

**THE CENTRAL ROLE OF THE FENUGREEK
(*TRIGONELLA FOENUM-GRÆCUM L.*) IN THE
REGULATION OF THE GLYCEMIA IN NORMAL RATS:
A STEREOTAXIC STUDY**

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Abstract: The fenugreek or "*Trigonella foenum-graecum*" was used for a long time like remedy for the diabetes and the problems of appetite. By its amino-acid the 4-hydroxy-isoleucine, this plant made evidence in several phytopharmacologic studies and in particular by stimulating the production of insulin and by improving the sensitivity of the organization to this hormone.

The aim of the present work is to undertake for the first time to our knowledge a study on the central effect of this plant on the brain for regulating the glycaemia. In order to make this study, we carried out the

intracerebroventricular injection of two extracts of the fenugreek; one is an aqueous extract while the other is extracted with methanol. Measurements of the rate of glucose in blood were made regularly from the injection and each 10 minutes till the third hour.

The results obtained showed a decrease of 30% of the glycaemia two hours following the administration of the aqueous extract of the plant, this value returned to its initial value one hour later. Concerning the organic extract, a more important decrease was noted at the end of the second hour but remained stable until the third hour.

Keywords: *glycaemia, fenugreek extract, 4-hydroxy-isoleucine, intracerebroventricular injection, rats*

INTRODUCTION

In recent years, the role of alternative therapeutic approaches has become very popular [1]. Nature has been a source of medicinal treatments for thousands of years, and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries [2] and Herbal drugs are frequently considered to be less toxic and with less side effects than synthetic ones [3].

The hypoglycaemic property of fenugreek was observed in alloxan-induced diabetic rats and diabetic patients [4, 5]. It was shown to decrease the blood glucose level in normal rats as well as in non-insulin-dependent DM patients [3].

Trigonella foenum-graecum L. is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in Pakistan, India and China [6]. Its dried ripe seeds are variously referred to as *Trigonella* seeds or as Fenugreek. It is well known for its pungent aromatic properties [7], and is often used to add flavor in Malaysian homes [8].

Chronic oral administration of an ethanolic extract of *Trigonella foenum-graecum* significantly increased food take and the motivation to eat in rats [9], which might be related to the aromatic properties of the seeds [10]. *Trigonella foenum-graecum* seeds are used in India as condiment, in Egypt as a supplement to wheat and maize flour for bread-making and in Yemen it is one of the main constituents of the normal daily diet of the general population. *Trigonella foenum-graecum* leaves are consumed widely in India as a green, leafy vegetable and are a rich source of calcium, iron, β -carotene and other vitamins [11].

Trigonella foenum-graecum L. (in Arabic, Hulabah) is also employed as an herbal medicine in many parts of the world. Its seeds are used for their carminative, tonic and aphrodisiac effects [12]. The seeds of *Trigonella foenum-graecum* contain the alkaloid trigonelline with mucilage, tannic acid, yellow coloring matter, fixed and volatile oils and a bitter extractive, diosgenin, gitogenin a trace of trigogenin and Vitamin A [13].

Trigonella foenum-graecum seeds and leaves are also said to a hypocholesterolaemic effects [14 – 16].

Trigonella foenum-graecum exerts its hypoglycaemic effect by delaying glucose absorption and enhancing its utilization [17]; Seeds of *Trigonella foenum-graecum* are

therefore considered to be potentially useful for glucose control and for preventing hyperlipidaemia and atherosclerosis in diabetic subjects. The active component of *Trigonella foenum-graecum* seeds has been found to be associated with a defatted part, rich in fiber containing steroidal saponins and proteins comparable to those of soybean [18].

However, very little is known about its cellular and biochemical mechanisms of the hypoglycemic or antihyperglycemic effects. It has been shown that the plant (whole seed powder added to the diet) favorably affected glycolytic, gluconeogenic, and lipogenic enzymes to restore glucose homeostasis in alloxan-induced diabetic rats [19]. In the present investigation, we studied the effect of aqueous and methanolic extracts of *Trigonella foenum-graecum* seeds on fasting blood glucose levels in normal rats by intracerebroventricular injection.

MATERIALS AND METHODS

Plant material

Trigonella foenum-graecum seeds were purchased from the local market, cleaned, dried and finely powdered.

Extraction of aqueous plant material

Twenty-five grams of powdered *Trigonella foenum-graecum* seeds were extracted with 500 mL boiling distilled water for 5 min. The heated decoction was taken and allowed to cool for 30 min, at room temperature. The decoction was filtered twice; the filtrate was lyophilized and stored in refrigerator.

Extraction of methanolic plant material

Two-hundred and fifty grams of powdered *Trigonella foenum-graecum* seeds were soaked with 500 mL methanol for 6 days. After 6 days the decoction was filtered twice. The filtrate was concentrated by rotary evaporator and stored in refrigerator.

Animals and surgery

Male Wistar rats initially weighing 250 to 300 g were used. The animals were housed in groups of 4 per cage. The first cage contained the control animals; they received saline solution (NaCl 0.9%). The second cage contains animals that were treated by aqueous extracted and the third group was treated by methanolic extract. Animals had free access to standard laboratory chow (35% carbohydrates, 25% proteins, 7% lipids, and 3% vitamins) and tap water. The animal room was maintained at 22 ± 2 °C with timed lighting on from 7 AM to 19 PM.

Stainless steel cannula (o.d. 0.96, i.d. 0.58 mm; biotrol) for i.c.v. administration of drugs was stereotaxically implanted under general anesthesia using Pentobarbital in the right lateral ventricle of the brain, according to the procedure described by Koning and Klippel.

