across rat everted gut sacs *in vitro*.

## MATERIALS AND METHODS

### Preparation of the extract

The *Trigonella foenum-graecum* seeds were collected from Vellore District of Tamilnadu, India during March to June. Fresh seeds were washed with distilled water and shade dried. The shade dried seeds were powdered in an electrical blender and stored at 5°C until further use. The powdered seeds were taken 100 g and mixed with 200ml of distilled water and were stirred magnetically at room temperature. The residue was removed by filtration and the aqueous extracts were used for experiments.

### Experimental design and surgical procedure

Adult male Swiss Albino rats weighing 100-150g were housed at room temperature and were used in this experiment. Animals were maintained on commercial feed and tap water. Before each experiment, the animals were starved for twelve hours but allowed for tap water use. Rats were sacrificed by cervical dislocation. The abdomen was opened by a midline incision. The entire small intestine was removed quickly by cutting across the upper end of the duodenum and the lower end of the ileum, and by stripping the mesentery manually. The small intestine was then washed with normal saline solution (0.9% w/v NaCl) using a syringe equipped with blunt end.

### Preparation of everted gut sacs

Intestinal segments (10±2 cm) were everted according to the method described by Wilson & Wiseman<sup>13</sup>. The sacs were filled with 0.5 ml of the incubation medium (serosal fluid) and were placed in 25 ml Erlenmeyer flasks with 5 ml of the same medium (mucosal fluid). After oxygenation of the flasks with 100% O<sub>2</sub> for 1 min, they were tightly stoppered and kept in a shaker (90-110 oscillations/min) for 1 h at room temperature. The incubation medium was Krebs-Henseleit bicarbonate buffer (KHB). The composition of the buffer was (mM/L): NaHCO<sub>3</sub> 25; NaCl 118; KCl 4.7; MgSO<sub>4</sub> 1.2;

CaCl<sub>2</sub> 1.2; and Na<sub>2</sub>EDTA 9.7 mg/L. Glucose (5.5 mM) was added to the medium just before the start of appropriate experiments.

### Effect of *Trigonella foenum graecum* seed extract on the uptake of glucose transport

For studying the effect of the plant extract on the uptake of glucose (substrate), glucose was added into mucosal compartment fluid just before the start of the experiment. The seed extract was also added in the same compartment (3.62 mg/ml). At the end of the incubation period (1 h), the sacs were removed from the flask and these sacs were emptied and the serosal fluid from the sacs was used for the estimation of glucose. Similar estimations were also performed on samples of mucosal fluid in the flasks. The initial serosal fluid content was determined as the difference between the weight of the empty and the filled everted sac before incubation, and the final serosal fluid content was calculated by subtracting the weight of the empty sac from that of the filled sac, after incubation.

Glucose concentrations were measured using a commercially available glucose oxidase kit (Lifechem<sup>TM</sup> – Glucose-LR). The loss of glucose from the mucosal fluid assumed to represent the glucose taken up by the intestine, and the rise in glucose in serosal fluid, the glucose released. The difference is attributable to the glucose retained in the tissue. Uptake and release of glucose were expressed as μM/g tissue wet weight/h.

### Control experiments

In each series of experiments, control everted gut sacs derived from the same rat in a buffer containing no substrate were run in parallel. The controls were run either with or without plant extract and results were corrected accordingly.

### Statistical analysis

The difference between the mean± S.E.M. between the controls and the experimental groups were examined using the one way analysis of variance (ANOVA) test. P value less than 0.05 were considered as significant. All data were analyzed using Excel. The analysis of variance at 95% level of confidence was used to test for the significant differences in the concentration of intestinal glucose absorption.

## RESULTS

Incubation of the rat everted intestinal sacs with *Trigonella foenum-graecum* seed extract resulted in the inhibition of transport of glucose. With varying concentration of substrate it was found that *Trigonella foenum-graecum* seed extract significantly inhibited the uptake of glucose (p<0.05)

## DISCUSSION

The observed results substantiate earlier studies where the fenugreek seed extract depleted the blood glucose levels. The present findings show that aqueous seed extract of *Trigonella foenum-graecum* inhibits glucose absorption in the rat gut significantly. Earlier studies<sup>14,15</sup> have shown that fenugreek seeds lowered blood glucose levels and partially restored the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems. Therefore it is possible that the active compounds of *Trigonella foenum-graecum* seed extract decreased the blood glucose levels by inhibiting the absorption of the glucose from the alimentary tract.

Our findings would tend to indicate that glucose transport was significantly decreased in the presence of aqueous seed extract of *Trigonella foenum-graecum*. Therefore, it is most probable that active phytochemicals in the seeds of *Trigonella foenum-graecum* prevented the glucose transit across the gut membrane probably by reducing the glucose transporter protein activity which might have lead to wash out of glucose from the body. Based on this data obtained in this study we propose that *Trigonella foenum-graecum* seed aqueous extracts revealed hypoglycemic property by inhibiting the glucose transport at the site of intestinal brush border membrane of rats.

**Table 1. Effects of *Trigonella foenum-graecum* on the uptake of the varying concentrations of Glucose**

| Glucose concentration in the medium (mM) | Uptake ( $\mu\text{mol/g}$ Tissue wet wt/h) Control(n = 6) | Uptake ( $\mu\text{mol/g}$ tissue wet wt/h) <i>Trigonella foenum-graecum</i> (3.62 mg/ml) (n = 6) | p-value |
|--|--|---|---------|
| 5.5                                      | 30.52 $\pm$ 0.06   | 26.83 $\pm$ 0.06  | P<0.05  |
| 6.5                                      | 32.96 $\pm$ 0.03   | 28.65 $\pm$ 0.04  |         |
| 7.5                                      | 35.02 $\pm$ 0.05   | 30.41 $\pm$ 0.09  |         |
| 8.5                                      | 37.21 $\pm$ 0.06   | 32.61 $\pm$ 0.05  |         |

**Keywords:** The intestinal sacs were incubated in Kerbs-Henseleit bicarbonate buffer (pH=7.4) at 37°C. n=number of sacs used. Values are expressed as mean $\pm$ S.E.M. of six experiments.

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