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Antidiabetic Effects of Subfractions from Fenugreek Seeds in Diabetic Dogs (42322)

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Abstract. We have previously shown that the antidiabetic property of fenugreek seeds (*Trigonella foenum graecum* L.) is associated with the defatted seed material which is rich in fibers, saponins, and proteins. In the present work this defatted preparation was divided into two subfractions: subfraction "a" which contains the testa and endosperm and is rich in fibers (79.6%); and subfraction "b" which contains the cotyledons and axes and is rich in saponins (7.2%) and proteins (52.8%). We investigated the effects of each subfraction on hyperglycemia and the levels of pancreatic hormones when chronically administered to alloxan-diabetic dogs. Each subfraction was studied separately and was given to the dogs per os (mixed with the two daily meals), in addition to the insulin treatment (which was kept the same throughout the experiment) for a period of 21 days. The addition of subfraction "a" to insulin treatment resulted in a clear decrease of hyperglycemia and glycosuria accompanied by a reduction of the high plasma glucagon and somatostatin levels in diabetic dogs. The treatment also decreased the hyperglycemic response to the oral glucose tolerance test. In contrast the chronic administration of subfraction "b" had no effect on hyperglycemia or on the levels of pancreatic hormones in diabetic dogs. Our results show that the antidiabetic properties of fenugreek seeds are contained in the testa and endosperm. Although this subfraction is rich in fibers (high viscosity; 115 cP), it is not possible to exclude the existence of one or more unknown active pharmacological compounds in this subfraction of the seed.

The seeds of fenugreek (*Trigonella foenum graecum* L.), an annual plant from the family of Papilionaceae–Leguminosae, have been known for a long time for their antidiabetic action. More recently Shani *et al.* (1) and Ghafghazi *et al.* (2) have confirmed this property in alloxan-diabetic rats.

We previously carried out a study to determine which fraction of the seed had an antidiabetic activity and the mechanisms involved in this effect. Two fractions of the seed were studied: the lipid extract, and the defatted seed material which contains fibers, saponins, and proteins (3, 4). The lipid extract proved to be ineffective in dogs. In contrast, the defatted fraction, administered for 8 consecutive days to alloxan-diabetic dogs, provoked a decrease in hyperglycemia and hypercholesterolemia. It seemed therefore interesting to continue this study using fractions of the defatted seed material. The defatted seed material was divided into two subfractions: subfraction "a" which contains the testa and endosperm and is rich in fibers, and subfraction "b" which contains the cotyledons and axes and is rich in proteins and saponins. The effects of these two subfractions on hyperglycemia and plasma levels of pancreatic hormones in alloxan-diabetic dogs were determined.

Materials and Methods. *Plant material.* Fenugreek seeds (cultivar Gouka) were obtained from our experimental field in Montpellier, France (Université des Sciences et Techniques du Languedoc).

Fractionation of defatted fenugreek seeds. Mature seeds were ground in a Miag grinder so that the powder could pass through a 0.8-mm mesh sieve. The particles obtained were put into a solution containing trifluorotrichloroethane (Flugène 113, Rhône-Poulenc, France) and hexane (77/23, v/v). In this solution the different histological parts of the seed can be separated according to their density. In the case of fenugreek seeds, testa and endosperm (T + E) are separated from cotyledons and axes. The cotyledons and axes (C + A) being of lower density are concentrated

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in the upper layer of the solution. Testa and endosperm are found in the bottom layer and are separated from C + A by a liquid interphase containing only fine particles.

All samples were defatted with hexane using a Soxhlet apparatus.

Techniques used to determine the composition of the subfractions of defatted fenugreek seeds. Moisture content was determined by heating the plant material in an oven at 102°C for 24 hr. Ash content was determined after 3 hr incineration of the samples at 500°C. Protein content was determined by measuring the total nitrogen content according to the method of Kjeldahl (Table I).

