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Pharmacognostic Profile of Trigonella Seed and Its Hypoglycaemic Activity

Mariam Ahmad[1], Norhayati Ismail and Zhari Ismail
School of Pharmaceutical Sciences, Universiti Sains Malaysia,
11800 Minden, Penang, Malaysia

Abstract—Pharmacognostic study was carried out on the seeds of *Trigonella foenum-graecum* L. (fenugreek) in order to establish its pharmacognostic characteristics. In view of its renewed interest as a dietary supplement among the local inhabitants afflicted with diabetes, its hypoglycaemic activity in normal as well as diabetic rats was also investigated. Oral glucose tolerance test showed that a suspension of the powdered trigonella seed inhibited the increase in blood glucose levels in normal rats that had been treated with an oral glucose load prior to the test. A reduction in the blood glucose levels was also observed when a suspension of the powdered seed was given by gastric intubation to the streptozotocin-induced diabetic rats. These findings suggested that trigonella seeds possessed some hypoglycaemic activities that might be useful to the diabetics.

Keywords—Trigonella seed · pharmacognostic profile · hypoglycaemic activity · diabetes

Trigonella foenum-graecum L. is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in India and China (Morton, 1990). Its dried ripe seeds are variously referred to as trigonella seeds or as fenugreek.

The taste and smell of the seed account for its use as a spice and a flavouring agent in Malaysian homes. The Malays also poultice the seeds onto burns and use them for chronic coughs, dropsy, hepatomegaly and splenomegaly (Perry, 1980). In recent years, it has received much scientific attention as a potential source of steroidal sapogenins (Fazli and Hardman, 1971) as well as an antidiabetic agent.

In the present investigation, we studied the pharmacognostic profile of trigonella seed and also its effects on the blood glucose levels in normal and diabetic rats.

Experimental

Plant Material - Fenugreek seeds were purchased from the local market. These were cleaned and ground to a fine powder (40 mesh) in a cyclone mill. Powdered samples were stored at room temperature.

Pharmacognostic Study - The microscopic studies of the powdered seeds were carried out in the conventional manner.

Qualitative tests were performed according to the methods described in the Materia Medika Indonesia, Vol. I (1977). Filtrate from 500 g of powdered samples soaked overnight in ethanol was used for thin-layer chromatography. The plates were developed in appropriate solvent systems and treated in iodine. All the chemicals used were analytical grade.

[1]Author for correspondence.

Animals - Both normal and diabetic albino rats (200-250 g) used in this study were obtained from the animal house of the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang. They were housed in clean wire mesh cages and fed *ad libitum* except during fasting.

Diabetes was induced in male Wister albino rats by the intravenous injection of streptozotocin (65 mg/kg body weight) dissolved in citrate phosphate buffer with pH adjusted to 4.5 (Theodorou *et al.*, 1980). The diabetic state was confirmed on the fifth day after the administration of streptozotocin by determining the blood glucose concentration and only those with high blood glucose levels above 250 mg/dl were used in the experiments.

Oral Glucose Tolerance Test - Oral glucose tolerance test was conducted on normal albino rats which had been fasted for 24 hours. A group of these rats was given the aqueous suspension of the powdered seeds (1 g/kg) by gastric intubation. The control rats were given oral saline (5 ml/kg). An oral glucose load of 1.5 g/kg was administered 15 minutes later. Blood samples were drawn from the tail at 30 minutes before the test and every half an hour thereafter for 270 minutes.

Glucose levels were measured in 0.02 ml of whole blood using glucose oxidase kits (Boehringer-Mannheim, GmbH, Mannheim, Germany) and UV spectrophotometer (Shimadzu).

Hypoglycaemic Study - Diabetic rats weighing 200-250 g were fasted for 24 hours. They were then given an oral dose of the aqueous suspension of the powdered seeds (1 g/kg). Control rats were given oral saline (5 ml/kg). Blood samples were drawn from the tail vein at 0, 1, 2, 3, 5 and 7 hours after the administration of the powdered seeds. Blood glucose levels were determined using glucose oxidase kits.

Statistical Analysis - The experimental data were evaluated by employing Student's t-test.

Results and Discussion

Plant Morphology - An erect, strongly aromatic, annual herb, reaching 60 cm high, the leaves trifoliate with a large petiole and leaflets 2-2.5 cm long. A stipule is found at the base of the petiole. The small, yellowish-white flowers are borne singly or in pairs in the leaf axils. The fruit, a legume which arises from leaf axil, is 5-10 cm long, narrow, pointed, containing about 10-20 brownish-yellow seeds.

Pharmacognostic Profile - *Macroscopic characteristics of the seed*: Trigonella seeds of commerce are small, hard, angular, somewhat compressed, often more or less shrivelled, and with a light brown or brownish-yellow colour externally, and yellow internally. It has a smooth outer surface with a groove on one side which is the hilum (Fig. 1). The smaller part of the seed which seems folded is the radicle. The powdered seeds, which are yellowish-brown, have a somewhat oily and farinaceous taste, accompanied by a slight bitterness, and feeble melilot flavour, with a strong, peculiar odour which is also suggestive of melilot or of coumarin.

