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USE OF *GYMNEMA SYLVESTRE* LEAF EXTRACT IN THE CONTROL OF BLOOD GLUCOSE IN INSULIN-DEPENDENT DIABETES MELLITUS

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Summary

GS_4, a water-soluble extract of the leaves of *Gymnema sylvestre*, was administered (400 mg/day) to 27 patients with insulin-dependent diabetes mellitus (IDDM) on insulin therapy. Insulin requirements came down together with fasting blood glucose and glycosylated haemoglobin (HbA1c) and glycosylated plasma protein levels. While serum lipids returned to near normal levels with GS_4 therapy, glycosylated haemoglobin and glycosylated plasma protein levels remained higher than controls. IDDM patients on insulin therapy only showed no significant reduction in serum lipids, HbA1c or glycosylated plasma proteins when followed up after 10–12 months. GS_4 therapy appears to enhance endogenous insulin, possibly by regeneration/revitalisation of the residual *beta* cells in insulin-dependent diabetes mellitus.

Introduction

Leaves of *Gymnema sylvestre* R.Br. (family Asclepiadaceae), a woody climber growing in unkempt tropical forests of the central and southern parts of India, has been used as an adjunct in the treatment of diabetes mellitus in ancient India. The first biological investigations were reported by Mhaskar and Caius (1930) who found that the blood glucose lowering effect of the leaves was obvious only when there was residual pancreatic function. In total pancreatectomised animals, administration of the leaves did not bring about blood glucose homeostasis.

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G. sylvestre is known as *Periploca of the woods* in English, *meshashringi* (meaning ram's horn) in Sanskrit; *waldschlinge* in German; *podapatri* in Telugu and *shirukurinja* in Tamil. *G. sylvestre* leaves, when chewed, abolish the taste of sweetness (Hooper, 1887); hence the plant is called *gurmar* in Hindi and *sarkaraikolli* in Tamil and Malayalam. These names mean 'destroyer of sugar' (Nadkarni, 1954). The anti-saccharine property was identified in a fraction called gymnemic acid by Power and Tutin (1904). Gharpurey (1926) reported that oral administration of *G. sylvestre* to diabetics reduced urine glucose.

Earlier work in our laboratory (Shanmugasundaram et al., 1981) has shown that the oral administration of the dried leaves of *G. sylvestre* brings down blood glucose and raises serum insulin levels, recorded during an oral glucose tolerance test in alloxan diabetic rabbits and in healthy human volunteers. Administration of dried leaves for a period of 10 days lowered blood glucose and increased the glucose triggered rise in serum insulin levels in a single case of maturity onset diabetes, suggesting possible repair or regeneration of the *beta* cells in the Islets of Langerhans.

Extraction of the leaves of *G. sylvestre* has produced a concentrate of the active principle named GS_4. GS_4 administration for 4 to 6 weeks led to the blood glucose homeostasis in streptozotocin diabetic rats (Shanmugasundaram et al., 1988). GS_4 administration was followed by normalisation of glycosylated haemoglobin and plasma proteins. Our observations are supported by the work of Higi (1986) and a crude preparation of the leaves of *G. sylvestre*, marketed in Japan for the control of obesity and diabetes. Histopathological studies of the pancreas have confirmed that the *beta* cell number is increased considerably after GS_4 therapy (Shanmugasundaram et al., 1990).

In the present paper, the leaf extract GS_4 was administered to patients with insulin-dependent diabetes mellitus in order to assess its effectiveness in regenerating/repairing/revitalising the *beta* cells and blood glucose homeostasis. The assessment was made by indirect measurements of fasting blood glucose, daily insulin requirement and levels of glycosylated haemoglobin (HbA1c) and glycosylated plasma proteins before and after GS_4 therapy. A comparison was made with a similar group who continued on insulin therapy alone for a period of 10—12 months. Direct evidence of the action of GS_4 on *beta* cell regeneration/repair could be shown by C-peptide assay before and after GS_4 therapy. This could not be undertaken at the beginning of the study due to constraints on resources; however, C-peptides in serum were assayed at the end of the test period and were compared with 30 cases of insulin-dependent diabetes mellitus (IDDM) on insulin therapy alone and on 40 normal healthy volunteers.

Materials and Methods

Preparation of extract

GS_4 was obtained by extraction of authentic *Gymnema sylvestre* leaves in

95% ethanol and an acidic precipitation as described earlier (Shanmugasundaram et al., 1990). The precipitate was recrystallised from alcohol and 200 mg of the product was dispensed in gelatin capsules. The patients were given 2 capsules/day to be taken after breakfast and supper, while continuing on their insulin regimen.