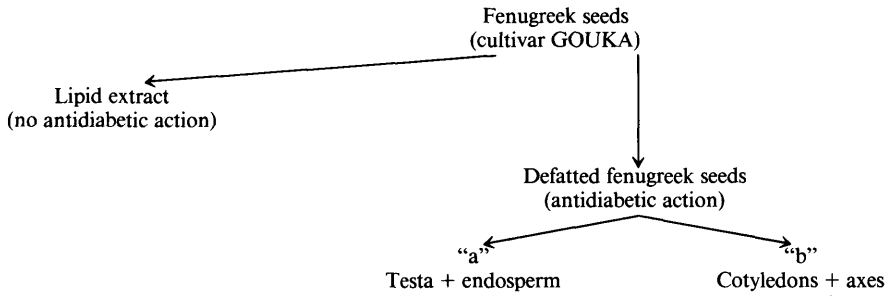
The total composition of the dietary fiber, including the undigestible sugars, was determined as follows. The gum was extracted from 1 g of sample with 100 ml of 5% CH_3COOH with stirring at 17°C for 20 min. The suspension was centrifuged for 20 min at 25,000*g* and the supernatant was recovered. The process was repeated four times and the gum (galactomannan) was precipitated by addition of ethanol until a final titer of about 50% was reached. The white hair-like precipitate was filtered through a glass-fiber filter. The precipitate, washed with ethanol and acetone, was oven-dried *in vacuo* at 40°C for 12 hr and then weighed.

The other components of the dietary fiber, i.e., hemicelluloses, cellulose, and lignin, were successively determined with a neutral detergent, an acid detergent, $KMnO_4$, and 72% H_2SO_4 according to the method of Van Soest and Goering as described by Southgate (5) by means of a Tecator Fibertec. The glucose content was determined according to the method of Trinder (6).

Steroidal sapogenins were determined by a modified specific spectrophotometric analysis (7) after acid hydrolysis of the saponins (8, 9). The results are expressed as saponins which are the native form in the seeds. Viscosity measurements were made with a Brookfield Synchro-Lectic viscometer (Beckman) at 32°C according to the method of O'Connor *et al.* (10). All the determinations were made in duplicate or triplicate.

Alloxan-diabetic dogs. Our experiments were carried out on conscious alloxan-diabetic dogs. Nine dogs were made diabetic by an intravenous injection of alloxan (50 mg/kg) at least 45 days before the experiment. The an-

TABLE I. COMPOSITION OF TWO SUBFRACTIONS OF DEFATTED FENUGREEK SEEDS (in g/100 g)



Moisture	10.0	9.6
Ash	3.0	4.9
Crude proteins ($N \times 6.25$)	6.8	52.8
Dietary fiber		
Gum	32.4	traces
Hemicelluloses	28.6	4.0
Cellulose	14.6	2.1
Lignin	3.8	0.6
Total	79.4	6.7
Steroid saponins	0.6	7.2
Other constituents	traces	18.8
Viscosity (cP)	115	0

imals were kept in individual cages. They were fed a standard constant diet (UAR, 121, Villemoisson-sur-Orge, France) divided into two meals. Each animal received two daily injections of soluble insulin (Endopancrine, Organon, France) so that they were clearly glycosuric but not ketotic. For each animal the amount of insulin injected was kept constant throughout the experiment. The doses ranged from 14 to 25 IU depending on the animal.

Experimental protocols. The effects of the chronic administration of the two subfractions from the defatted fenugreek seed material on hyperglycemia and pancreatic hormone levels in alloxan-diabetic dogs were determined. Subfraction "a" (testa + endosperm) or "b" (cotyledon + axes) was mixed with the animals' two daily meals. Five dogs received subfraction "a" at 1.145 g/kg daily. Four dogs received subfraction "b" at 1.126 g/kg daily. The doses were chosen so that the animals received an amount of fiber (subfraction "a") or saponins (subfraction "b") corresponding to that of the total defatted fraction (3, 4). The dogs were treated for 21 days. Blood samples were taken from the jugular vein every morning in the 16-hr fasted dogs, in order to measure blood glucose levels and plasma levels of insulin, glucagon, and somatostatin. The 24-hr glucosuria as well as the weight of the animals was regularly checked.