The cannula was inserted at a point 1mm anterior to lambda, 2mm lateral to the midline and 5mm below the skull. Amoxiciline powder (Clamoxyl, Smithkline Beecham) was used prior to stitching. After surgery, the animals were allowed to recover for one week before being subjected to pharmacological investigations.

All animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals”. Experiments were carried out in accordance with the European Community Council Directive of November 24, 1986 (86=609=EEC).

Experimental procedure

The effect of intracerebroventricular (i.c.v.) fenugreek injection on glycaemia was studied at 25 °C Ta. At the end of the period the rats were usually very quiet.

Blood sampling

After the administration of aqueous and methanolic extracts the blood samples were drawn from the tail at 0, 1, 2, and 3 h. Blood glucose levels were determined using glucometer (*ACCU-CHEK® Active* from the company *Roche Diagnostics*).

Blood glucose levels at zero time were considered as control values.

RESULTS AND DISCUSSION

Hypoglycaemic activities of the extract of *Trigonella foenum-graecum* seed

Blood glucose level in control animals

From our studies, the mean of blood glucose level was 80 mg.dL⁻¹ and after the intracerebroventricular administration of 0.2 mL saline solution.

Hypoglycaemic effect of aqueous extract of *Trigonella foenum-graecum* in normal animals

It has been observed that the intracerebroventricular administration of aqueous *Trigonella foenum-graecum* seeds extract at a dose of 0.2 mL produces significant hypoglycaemic effect in normal animals 2 hours after injection (Table 1). Glycaemia showed a fall of 30% (from 99 mg.dL⁻¹ to 65 mg.dL⁻¹) then returned back to its initial value after the third hour.

Table 1. Blood glucose level of normal mice after ICV injection of aqueous “*Trigonella foenum-graecum*” (Fenugreek) seed at a dose of 0.2 mL

| Time (h) | Glycaemia of Controls (mg.dL⁻¹) | Glycaemia of animals treated by aqueous extract (mg.dL⁻¹) |
|-----------------|---|---|
| 0 | 80 | 99 |
| 1 | 80 | 98 |
| 2 | 80 | 65 |
| 3 | 80 | 97 |

Hypoglycaemic effect of methanolic extract of *Trigonella foenum-graecum* in normal animals

It has been observed that the ICV administration of methanolic extract of *Trigonella foenum-graecum* seeds at a dose of 0.2 mL produced only 10% decrease glycemia after two hours (from 73 mg.dL⁻¹ to 64 mg.dL⁻¹) (Table 2).

Table 2. Blood glucose level of normal mice after ICV injection of methanolic “*Trigonella foenum-graecum*” (Fenugreek) seed at a dose of 0.2 mL

| Time (h) | Glycaemia of Controls (mg.dL⁻¹) | Glycaemia of animals treated by methanolic extract (mg.dL⁻¹) |
|-----------------|---|--|
| 0 | 80 | 73 |
| 1 | 80 | 72 |
| 2 | 80 | 64 |
| 3 | 80 | 62 |

It is well known that health problems are multiplying in threatening enormity in almost all the countries. In past few years, it has been observed that diabetes is one of the most common diseases in both sexes. This disease is characterized by hyperglycemia, glycosuria and other symptoms that differ in importance. Its basic cause is a relative or absolute lack of insulin, which results in profound changes in the intermediary metabolism of carbohydrates, lipids and proteins. Ever since the isolation of insulin work has been going on to find out at what point in the metabolism of carbohydrates it acts and to elucidate the pathology of diabetes. Insulin, which is secreted from beta cells of the islets of Langerhans in the pancreas, is responsible to make possible adequate utilization of glucose by the tissue at normal blood sugar levels. This produces a diminished glucose tolerance with a raised blood sugar level, which is often well above the renal threshold for glucose. Impaired utilization of carbohydrates is associated with increased secretion of anti-storage hormones such as glucagon and growth hormone in an attempt to provide alternative metabolic substrate. Glycogenolysis, gluconeogenesis and lipolysis are all increased. The latter results in excess formation of ketone bodies turn leads to acidosis. If untreated, this will eventually cause coma (ketoacidosis diabetic coma) and death.

Much work has been done and is still going on for the cure of this disease. Many synthetic hypoglycaemic agents have been produced to overcome this problem. Before the advent of insulin and oral hypoglycaemic agents the major form of treatment involved the use of plants. More than 400 plants are known to have been recommended, and recent investigations have affirmed the potential value of some of these treatments [21, 22].

According to literature on *Trigonella foenum-graecum* this plant has had a long history of medicinal uses in most of the Asian and African countries. Its uses have been well known for a long time for their antidiabetic action [23 – 27]. *Trigonella foenum-graecum* seeds are found to remarkably suppress the clinical symptoms of diabetes such as polyurea, polydypsia, weakness and weight losses [11].

This study has shown for the first time the central hypoglycaemic effect of *Trigonella foenum-graecum* in normal rats. The aqueous extract of *Trigonella foenum-graecum* seeds show a significant fall in blood glucose level in normoglycaemic mice administrated centrally. Although the methanolic extract of *Trigonella foenum-graecum*

at the same dose showed no significant fall in blood glucose level. This may be due to the fact that the substance responsible for hypoglycaemic activity is probably polar in nature, being more soluble in water than in methanol. Another reason for the observed results could be the presence of two different substances present in *Trigonella foenum-graecum* but with different hypoglycaemic activities.

The hemathoencephalique barrier may play a selective role for substances that cross it by delaying the passage of methanol.

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