Effect of Trigonella Foenum Graecum Seed Extract on Entero-Insular Axis by Oral Glucose Tolerance Test in Albino Rats

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ABSTRACT

The research study evaluates the effect of *Trigonella foenum graecum* seed extract on the entero-insular axis and the hyperglycemia due to oral glucose tolerance test in albino rats. Methanolic seed extract of *Trigonella foenum graecum* was prepared by Sami labs, Bangalore and used for the study. Institutional Animal ethical committee clearance obtained. Male albino rats (155–215 g) were divided into 4 groups each having 6 albino rats were randomly assigned Control (Normal saline) group, Standard (Vildagliptin 50 mg/kg group), *Trigonella foenum graecum* seed extract (100 mg/kg) and (200 mg/kg) groups. After overnight fasting the baseline biochemical evaluation in the fasting state blood glucose, Insulin level, Glucagon level, GLP-1 (Glucagon like peptide-1), GIP (Glucose dependent Insulinotropic peptide) level were measured at -30 minutes for all the above groups. Control, Vildagliptin (STD) and the extracts were orally administered by using rat oral feeding tube. 30 minutes later oral glucose tolerance test (OGTT) was done. Blood samples were evaluated for blood sugar, Insulin, Glucagon, GIP at -30, 0, 15 and 45 minutes after oral glucose load. *Trigonella foenum graecum* (2 gm/kg) showed significant reduction in blood glucose (*p<0.001*) at 45 minutes when compared to control. *Trigonella foenum graecum* (1 gm/kg & 2 gm/kg) showed significant (*p<0.001*) increase in GIP levels at 45 minutes of OGTT and (1 gm/kg) showed reduction in glucagon levels (p=< 0.001) at 15 minutes and at 45 minutes (p=<0.05). *Trigonella foenum graecum* extracts showed significant antihyperglycemic and incretin enhancing effects through entero-insular axis. Further in-depth animal and clinical studies are necessary to bring out the therapeutic potential of this seed extract for the treatment of type 2 diabetes mellitus.

Key words: *Trigonella foenum graecum*, Entero-insular axis, Antihyperglycemic, Insulin, Glucagon, GIP-1, GIP

INTRODUCTION

As India is leading the world with a greater number of diabetic patients, most of the research studies are directed towards diabetes. Still there is lacunae in many aspects especially the pathophysiology and treatment modalities of type 2 diabetes mellitus. Diabetes mellitus is a chronic metabolic syndrome, characterized by hyperglycemia, and abnormality of carbohydrate, protein and fat metabolism due defect in insulin secretory mechanism or defect in action on the target organs. International Diabetes federation data shows currently 366 million people are suffering from diabetes and this may double in number by 2030. 1 In India presently 40 million people are living with diabetes which will increase to 60 million by 2025. In India the presentation of type 2 diabetes is postprandial hyperglycemia attributed to high carbohydrate intake. Diet and lifestyle modification play an important role in the management of Diabetes. Medical nutrition therapy consists of herbs which reduces hyperglycemia, hyperlipidemia will arrest the progression of the disease resulting in micro and macrovascular complications of diabetes. 2 Dietary polyphenols has anti-hyperglycemic effects with regard to carbohydrate metabolism, beta cell function and insulin resistance. 3

Indian dietary pattern constitutes of more than 75% carbohydrates, 4 hence making drastic change in diet is not possible. Alternatively, we can use edible herbals in diet which can reduce carbohydrate absorption from gut and act in the entero-insular axis by increasing incretins and Insulin. 5 This is an interesting novel area of research needs to be explored. In this study we evaluated the anti hyperglycemic and incretin enhancing effect of commonly used edible herbal *Trigonella foenum graecum* on the entero-insular axis in albino rat model. This will pave way for further in-depth research towards the pharmacotherapy of type 2 diabetes in future.