Before and at the end of both chronic treatments with fenugreek subfractions (18th day) the animals were submitted to an oral glucose tolerance test. Oral glucose tolerance tests were performed in the morning on the animals fasted for 16 hrs. Glucose (1 g/kg) was dissolved in 100 ml water and administered, by ingestion for 3 min. Thirteen blood samples were taken from a jugular vein: −10, −5, −1, +4, +8, +15, +30, +45, +60, +90, +120, +180, and +240 min, and the effects on blood glucose and plasma insulin, glucagon, and somatostatin levels were determined.

Methods of assays. Blood glucose was measured with a Technicon autoanalyzer using the potassium ferricyanide procedure for hemolyzed blood (11). Radioimmunological methods were used for the evaluation of pancreatic hormones. Plasma insulin was assayed by the method of Hales and Randle (12). The anti-insulin antibody used was the antibody INSIK-1 from CEA (Commissariat à l'Energie Atomique). Plasma glucagon was evaluated by the method of Unger *et al.* (13) using the BR 124 glucagon antiserum, a gift from the Institut de Biochimie Clinique, Geneva (Switzerland) (14). Plasma immunoreactive somatostatin was assayed according to the technique previously described (15) using the 80C antiserum of Unger. The antiserum was a gift from Dr. Unger (Health Science Center, Dallas, Tex.).

Statistical analysis. Results are expressed as means ± SEM. They were submitted to an analysis of variance using the multiple comparison test (16).

Results. *I. Effects of fenugreek subfraction "a" (T + E). (a) Chronic administration of subfraction "a" (T + E); Effects on glycemia and glucosuria.* Before the alloxan injection the glycemia of the dogs was 76 ± 2 mg/100 ml. Just before treatment with subfraction "a," the glycemia of the alloxan-diabetic dogs was 329 ± 27 mg/100 ml, and glucosuria was 50.5 ± 14 g/24 hr (Fig. 1).

Treatment with subfraction "a" resulted in a progressive reduction of hyperglycemia. The reduction became significant from the ninth day ($P < 0.05$). At the end of the treatment glycemia decreased to 258 ± 27 mg/100 ml

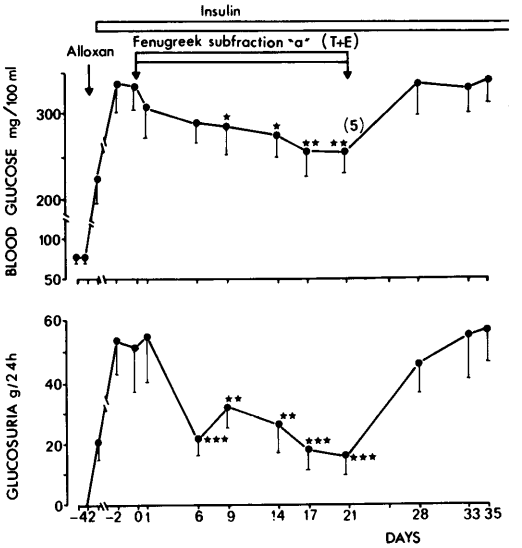


FIG. 1. Effects of the chronic administration of fenugreek testa + endosperm (T + E, subfraction "a") combined with insulin treatment on blood glucose levels and glucosuria in alloxan-diabetic dogs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

($P < 0.02$). Eight days after cessation of treatment, glycemia reached values comparable to pretreatment levels.

Glucosuria followed the same pattern as glycemia. It progressively decreased under subfraction "a" treatment (from 50.5 ± 14 to 15.7 ± 6.4 g/24 hr on the 21st day) ($P < 0.001$). Eight days after the end of treatment, it rose again to 46.3 ± 8.6 g/24 hr.

