STUDIES ON THE ISOLATION AND EFFECT OF AN ORALLY ACTIVE HYPOGLYCEMIC PRINCIPLE FROM THE SEEDS OF FENUGREEK (Trigonella Foenum Graecum)

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The seeds of *T. foenum graecum* have been credited with many medicinal properties. The extracts of fenugreek seeds as well as, its major alkaloid trigonelline exert a mild hypoglycemic activity (Nadkarni and Nadkarni, 1954). However, according to Mishkinsky et al (1979), hypoglycemic effect of the nicotinic acid derivative trigonelline was transient. *Trigonella* seeds have also been reported to contain coumarin, some derivatives of which are carcinogenic. This aspect should be kept in mind while treating with whole fenugreek seeds, which have been recently tried in humans. The present studies have been undertaken with a view to isolate an active principle from *T. foenum graecum* seeds, study its effect and understand its mechanism of action.

Materials and Methods

*Alloxan recovered (AR) and severely diabetic (SD) rabbits:*

Alloxan (80mg/kg) was given intravenously to rabbits and fasting blood glucose (FBG) values were estimated at interval of 5 days for one month. The rabbits with FBG values above 250 mg/dL and stabilised diabetes were designated as severely diabetic (SD) rabbits. However, the rabbits which recovered by one month and showed normal or slightly elevated FBG values had an abnormal glucose tolerance pattern when glucose tolerance test (GTT) was performed. These apparently normal-rabbits but with impaired glucose tolerance were called alloxan recovered AR rabbits. The details have been reported (Venkanna Babu et al 1988).

*Assessment of hypoglycemic effect*

(a) With *SD rabbits*: After oral administration of the drug to the overnight fasted rabbits, fall in FBG with time was taken as a measure of hypoglycemic effect.

(b) With *AR rabbits*: Basal GTT was first established in AR rabbits with water (glucose, 3g/Kg) in place of the drug. One week later, the same group of animals were administered the test fraction orally and GTT performed 90 min. later. Improvement in glucose tolerance as indicated by a decrease in peak blood glucose (usually at 90 min), decrease in blood glucose at 2½ hrs, and an overall suppression of the curve was taken as a measure of hypoglycemic effect. The effect could also be assessed quantitatively by measuring the area under the curve (AUC). In this test the same animal served as its own control. Details were described earlier Venkanna Babu et al, (4).

*Blood glucose estimation*: Blood glucose was estimated by the method of Hugget and Nixon, 1957 (3).

*Isolation of hypoglycemic principle*

Fenugreek seeds were soaked in minimal amount of water and the extract subjected to DEAE cellulose chromatography. The yellow active fraction (FI) was cut from the column, eluted and passed through Sephadex G-100 column. The light yellow fraction (GII) was active. It was further purified by preparative thin layer chromatography using silica gel-6 and...
butanol-acetic acid: water (5:1: 4 v/v) as solvent. This gave two fractions out of which the second (TII) was active. Due to low yield of the TII fraction, GII and FI fractions were also used in the experiments as indicated.

**Results**

Preliminary studies with water extract gave only an indication of the hypoglycemic effect of fenugreek seeds. The results were uncertain. Sometimes there was hyperglycemic effect also.

*Assessment of hypoglycemic activity in AR rabbits with Tolbutamide*

Before testing the hypoglycemic effect of purified fractions of *T. foenum graecum* the effect of the standard drug tolbutamide, which is known to act initially through pancreas, was tried using the new model of AR rabbits. Table 1 shows the GTT pattern in normal, untreated AR and tolbutamide treated AR rabbits.

The above results show that the standard drug—tolbutamide—could suppress GTT curve and decrease AUC by 30%.

**The effect of the hypoglycemic principle on FBG, and GTT in AR rabbits**

The purified principle was found to be different from the alkaloid trigonelline and nicotinic acid based on UV and IR absorption Spectra, TLC and HPLC criteria. Having shown that it is different from these principles, the effect of the purified principle on FBG, GTT pattern, glucose induced serum insulin levels serum lipid profile, serum glycosylated haemoglobin, and some of the glycolytic and gluconeogenic enzymes using single as well as multiple doses was studied.

### Table 1

#### Blood glucose mg/dL (Means S. E. M.)

<table>
<thead>
<tr>
<th>Type of Fasting Rabbits (5 in each 1 group)</th>
<th>During GTT minutes after glucose</th>
<th>AUC</th>
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<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>95±8</td>
<td>142±11</td>
</tr>
<tr>
<td>AR untreated</td>
<td>103±9</td>
<td>204±18</td>
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<td>AR tolbutamide (300mg/kg)</td>
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<td></td>
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<tr>
<td>(a) pre-treatment</td>
<td>137±7</td>
<td>221±19</td>
</tr>
<tr>
<td>(b) after treatment</td>
<td>138±7</td>
<td>165±11</td>
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</tbody>
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April, 1989
Single dose up to 100 mg/kg of sephadex fraction G-II had no significant effect on FBG but even 25 mg/kg suppressed GTT curve and brought about reduction in both the peak glucose level at 90 min and the area under the curve (AUC) by 22%. With a higher dose of 50 mg/kg the fall was 31%. But higher doses up to 175 mg/kg did not enhance the effect.