*Trigonella foenum graecum* (Fenugreek)

*Trigonella foenum graecum* known as fenugreek, locally called as methi, is a readily available household medicine in India. The seeds are called as methi dana in Hindi posses many medicinal value. It is rich in soluble fibre and can help in controlling blood sugar by slowing down the absorption of carbohydrates. 6 Traditionally used to treat boils, abscesses and ulcers. Fenugreek is natural soluble protein which reduces hair fall and promotes the keratin for hair growth. It has many biological as well as pharmacological actions in reducing various ailments. It reduces the biosynthesis of cholesterol, absorption of cholesterol and reduces inflammatory mediators. 7

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studies showed the hypolipidemic, anti-inflammatory activity especially as local application in the form of gel. Fenugreek seeds functions as an appetite suppressant and gives a feeling of satiety, which help in reducing obesity. Latest studies showed that the herb contains lecithin, a natural emollient known to strengthen and moisturize hair. Earlier animal studies suggested hypoglycemic and anti hyperglycemic action of oral fenugreek seed powder in diabetic rat models. Most of the studies done previously showed anti diabetic activity and prevention of complications but exact mechanism of action is yet to be investigated. This study is conducted to explore mainly the anti hyperglycemic and incretin (viz., GIP, GLP -1) enhancing effect of Trigonella foenum graecum on the entero-insular axis by oral glucose tolerance test. This may through some light for the herbal based new drug development of type 2 diabetes mellitus.

**MATERIALS AND METHODS**

Randomized controlled study with animals (albino rats) to evaluate the antihyperglycemic effect of seed extract of Trigonella foenum graecum by using Oral glucose tolerance as a basis. It evaluates the glycemic and hormonal influences of OGTT on the entero-insular axis.

Institutional research committee and animal ethical committee approval (Protocol No.002/09/2015/IAEC/SRMCH) obtained through proper channel. The extract was manufactured and supplied by Sami labs Limited, Bangalore. Product Code 2045. Batch No. C170698EM. Date of manufacture April 2017. Trigonella foenum graecum (Fenugreek) methanolic seed extract (Fenustersols). Product Code 0566. Batch No. H170111. Date of manufacture January 2017. Content of steroidal sapopins by gravimetry 52.04 % w/w, Alkaloids 0.63% and Diosgenin by HPLC 1.51% w/w was used for this study.

Physical, Chemical and Microbiological testing for the above extract was done and certificate of analysis was issued by the Sami Labs Limited. Vildagliptin 50 mg tablets were obtained from the local pharmacy, powdered and made into a suspension (10 mg/ml) and administered to rats by oral feeding tube.

Male and female albino rats (weighing 150-250 gram) total 70 numbers were purchased from King Institute, Guindy, Chennai. The rats were provided with a commercial diet and maintained under controlled temperature, humidity, and lighting (22 ± 2°C, 55 ± 5%), and a 12-hr light/dark cycle with lights on at 7:00 AM. All procedures were conducted according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines and as per the Animal ethical Committee regulations.

**Preliminary acute toxicity**

Single oral dose of 2000 mg/kg for the extract groups of 6 albino rats (3 males and 3 females in each group) weighing 155-215 gm. Similarly, one control group of 6 rats was administered with distilled water (1 ml /100 g) orally.

The animals were observed for clinical manifestations and mortality for first 6 hours and then for next 14 days. The results and conclusion is that the single oral dose of 2000 mg/kg Trigonella foenum graecum seed extract did not cause death or any abnormal clinical manifestations in male or female rats.

The effective dose was arrived at by studying previous similar research articles. 2 dose levels (low and high) were chosen for the extract. Trigonella foenum graecum extract 1 gm/kg (low dose) and 2 gm/kg (High dose). Vildagliptin (Standard drug) (50 mg /kg).

**Grouping of animals**

Male albino rats (155–215 g) were randomly divided into 4 groups each having 6 albino rats as follows:

- Group 1: control (Normal saline) group (n=6)
- Group 2: Standard (Vildagliptin 50 mg/kg) group (n=6)
- Group 3: Trigonella foenum graecum (Low dose 1 gm/kg) (n=6)
- Group 4: Trigonella foenum graecum (High dose 2gm/kg) (n=6)

**Rationale of Oral glucose tolerance test**

This method is recognized as the most widely used physiological test as it correlates the correct route of consumption of carbohydrates. The ingested glucose (usually instilled into the stomach) is absorbed in the intestinal tract and enters the splanchnic circulation and then into the systemic circulation.

Increase in the blood glucose level leads to the release of Insulin from pancreatic beta cells. In turn Insulin increases glucose uptake by the liver, peripheral tissues and other metabolic functions. The entry of the nutrients through the early part of the intestine stimulates the release of the gut hormones (e.g., Glucoside dependent insulinotropic polypeptide-GIP, and Glucagon like peptide-1 GLP-1), which in turn physiologically activate the beta cells to synthesis and release insulin.