Effects on plasma insulin, glucagon, and somatostatin levels. In diabetic dogs treated only with insulin injections, circulating insulin levels were significantly decreased ($P < 0.05$), and plasma glucagon and somatostatin levels were significantly increased ($P < 0.02$), respectively, from 137 ± 7 to 197 ± 11 pg/ml and 53 ± 9 to 91 ± 7 pg/ml (Fig. 2).

The daily intake of subfraction "a" did not alter plasma insulin levels. The hyperglucagonemia progressively decreased to 161 ± 6 pg/ml at the end of treatment ($P < 0.05$). The high plasma somatostatin levels decreased as well and reached 63 ± 6 pg/ml at the end of treatment ($P < 0.05$).

No significant change in the weight of the dogs was observed during the chronic administration of fenugreek subfraction "a" (21.8 ± 3.1 kg versus 21.8 ± 2.9 kg).

(b) Oral glucose tolerance test (OGTT): Effect on glycemia. The oral administration of glucose to the diabetic dogs induced a progressive increase in blood glucose levels from 285 ± 2 to 421 ± 27 mg/100 ml within 60 min ($P < 0.001$). After these 60 min glycemia progressively returned close to starting values. After 18 days of treatment with subfraction "a," blood glucose levels were less increased by OGTT. The slope of this increase was similar till 30 min. At 30 min the maximum increase was reached (325 ± 15 mg/100 ml, Fig. 3).

Similarly, the calculation of the glycemic increments (Fig. 4A) showed that for the first 30 min the kinetics of the increase was the same for both tests. However, under subfraction "a" impregnation the maximum (+83 ± 6.3) was reached within 30 min and blood glucose did not further increase, whereas in the control tests the glycemic increment per-

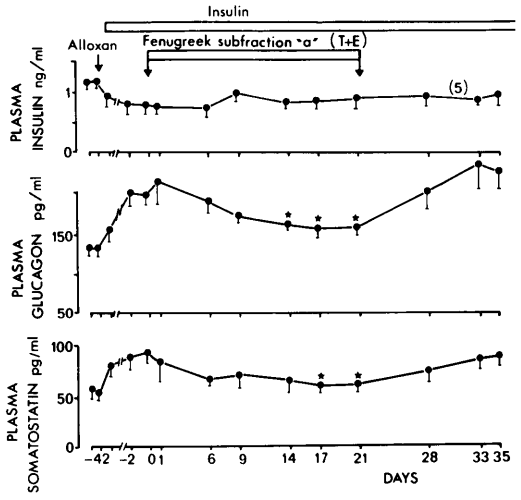


FIG. 2. Effects of the chronic administration of fenugreek testa + endosperm (T + E, subfraction "a") combined with insulin treatment on plasma insulin, glucagon, and somatostatin levels in alloxan-diabetic dogs. *$P < 0.05$.

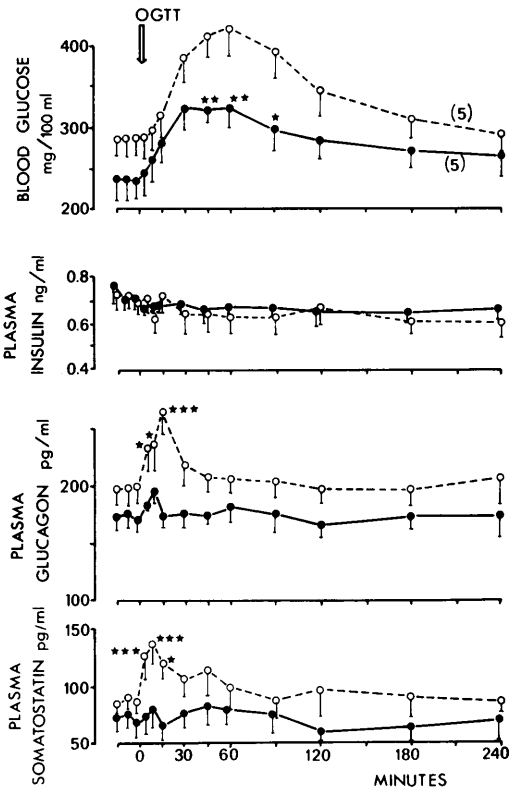


FIG. 3. Oral glucose tolerance test (OGTT) in alloxan-diabetic dogs before treatment (○) and on the 18th day of chronic treatment with fenugreek testa + endosperm (T + E, subfraction "a") (●). Effects on blood glucose, plasma insulin, glucagon, and somatostatin levels. Statistical comparison during treatment versus before treatment: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