Clinical subjects

GS_4 was administered to 23 Type I diabetics (14 males and 9 females) 10–31 years of age and 4 cases of Type I diabetics (3 males and 1 female) 44–50 years age for periods ranging from 2 to 30 months along with daily insulin injections. They were compared with 37 controls 8–30 years of age on insulin therapy alone. All patients were free from symptoms of renal damage, retinopathy or cardiovascular disorders. The patients were attending the Dr. Ambedkar Institute of Diabetes, Kilpauk Medical College Hospital, Madras and were being continuously monitored and under insulin therapy. Information on the family history and history of the course of diabetes and the treatment in the past were obtained through a questionnaire and by personal interview. The dose of insulin, usually a mixture of regular short acting (P) and protamine zinc (Pz) insulin was fixed after hospitalisation and by trial and error over periods not less than a fortnight. During this period, the patients were given adequate instructions on the necessity for continuous therapy and testing for glucosuria (Benedict's test). They also learned to recognise hypoglycaemic episodes and were advised to consume one teaspoon of sugar with water or drink any glucose-containing beverages available, when they experienced the symptoms of hypoglycaemia (a tendency for fainting, dry feeling in the mouth and weakness in the limbs). The insulin dosage was fixed so that hypoglycaemic episodes were avoided, but this did not provide complete control of blood glucose or glucosuria. The patients were included in the trial only after informed consent.

The group of 37 patients with Type I diabetes who continued conventional therapy (insulin alone) were investigated for fasting blood glucose, HbA1c, glycosylated plasma protein, serum amylase, plasma lipids and daily insulin dosage at the beginning of the study and also after a lapse of 6–8 months. This group served as the control and were given the same care and education as the GS_4 therapy group.

In the course of time, the GS_4-treated patients developed hypoglycaemic episodes and their insulin dose was reduced by 10 units at a time. Urine was tested using Benedict's solution for detection of reducing sugar on a weekly basis. The patients of both groups had regular attention at the hospital, which they visited daily. Blood analysis was carried out initially, after 6–8 months and at the end of 16–18 months, 20–24 months and 26–30 months. The patients on GS_4 therapy were enlisted over a period of 3 years and all completed 6–8 months of therapy (6 completed 26–30 months; 11 completed 20–24 months; 22 completed 16–18 months). It was not possible to obtain fasting blood samples from patients on a predetermined date, since some

were administering insulin at home and avoided reaching the hospital in the fasting state. A very intensive drive including home visits was arranged to draw fasting blood samples. There were 11 drop outs in the first six months.

Clinical testing

Venous blood samples were drawn under fasting conditions into tubes containing the disodium salt of ethylene diamine tetraacetic acid (1 mg/ml) as the anticoagulant. Another sample was collected with sodium fluoride as the anticoagulant for blood glucose estimation by the *o*-toluidine colour reaction (Dubowski, 1962) as modified by Sasaki and Matsui (1972) and urea by the method of Marsh et al. (1965). Glycosylated plasma proteins, cholesterol, triglycerides and free fatty acids were assayed in plasma samples obtained by sedimentation of blood samples collected with EDTA as anticoagulant using the respective methods of Merelyn et al. (1981), Parekh and Jung (1970), Rice (1970) and Hron and Menahan (1981).

The sedimented cells were resuspended and erythrocytes were isolated, lysed and HbA1c was estimated in the haemolysate by the method of Wang and Yang (1982).

Serum C-peptide assays were made using fasting blood samples from 40 healthy volunteers (18 males and 22 females in the age group of 18 to 30 years), 30 cases of Type I diabetics (16 males and 14 females of 1–18 years duration) on conventional insulin therapy and 20 cases of Type I diabetes mellitus after GS_4 therapy (for 16–18 months) together with insulin as described earlier. Serum was separated from the cells within 2 h of blood collection, labelled and stored under liquid nitrogen until assays were made. Radioimmunoassay of serum C-peptides were made using antiserum K_6, a kind gift (as a part of the RIA kit) from Dr. Lisa G. Heding, Novo Research Laboratories, DK-2880 Bagsvaired, Denmark. The procedure followed was that recommended by Novo with the additional step of first precipitating all samples with polyethylene glycol (25% w/v). The blanks and standards also contained polyethylene glycol at the same level.

Results

Patients on GS_4 therapy did not report any undesirable side effects such as nausea, vomiting, lassitude, insomnia, alopecia or any gastrointestinal disturbances. In a few female patients, pre-existing pain in the limbs was abolished within 2 weeks of GS_4 therapy. Five patients reported a sense of greater well-being characterised by alertness of mind and body during their daily chores such as catching a bus at a crowded bus stop, playing games and writing examinations. Height and weight gain was recorded in those below 16 years and also in a single instance of a male aged 22 years with a history of diabetes for over 8 years.