**PROCEDURE**

After overnight fasting the baseline (-30 minutes) biochemical evaluation such as fasting blood glucose, Insulin, Glucagon, GLP-1 and GIP blood levels were estimated for all the 4 groups.

The extract of Trigonella foenum graecum (Low dose 1 gm/kg) and (High dose 2 gm/kg), Vildagliptin 50 mg/kg, and control (Normal saline) were administered orally to the respective groups of rats using rat feeding tube. 30 minutes later oral glucose load (dose 2.2 gm/kg) was administered by oral feeding tube to all animals. Blood samples were collected at 0,15 and 45 minutes after oral glucose load from the tail vein of the rat (0.2-0.25 ml) and transferred into heparinized tubes. Blood glucose test and hormonal assays (Insulin, Glucagon, GLP-1 and GIP) were performed as per the procedure.


**Blood glucose determination**

Blood samples were collected as per the approved blood collection techniques for laboratory animals. Institutional animal ethical committee permission obtained to collect blood with appropriate technique. Blood samples (0.2-0.25 ml) were collected from the rat tail vein at -30, 0,15 and 45 minutes. It was mixed with 140 µl of 0.6 M perchloric acid. After centrifugation, the supernatants were assayed for glucose using an enzymatic assay kit.

**Plasma Insulin, GLP-1, GIP determination**

Approximately 250 µl of blood samples were collected from the tail vein at -30, 0,15 and 45 minutes of the test in the heparinized capillary tubes. After centrifugation, supernatants were assayed for plasma insulin, glucagon, active GLP-1 and GIP levels.

Plasma insulin and glucagon levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Plasma active GLP-1 & GIP levels were determined using an ELISA kit (GLP-1) Active ELISA kit.
**Statistical analysis**

Biochemical and hormonal test result data are expressed as the mean ± S.E.M. Differences in the values of blood glucose in an OGTT between the groups treated with control, standard drug and the extract of 2 doses were determined by one-way ANOVA, followed by Dunnet’s multiple comparison test.

The data of plasma insulin, Glucagon, GLP-1 and GIP values at -30, 0, 15 and 45 minutes were compared with the standard drug Vildagliptin. The data were analyzed using one-way ANOVA, followed by Dunnett’s multiple comparison test.

*p value of < 0.05 (two-sided) was considered statistically significant.*

Statistical analyses were performed using Graph Pad software (Prism Windows 5).

**RESULTS AND DISCUSSION**

VG (STD 50 mg/kg) and FG (2 gm/kg) shows significant reduction in blood glucose (p = <0.001) at 45 min when compared to control (Table 1 and Graph 1).

Vildagliptin (50 mg/kg) shows significant increase in GIP (p = <0.001) at 45 minutes when compared to control. FG (1 gm/kg & 2 gm/kg) shows significant (p = <0.001) increase in GIP levels at 45 minutes.

Vildagliptin (50 mg/kg) shows significant increase in Insulin level (p = <0.001) at 15 and 45 minutes when compared to control. FG (1 gm/kg) shows significant reduction in glucagon levels (p = < 0.05) at 15 and (p = <0.05) at 45 minutes.

**DISCUSSION**

Plants play an important role in the treatment of diabetic patients from time immemorial. Most of the modern drugs available is derived either directly or indirectly from the herbas. Scientific studies done so far were directed towards the evaluation of anti diabetic effect but in our study, it evaluates the antihyperglycemic and incretin enhancing effect of herbal extract in the entero-insular axis. Favorably the Trigonella foenum graecum extract showed significant anti hyperglycemic and incretin enhancing effect. Vildagliptin (STD 50 mg/kg) and TFG (2 gm/kg) showed significant reduction in blood glucose (p = <0.001) at 45 min (Table 1) when compared to control. Similar studies proved the effect of TFG in reduction of blood glucose levels in alloxan induced diabetic models. But in our study, it demonstrated the antihyperglycemic effect by OGTT in the entero-insular axis of normal rats. The effect of Trigonella foenum graecum on carbohydrate absorption and role of insulin secretion was also investigated by some research studies. The antihyperglycemic effect of the edible extracts especially Trigonella foenum graecum seed extract shows reduction in blood glucose level (p =<0.001) at 45 min of OGTT on par with Vildagliptin group (Table 1). This anti hyperglycemic action through the entero-insular axis of Trigonella foenum graecum extract will contribute significantly in controlling the postprandial hyperglycemia in Type 2 diabetes mellitus.