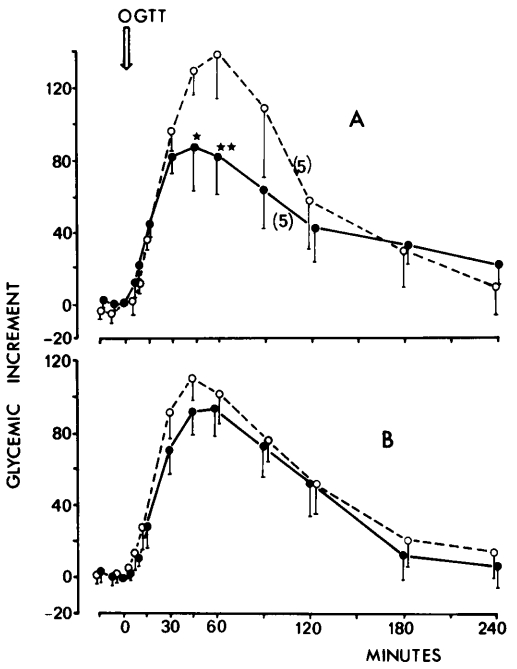


FIG. 4. In alloxan-diabetic dogs, increments in glycemia after oral glucose tolerance tests (OGTT) performed (A) before (○) and on the 18th day of chronic treatment with fenugreek testa + endosperm (T + E, subfraction "a") (●), and (B) before (○) and on the 18th day of chronic treatment with fenugreek cotyledons + axes (C + A, subfraction "b") (●). Statistical comparison, during treatment versus before treatment: $*P < 0.05$, $**P < 0.01$.

sisted, and the maximum (+136 ± 32.9) was reached at the 60th minute.

Effect on plasma insulin, glucagon, and somatostatin levels. The low plasma insulin levels were not affected by OGTT in diabetic dogs. The oral administration of glucose to these dogs triggered a rapid and considerable but transient increase in hyperglucagonemia from 200 ± 14 to 274 ± 19 pg/ml within 15 min ($P < 0.001$).

Chronic treatment with subfraction "a" caused a clear reduction of the increase in hyperglucagonemia, which rose from 172 ± 9 to 196 ± 8 pg/ml ($P < 0.05$) (8th minute).

Oral glucose intake induced a rapid but transient elevation of circulating somatostatin levels from 85 ± 6 to 138 ± 14 pg/ml at 8 min ($P < 0.001$). This increase was not observed after chronic treatment with subfraction "a."

II. Effects of fenugreek subfraction "b" (C + A). (a) Chronic administration of subfraction "b" (C + A). Effects on glycemia and glycosuria. Before the alloxan injection, the glycemia of the dogs was 79 ± 2 mg/100 ml. Before the treatment with subfraction "b," the glycemia of the diabetic dogs was 309 ± 18 mg/100 ml and glucosuria was 49 ± 4 g/24 hr (Table II).

Chronic treatment with subfraction "b" caused a transient but not significant diminution of hyperglycemia which was 252 ± 24 mg/100 ml on the sixth day. Then, glycemia rose again to values close to those recorded before subfraction "b" intake. A diminution of glucosuria was also noted on the sixth and ninth days of treatment. However, at the end of treatment glucosuria was still elevated (43 ± 3 g/24 hr).

Effects on plasma insulin, glucagon, and somatostatin levels. In these alloxan-diabetic dogs treated by insulin, there was a clear increase in plasma glucagon and somatostatin levels, respectively from 123 ± 7 to 319 ± 33 pg/ml ($P < 0.001$) and from 44 ± 5 to 89 ± 21 pg/ml ($P < 0.05$). Circulating insulin levels were significantly decreased.