The clinical trial on GS_4 was carried out in a hospital of orthodox medicine and prejudices, scepticism and apathy for herbal therapy were not unusual.

There were 11 drop outs in the first 6 months from among the earliest recruited for GS_4 therapy. Among them, 5 left the city for their native villages and could not obtain monthly supplies of GS_4. In one case, the response was irregular and the patient had frequent episodes of hypoglycaemia. He was found to have brittle diabetes and was counselled not to continue in the trial. Five did not get continued support from the family who believed in the superiority of orthodox medicine. Seven out of 11 in this group were females and the parents were no longer interested in their welfare. Hence, the therapy was discontinued. Table 1 gives the data on individual patients studied. The actual body weight was less than their ideal body weight.

It can be seen that the insulin dose has progressively reduced in every instance. Patient 2 had combined Lente insulin with Euglucon (oral) before GS_4 therapy and could discontinue Euglucon after six months. GS_4 therapy showed similar effects irrespective of the duration of the disease, as exemplified in Case 4, a patient with a 25-year history of the disease. Patient 3 was on NPH insulin and showed a quick response as can be seen by the reduction in insulin requirement and HbA1c. During the 3-year period, he moved his home and the moves were coupled with aberrations in blood glucose homeostasis. As shown in Table 2, there were no increases in blood urea and haemoglobin. Fasting blood glucose, glycosylated haemoglobin and plasma proteins were reduced in all instances. Reducing sugar in the urine, which was abundant at the initiation of GS_4 therapy, was reduced in all cases.

Table 2 gives the data on blood analysis made on 37 IDDM diabetics who were on insulin therapy alone over a period of 10—12 months. From Table 2, it can be seen that, in spite of insulin therapy, fasting blood glucose and HbA1c were not lowered significantly. Table 3 gives the overall effect of GS_4 therapy on insulin requirements, blood glucose, glycosylated protein, plasma lipids and serum amylase level compared to a group of 100 age- and sex-matched healthy volunteers. Insulin dosage was reduced to nearly half of the initial amount, while mean blood glucose was reduced from 232 to 152 mg/dl. Blood urea remained within the normal range, before, during and after 18 months of GS_4 therapy. However, a reduction in blood urea with the onset of GS_4 therapy was recorded. There was a statistically significant reduction ($P < 0.001$) in HbA1c level in the first 6—8 months therapy, but the levels remained significantly higher than the normal values. Similar reductions were observed in the glycosylated plasma protein levels. Cholesterol, which is elevated in diabetes, was significantly reduced and brought to near normal levels during GS_4 therapy. Triglycerides were also lowered together with FFA. Serum amylase, which is more than doubled in diabetics, was brought down by GS_4 therapy, but not to normal levels.

In patients on insulin therapy alone studied over a period of 10—12 months, blood lipids remained high, together with fasting blood glucose, HbA1c and glycosylated plasma proteins, in spite of insulin therapy. Serum amylase remained high, despite insulin therapy.