Vildagliptin group showed incretin enhancing effect by increasing GLP-1 (p =<0.05) after 15 minutes and GIP (p =<0.001) at 45 minutes of OGTT (Table 2). A similar study showed increase in GLP-1 level at 5 minutes after OGTT. In our study the GLP-1 level increased at 15 minutes and significant increase in GIP levels (p =< 0.001) at 45 minutes of OGTT (Table 2) were also demonstrated.

The TFG herbal extract at low and high doses showed significant increase in GIP levels (p =< 0.001) at 45 minutes of OGTT (Table 2) which is an important finding in our study to prove that Trigonella foenum graecum seed extract is having effect on the entero-insular axis. An interesting in-vitro study done on the pancreatic beta cells showed that GIP acts as a mitogenic and anti-apoptotic factor for beta cells. In concordance with the similar action our herbal extracts may also have the mitogenic and beta cell proliferating action through GIP. However further in-depth research in this aspect will be highly rewarding to discover an ideal antidiabetic drug for diabetes mellitus.

Trigonella foenum graecum extract (1 gm/kg) decreased glucagon (p =<0.001) at 15 minutes and 45 minutes of OGTT (Table 3). A similar study done in dogs established the effect of Trigonella foenum graecum in reducing plasma glucagon and somatostatin which supports the finding of our study.

![Graph 1: Shows effect of Vildagliptin (VG) and Trigonella foenum graecum (TFG-L) 1g/kg, (TFG-H) 2gm/kg on blood glucose levels during OGTT in albino rats.](image-url)
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Table 1: Effect of Trigonella foenum graecum on Glucose tolerance test in albino rats.

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-30mts ± SD(n=6)</td>
</tr>
<tr>
<td>Control</td>
<td>76.4 ± 6</td>
</tr>
<tr>
<td>VG (50 mg/kg)</td>
<td>77.1 ± 2</td>
</tr>
<tr>
<td>TFG (1gm/kg)</td>
<td>72.2 ± 15</td>
</tr>
<tr>
<td>TFG (2gm/kg)</td>
<td>73.3 ± 16</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6

Table 2: Effect of Trigonella foenum graecum, on GLP-1, GIP during Glucose tolerance test in albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLP-1 (pg/ml)</th>
<th>GIP (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD (mean n=6)</td>
<td>Time (minutes)</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 1</td>
<td>15 ± 0.2</td>
</tr>
<tr>
<td>VG (50 mg/kg)</td>
<td>15 ± 0.5</td>
<td>16 ± 0.5</td>
</tr>
<tr>
<td>TFG (1gm/kg)</td>
<td>15 ± 1</td>
<td>13 ± 0.4</td>
</tr>
<tr>
<td>TFG (2gm/kg)</td>
<td>14 ± 0.1</td>
<td>14 ± 1.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD; (n=6) VG- Vildagliptin, TFG- Trigonella Foenum graecum

Table 3: Effect of Trigonella foenum graecum on Insulin and Glucagon during Glucose tolerance test in albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Insulin (ng/ml)</th>
<th>Glucagon (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD (mean n=6)</td>
<td>Time (minutes)</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td>Control</td>
<td>0.2 ± 1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>VG (50 mg/kg)</td>
<td>0.21 ± 1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>TFG (1gm/kg)</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>TFG (2gm/kg)</td>
<td>0.20 ± 1</td>
<td>0.20 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD; (n=6) VG- Vildagliptin, TFG- Trigonella Foenum graecum

CONCLUSION
Trigonella foenum graecum seed extract shows significant antihyperglycemic and incretin enhancing effects through enteroinsular axis. Further scientific and clinical studies are necessary to establish the therapeutic potential of the extract in the treatment of type 2 diabetes mellitus.

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CONFLICTS OF INTEREST
Conflicts of interest declared none.

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AUTHORS CONTRIBUTION STATEMENT
Prof.M. Muniappan conceived the idea and guided me in conducting this research study and also reviewed the manuscript. Dr. S. D. Inbaraj myself carried out the research study, evaluated the results and written the manuscript.

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