Microscopic characteristics of the seed powder: The fragments of the epidermis of the testa

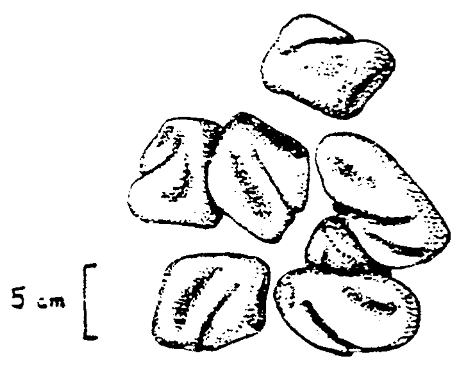

Fig. 1. Seeds of *Trigonella foenum-graecum* Linn.

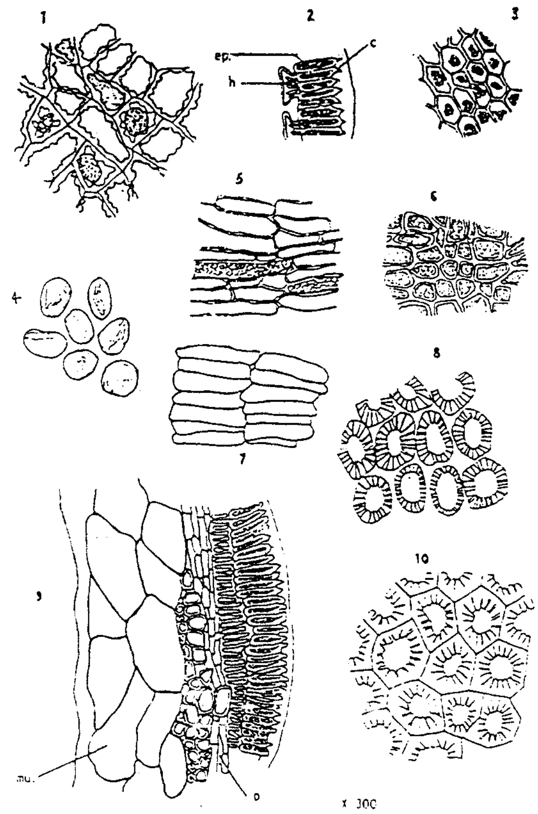

Fig. 2. Microscopic characteristics of trigonella seeds powder.

1. Outer layer of endosperm from surface view
2. Testa showing cuticle layer
3. Epidermis of testa
4. Starch granules
5. Parenchyma cells
6. Epidermis of testa viewed from below
7. Palisade cells
8. Hypodermis of testa from surface view
9. Section showing epidermis, hypodermis and parenchyma cells (p) for testa, followed by the outermost endosperm layer and mucilage cells.
10. Epidermis of testa viewed form below

consist of cells containing yellowish-brown pigments. They are longitudinally elongated, conical towards the outside. In surface view, the cells are polygonal and regular with thick walls and small lumen from which radiate distinct pits; when viewed from below the cells are similar in outline but the lumen is larger and filled with dense pigments (Fig. 2).

The hypodermis of the testa composed of a single layer of colourless cells with a very characteristic appearance. In sectional view the cells seem to be narrower at the end and are thickened on the radial wall with evenly spaced, rod-like thickenings which run vertically. If viewed from above, the rounded outline of the upper wall of the cells is seen with the top of the rods thickening; when viewed from below the polygonal outline is apparent.

The parenchyma of the testa composed of several layers of thin-walled cells which appear similar in sectional view but in sufrace view the various layers show differences in structure; some of the layers are composed of elongated rectangular cells with slightly thickened and beaded walls; other layers are composed of thin-walled polygonal cells which may be very irregular in size or may enclose irregular intercellular spaces.

The abundant parenchymal cells are found in the cotyledon. The cells are different from the parenchymal cells of testa. The cells are differentiated to form an epidermis and palisade while others are rounded or polygonal and undifferentiated. The mucilage cells are found in the endosperm. They are large thin-walled cells. The very few starch granules are round to oval with faint striations. Lumen are sometimes found in the centre.

Color test: The color tests of the powdered seeds with concentrated sulphuric acid, concentrated hydrochloric acid, sodium hydroxide (5%), potassium hydroxide (5%), ammonium hydroxide (25%) and ferric chloride (5%) resulted in colorless to brown, light yellow, light yellow to yellow, colorless to yellow, colorless to light yellow, and yellow to greenish-brown, respectively.

Moisture content: Not more than 7%.

Ash content: Not more than 3.5%.

Water soluble ash content: Not less than 1%.

Acid insoluble ash content: Not less than 2%.

Ethanol extractive value: Not less than 3%.