Chronic treatment with subfraction "b" did not significantly affect plasma insulin, glucagon, and somatostatin levels of the diabetic dogs. The weight of the animals did not change (22.7 ± 3.8 kg versus 22.3 ± 3.9 kg).

(b) Oral glucose tolerance test (OGTT) (Fig. 5). The oral administration of glucose produced a progressive increase in blood glucose level from 321 ± 35 to 429 ± 32 mg/100 ml within 45 min ($P < 0.001$). After 18 days of treatment with subfraction "b," OGTT induced a similar increase in blood glucose levels from 311 ± 37 to 382 ± 40 mg/100 ml at 45 min. The increment in glycemia was not different from that of controls (Fig. 4B). No change was observed in plasma insulin during OGTT.

The oral administration of glucose to these diabetic dogs triggered a rapid and transient increase in plasma glucagon and somatostatin levels ($P < 0.02$). OGTT performed at the end of treatment with subfraction "b" (18th day) produced comparable responses.

Discussion. Our experiments show that the chronic administration of the defatted subfraction of fenugreek seeds corresponding to testa and endosperm (subfraction "a") can reduce hyperglycemia and glucosuria in alloxan-diabetic dogs. Furthermore, this treat-

TABLE II. EFFECTS OF THE CHRONIC ADMINISTRATION OF FENUGREEK COTYLEDONS + AXES (SUBFRACTION "b", C + A) COMBINED WITH INSULIN TREATMENT ON GLYCEMIA, GLUCOSURIA AND PLASMA INSULIN, GLUCAGON, AND SOMATOSTATIN LEVELS IN ALLOXAN-DIABETIC DOGS

	Day												
	−44	−42	−41	−2	−1	1	6	9	14	17	21	28	33	35
Glycemia (mg/100 ml)	80 ± 3	79 ± 2	210 ± 29	311 ± 20	309 ± 18	267 ± 9	252 ± 24	269 ± 20	290 ± 19	288 ± 26	284 ± 24	298 ± 30	322 ± 33	296 ± 35
Glucosuria (g/24 hr)	0	0	19 ± 5	44 ± 5	49 ± 4	55 ± 5	28 ± 10	35 ± 4	50 ± 3	44 ± 5	43 ± 3	46 ± 5	44 ± 5	49 ± 7
Plasma insulin (ng/ml)	1.19 ± 0.03	1.29 ± 0.08	0.89 ± 0.05	0.67 ± 0.09	0.67 ± 0.11	0.64 ± 0.10	0.67 ± 0.09	0.70 ± 0.10	0.68 ± 0.11	0.72 ± 0.06	0.70 ± 0.09	0.67 ± 0.10	0.71 ± 0.09	0.69 ± 0.08
Plasma glucagon (pg/ml)	126 ± 8	123 ± 7	146 ± 7	280 ± 7	319 ± 33	328 ± 49	297 ± 15	285 ± 27	318 ± 23	327 ± 45	323 ± 47	317 ± 28	399 ± 81	426 ± 110
Plasma somatostatin (pg/ml)	52 ± 6	44 ± 5	85 ± 6	79 ± 12	89 ± 21	79 ± 6	67 ± 8	85 ± 15	82 ± 20	80 ± 13	77 ± 8	85 ± 15	106 ± 23	78 ± 3

Note. Results are expressed as means + SEM.

ment lowers the high plasma glucagon and somatostatin levels of these animals. Chronic administration of the subfraction containing cotyledons and axes (subfraction "b") did not induce these beneficial effects. Therefore, our results clearly show that the subfraction containing testa and endosperm is responsible for the antidiabetic action of fenugreek. However, during the treatment with subfraction "b" a transient decrease in glycemia and glucosuria was noted (sixth day of treatment). It is possible that these effects, which disappeared on the ninth day of treatment, may be due to the acceleration of the intestinal transit (softening of the feces), observed at the beginning of treatment. When the transit became normal again, these effects were not observed despite the daily intake of subfraction "b." The antidiabetic effects of the subfraction containing testa and endosperm (subfraction "a") were confirmed by the OGTT. Thus, the hyperglycemia induced by OGTT was clearly reduced in the diabetic dogs treated with this subfraction. In contrast, treatment with the subfraction containing cotyledons and axes (subfraction "b") did not modify the hyperglycemia induced by glucose intake. Circulating insulin levels were not increased by either subfraction, as was the case during the treatment with the whole defatted fraction of fenugreek (3).

