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

Moorthy R, Prabhu KM, Murthy P. S.

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) *Wtth 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

Department of Biochemistry, University College of Medical Sciences, Shahdara, Delhi 110032.

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.)

Type of Fasting Rabbits (5 in each 1 group)	During GTT minutes after glucose						
	30	60	90	120	150		AUC
Control	95±8	142±11	165±10	118±12	100±11	92±7	18960±872
AR untreated	103±9	204±18	230±21	290±12	254±19	218±10	33431±1140 76% increase p<0.001
AR tolbutamide (300mg/kg) (a) pre-treatment	137±7	221 ±19	275±6	305±27	282±16	248±6	38870 + 930
(b) after treatment	138±7	165±11	210±9	181±14	162±15	146±13	27043±230 (30% decrease p<0.001)

Single dose upto 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 50mg/kg the fall was 31%. But higher doses upto 175 mg/kg did not enhance the effect.

With two doses of 50mg/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 100mg/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 100mg/kg of the sephadex **GII** fraction for 21 days. **FBG** came down to 125±llmg/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 intermittant administration with 50mg/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±8mg/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 lipis and glycogen and enzymes of glycolysis and gluconeogenesis

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 (glucoknase (**GK**) phosphofructoknase (**PFR**) and pyruvate knase (**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 lipis 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 phosphatose, 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

1. Nadkarni, Nadkarni, K M (1954). Indian Materia Medica. Popular Book Depot 1954; p. 1240.

2. Mishkinsky J, Joseph B, Sulman FG, Golschmaid AL. Lancet 1967; 1, 1311.

3. Huggett, A. St G, Nixon M A. Use of glucose oxidase peroxidose, o-dianisidine in determination of blood and urinary glucose. Lancet 1957; 2, 368.

4. Babu B V, Moorti R, Pugazhenthi S, Prabhu KM, Murthy P S. Alloxan recovered rabbits as an animal model for screening for hypoglycemic activity of compounds Ind. J. Biochem. Biophys, 1988 (in Press).