Fig. 3. TLC pattern of trigonella seeds.

Water extractive value: Not less than 18% (by hot method) and 35% (by cold method).

Thin-layer chromatography: The TLC fingerprints (solvent system-chloroform: methanol: water=6:1:0.5) were shown in Fig. 3.

Hypoglycaemic Study - Glucose tolerance test conducted on normal rats showed that the oral administration of the powdered trigonella seeds (1 g/kg) prior to an oral glucose load resulted in the inhibition of the glucose-induced hyperglycaemia (Table 1). The blood glucose level was significantly reduced by as much as 13% when compared to the control group at 120 minutes and remained significantly lower thereafter throughout the duration of the experiment. In addition, the blood glucose levels in streptozotocin-induced diabetic rats were also significantly reduced ($p<0.05$) after the oral administration of the seeds at a dose of 1 g/kg (Table 2). This occurs at the third, fifth and seventh hour of the experiment during which the glucose leves in the blood of the diabetic rats were lowered by as much as 16%, 24% and 29%, respectively.

In the past decade, there has been a considerable interest in the use of high-fiber diets for improving blood glucose levels in diabetic patients. With some exceptions (Wahlqvist *et al.*, 1979), most of the studies have reported beneficial effects. The addition of fiber to the diet was found to lower postprandial glucose levels in normal subjects (Jenkins *et al.*, 1977) as well as in patients with non-insulin dependent diabetes mellitus, NIDDM (Kanter *et al.*, 1980). Since the analysis of the trigonella seed has revealed that 53.9% of its component is dietary fibre (Valette *et al.*, 1984), it might be incorported as a supplement to the diabetic diet. The trigonella fiber consists of 19.0% gum, 23.6% hemicellulose, 8.9% cellulose and 2.4% lignin.

Dietary fibre improves carbohydrate metabolism by several mechanisms. First of all, water-soluble fibers (gums, pectin, some hemicelluloses) delay gastric emptying time and transit time (Holt *et al.*, 1979). When soluble fibres are incorporated into standard meals, postprandial hyperglycaemia is less striking than after controlled meals, because fiber slows down the release of glucose from the stomach. Second, D-xylose tolerance tests and hyperglycaemic clamp techniques performed with the simultaneous administration of fiber have demonstrated that the glucose-lowering effect of dietary fibre is a consequence of decreased carbohydrate absorption at the gut level (rather than increased total glucose utilisation or augmented uptake of glucose by the

Table 1. Blood glucose levels of normal rats at half-hourly intervals after oral administration of powdered trigonella seed (1 g/kg) during oral glucose tolerance test.

	Blood glucose levels (mg/dl)								
Treatment	Initial	Post-treatment (min)							
	0	30	60	90	120	150	180	210	270
Control	131.1±10.7 (100)	192.3±5.7 (100)	184.4±6.3 (100)	180.0±7.2 (100)	167.1±5.9 (100)	176.5±7.8 (100)	167.2±4.5 (100)	171.5±6.6 (100)	153.5±6.5 (100)
Trigonella seed	139.3±5.9 (106)	177.1±15.1 (92)	190.1±16.4 (103)	171.9±4.1 (96)	144.6±14.8* (87)	133.0±8.4* (75)	136.2±10.9* (81)	140.5±9.4* (82)	136.4±9.7 (89)

Each value represents the mean±S.E. for six rats (n=6).
Experimental group has been compared with control group.
*Significantly different from the control value: $p<0.05$
Figures in parenthesis are percentages of the control value.

Table 2. Blood glucose levels of streptozotocin-induced diabetic rats at various time intervals after oral administration of powdered trigonella seed (1 g/kg)

	Blood glucose levels (mg/dl)					
Treatment	Initial	Post-treatment (h)				
	0	1	2	3	5	7
Control	371.1±6.5 (100)	397.4±11.5 (100)	394.3±9.2 (100)	400.7±11.8 (100)	409.3±12.1 (100)	426.2±21.4 (100)
Trigonella seed	380.3±8.2 (102)	381.8±11.6 (96)	372.8±21.8 (95)	338.4±12.0* (84)	309.9±10.6* (76)	304.5±14.9* (71)

Each value represents the mean±S.E. for six rats (n=6).
Experimental group has been compared with control group.
*Significantly different from the control value: $p<0.05$
Figures in parenthesis are percentages of the control value.

liver) (Jenkins *et al.*, 1978; Wahren *et al.*, 1982). It appears that fiber may package the carbohydrate molecules and insulate them from both digestive enzymes and direct contact with the intestinal mucosa. Third, in several studies the lower blood glucose levels observed after the consumption of dietary fibre have been associated with lower serum insulin (Kay *et al.*, 1981; Monnier *et al.*, 1982) and urinary C-peptide levels (Burke *et al.*, 1982). Therefore, the findings in the present study might be due to the fiber in trigonella seeds which possibly exerts its hypoglycaemic effect by any of these mechanisms.

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