In the alloxan-diabetic dogs, oral glucose intake induced a paradoxal hyperglucagonemia. This effect has already been reported in insulin-dependent diabetics (17–19). Chronic administration of subfraction "a" (T + E) but not subfraction "b" (C + A) markedly reduced this hyperglucagonemia, an aggravating factor in diabetes. The hypersomatostatinemia observed after OGTT was also reduced. The reduction of the elevated plasma glucagon and somatostatin levels can be related to better carbohydrate regulation observed during the treatment with subfraction "a" (T + E).

It is known that the addition of dietary fiber to the diet of diabetics results in a reduction of blood glucose during OGTT (20–22) and also in a decrease in total plasma glucagon-like immunoreactivity (23). Subfraction "a" (T + E) of fenugreek is rich in fibers (79.6%) while subfraction "b" (C + A) does not contain high levels of fiber (6.7%). Furthermore viscosity measurements made *in vitro* (Table I) have revealed that subfraction "a" had a high

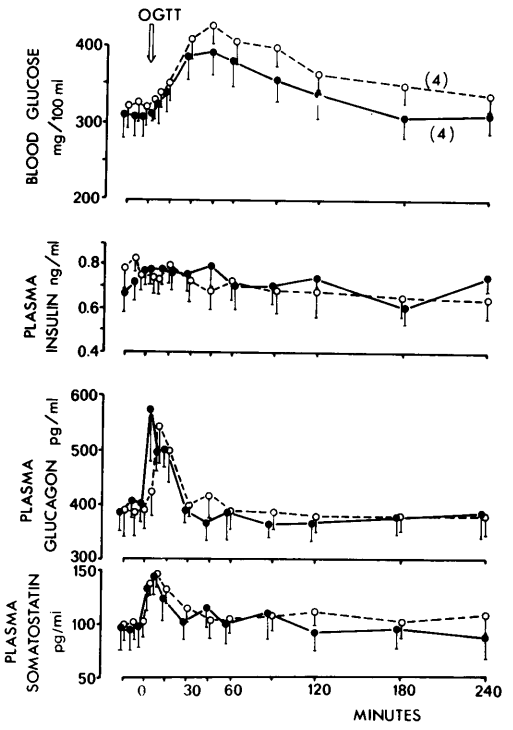


FIG. 5. Oral glucose tolerance test (OGTT) in alloxan-diabetic dogs before treatment (○) and on the 18th day of chronic treatment with fenugreek cotyledons + axes (C + A, subfraction "b") (●). Effects on blood glucose, plasma insulin, glucagon, and somatostatin levels.

viscosity (115 cP for 1% of the substance in distilled water). Johnson (24) has reported that a high viscosity substance can inhibit the intestinal absorption of glucose. It must, however, be noted that O'Connor *et al.* (10), using guar preparations of different viscosity, have found that they were equally effective in significantly reducing the mean postprandial blood glucose and insulin curve. This property has been attributed to the viscosity of the hydrated guar gum which reduces the rate of gastric emptying (25). In a study performed in streptozotocin-diabetic rats, Madar (26) recently suggested that the blood modulating effect of fenugreek was mainly due to delayed gastric emptying coupled with direct interference with intestinal glucose absorption.

In conclusion, our results show that the antidiabetic properties of fenugreek seeds are contained within the testa and endosperm subfraction. Although this subfraction is rich in fibers, it is not possible to exclude the existence of one or more effective principles in this subfraction of the seed.

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