With two doses of 50 mg/kg at an interval of 1 week, the suppression in GTT was seen for a period of another one week. Even when given for 1 week (daily 50 mg/kg), there was no fall in FBG when the initial FBG was less than 100 mg/dL indicating that it did not have any adverse effect on the mechanism of glucose homeostasis. But there was a fall in FBG when the initial FBG was nearly 100 mg/dL. This favourable effect of bringing down FBG when the initial fasting level was higher was observed in AR rabbits. AR rabbits with initial FBG of 185 ± 14 mg/dL was given daily once 100 mg/kg of the sephadex GII fraction for 21 days. FBG came down to 125 ± 11 mg/dL (32% fall) and this was maintained at this level for one week more after stopping treatment. In a still prolonged trial of 2 months with intermittent administration with 50 mg/kg/day (days of treatment were 0-5, 11-15, 26-30 and 50-60). There was a remarkable improvement in FBG which came down from an initial value of 192 ± 12 to a near normal value of 95 ± 8 mg/dL with a similar improvement in GTT also. The AUC was brought down from 59850 to 36453 mg/dL/min (39% fall). This indicates that the drug exhibited very good glycemic control even though the interval between each period of treatment was progressively increased. At a dose of 100 mg/kg daily once for 3 days in AR rabbits with an initial FBG of about 115 mg/dL sephadex fraction GII, the alkaloid trigonelline and tolbutamide brought about reduction in AUC of GTT by 32%, 34% and 28% respectively, (P<0.01 in all cases). Therefore its action was comparable to that of tolbutamide and trigonelline. However, when the initial FBG value in AR rabbits was 185-210 mg/dL, trigonelline did not show any significant fall in FBG but only slight (16%) improvement while sephadex GII fraction produced both fall in FBG (30%) and improvement in GTT (36% reduction in AUC) pointing out that sephadex GII fraction is different from and more potent than trigonelline. The fact that there was an improvement in a group of rabbits with FBG ranging from 185-210 mg/dL in which there would be significant destruction of B cells of pancreas perhaps indicates that it has extra pancreatic effect too.

Effect of the hypoglycemic principle on severely diabetic (SD) rabbits

With a single oral dose varying from 25 to 175 mg/kg to SD rabbits with an initial FBG of 400 mg/dL and above, there was no significant fall in FBG. Six dose of 50 mg/kg daily once for 1 week also did not produce any significant fall in FBG. However, when fed for one month, the FBG values came down from about 400 mg/dL to nearly 220 mg/dL and even GTT could be performed in such rabbits. Untreated SD rabbits usually die if glucose is given. This indicates the use of the hypoglycemic principle on SD rabbits and also its extra pancreatic effect.

Studies on the mode of action

Since the hypoglycemic principle gave indication in the above studies of exhibiting pancreatic as well as extra pancreatic effects, detailed biochemical studies were also carried out. A dose of 100 mg/kg of sephadex GII fraction was given once a day orally for 15 days to both AR and SD rabbits (5 in each group). FBG, glycosylated haemoglobin, serum lipid profile, tissue lipids and glycogen and enzymes of glycolysis and gluconeogenesis were assayed. However, the results are not shown in this paper. In a still prolonged trial of 2 months with intermittent administration with 50 mg/kg/day, there was a remarkable improvement in both FBG and GTT also. The AUC was brought down from 59850 to 36453 mg/dL/min (39% fall). This indicates that the drug exhibited very good glycemic control even though the interval between each period of treatment was progressively increased. At a dose of 100 mg/kg daily once for 3 days in AR rabbits with an initial FBG of about 115 mg/dL sephadex fraction GII, the alkaloid trigonelline and tolbutamide brought about reduction in AUC of GTT by 32%, 34% and 28% respectively, (P<0.01 in all cases).
were estimated. There was a significant increase in glucose induced serum insulin by 136% in AR rabbits with improvement in glucose tolerance of 28%. The glycosylated hemoglobin, which is being used to monitor long term glycemic control decreased only by 14% in AR rabbits. These results suggested good glycemic control even in SD rabbits on treatment with the putative hypoglycemic principle.

Treatment for 15 days did not enhance the activity of key glycolytic enzymes (glucokinase (GK) phosphofructokinase (PFR) and pyruvate kinase (PK) in liver which reduced in AR and SD rabbits but in muscle there was an increase in GK (35%) and PFK (89%) activities. Likewise two gluconeogenic enzymes-glucose-6-phosphatase and fructose-1, 6-diphosphatase in liver and kidney which were elevated showed some reduction after treatment, though not highly significant. Increased serum lipids namely cholesterol, triglycerides, phospholipids and free fatty acids were brought back in both AR and SD rabbits to near normal values. This shows that sephadex GII fraction acts first by correcting the abnormalities in lipid metabolism. Then enzymes of gluconeogenesis and enzymes of glycolysis in muscle are influenced. Perhaps changes in liver enzymes are to be anticipated only later. In support of the above view it was observed from histopathology of pancreas that after 15 days treatment there was less fatty infiltration in the islet cells.

There were no toxic effects after 15 days treatment as judged by the liver and kidney function tests like the levels of serum alkaline phosphatase, transaminases, bilirubin, proteins, creatinine and blood urea.

Discussion

A highly active hypoglycemic principle has been isolated from *T. foenum graecum*. It appears to be different from and more potent than trigonelline, the major alokaloid. In AR rabbits it increased glucose induced serum insulin levels and improved glucose tolerance in GTT. After treatment with a dose of 100 mg/ kg, 15 days in both AR and SD rabbits, there was an improvement in glycosylated hemoglobin and serum lipid profile. In muscle but not in liver there was an increase in the activity of the key enzymes of glycolysis. Slight inhibition in the activity of gluconeogenic enzymes was also noticed. That the active compound seems to act both at pancreatic and extra pancreatic sites.

References