Table 4 gives the fasting serum C-peptide levels in the healthy normals

TABLE 1

DATA ON 27 TYPE I DIABETIC PATIENTS BEFORE AND DURING GS_4 SUPPLEMENTATION

Patient	Age (years)	Sex	Height (cm)	Weight (kg)	Broca index (%)	Disease duration (years)	P + P_z insulin (U/day)[a]					Blood glucose (mg/dl)				
							Pre GS_4	6–8 months	16–18 months	20–24 months	26–30 months	Pre GS_4	6–8 months	16–18 months	20–24 months	26–30 months
1	10	M	112	15	125	2	40 + 40	30 + 30	20 + 20	10 + 10	20 + 10	335	272	185	145	130
2	28	F	161	45	74	1	(L20 + Euglucon)	(L20)	(L20)	(L20)	(L10)	250	81	100	208	185
3	20	M	162	43	69	8	(NPH 70)	(NPH 40)	(NPH 40)	(NPH 40)	(NPH 40)	325	120	165	250	220
4	28	F	158	47	81	25	40 + 40	30 + 30	20 + 20	20 + 20	20 + 20	189	143	100	85	125
5	10	M	125	24	96	1	30 + 20	20 + 20	20 + 10	10 + 10	10 + 10	145	100	120	125	120
6	31	M	166	62	94	10	10 + 10	Nil	Nil	Nil	Nil	178	156	100	149	129
7	21	F	152	46	88	21	30 + 30	20 + 20	20 + 10	10 + 10		310	95	105	100	
8	20	M	158	48	83	3	20 + 20	20 + 10	10 + 10	10 + 10		200	190	165	145	
9	16	F	148	45	94	1	40 + 40	30 + 30	20 + 10	20 + 10		295	210	190	186	
10	24	M	170	50	71	5	40 + 40	30 + 30	20 + 20	10 + 10		170	213	126	110	
11	30	M	169	65	94	4	20 + 20	(L20)	(L20)			125	108	108		
12	22	M	153	41	77	2	30 + 30	20 + 20	20 + 20			165	130	100		
13	20	F	142	26	62	2	40 + 40	20 + 20	20 + 20			240	260	238		
14	24	M	170	50	71	2	40 + 40	30 + 30	20 + 20			150	115	107		
15	20	F	155	35	64	2	40 + 40	30 + 30	20 + 20			270	185	138		
16	26	F	170	39	56	2	40 + 40	20 + 20	10 + 10			239	177	142		
17	14	M	135	27	77	2	40 + 40	30 + 30	30 + 30			240	434	320		
18	28	M	167	55	82	2	10 + 10	Nil	Nil			143	127	128		
19	17	F	156	39	70	7	40 + 40	20 + 20	10 + 10			285	160	142		
20	21	M	159	46	78	6	40 + 40	30 + 30				260	156			
21	20	F	158	42	72	2	(L20)	(L20)				275	119			
22	22	M	162	45	73	5	40 + 40	30 + 30				325	296			
23	29	M	165	65	100	3	20 + 20	20 + 20				320	230			
24	50	M	150	61	122	5	20 + (L20)	10 + (L20)	20 + 10	10 + 10		234	191	187	170	
25	49	M	160	59	98	16	30 + 30	20 + 20	20 + 20			220	155	140		
26	44	M	168	60	88	7	30 + 30	30 + 30	20 + 10			213	200	195		
27	48	F	160	53	88	4	(L30)	(L30)				160	159			

TABLE 1 (*continued*)

Patient	Glycosylated haemoglobin (%)					Glycosylated plasma proteins (μg hexose/mg proteins)					Glucosuria[b]				
	Pre GS_4	6–8 months	16–18 months	20–24 months	26–30 months	Pre GS_4	6–8 months	16–18 months	20–24 months	26–30 months	Pre GS_4	6–8 months	16–18 months	24–26 months	26–30 months
1	14.6	12.0	9.2	7.1	7.0	2.36	2.08	1.69	1.50	1.40	4+	3+	2+	±	±
2	12.6	11.8	9.8	8.7	8.0	4.15	3.29	2.36	2.20	2.00	2+	1+	±	±	1+
3	10.3	8.7	7.2	16.0	12.0	3.38	2.29	2.36	3.00	2.90	4+	2+	±	3+	2+
4	14.1	7.5	5.7	10.3	9.0	4.14	2.36	1.82	3.00	2.00	4+	3+	±	±	±
5	11.5	8.6	7.8	7.5	7.0	2.39	2.37	2.00	2.35	2.20	2+	2+	1+	±	±
6	14.4	9.7	7.2	6.0	6.0	3.30	2.27	2.12	2.00	2.00	2+	1+	±	1+	±
7	12.6	5.2	5.8	6.0		2.48	1.90	2.10	2.00		2+	±	1+	±	
8	13.0	10.0	14.2	11.0		3.40	3.80	4.00	3.50		3+	2+	3+	2+	
9	10.5	13.8	6.0	6.2		3.38	2.21	2.00	2.00		3+	3+	2+	1+	
10	14.8	8.0	6.5	6.0		3.55	2.56	2.86	2.50		4+	3+	2+	±	
11	14.0	9.7	12.0			4.10	3.82	3.50			3+	2+	1+		
12	14.8	9.0	9.1			3.25	3.04	2.48			2+	2+	1+		
13	15.7	9.7	10.8			2.29	4.13	3.82			3+	2+	1+		
14	14.4	9.0	8.6			2.48	2.02	2.15			3+	3+	2+		
15	14.8	11.8	10.9			2.03	1.97	2.21			4+	3+	2+		
16	13.5	9.7	9.7			2.54	1.73	1.87			4+	2+	±		
17	13.9	11.3	12.8			4.86	3.75	2.95			4+	3+	4+		
18	13.6	8.2	10.0			4.29	1.36	2.22			2+	1+	±		
19	11.9	8.2	9.7			2.86	2.06	1.87			3+	2+	±		
20	13.5	8.3				3.15	2.48				3+	2+			
21	13.1	10.2				3.38	3.05				4+	1+			
22	14.4	10.8				2.55	2.96				4+	2+			
23	12.3	10.1				3.94	2.20				4+	2+			
24	9.8	8.3	7.8	6.5		3.72	3.86	3.50	2.30		3+	2+	2+	1+	
25	10.7	10.2	8.9			3.20	4.28	3.45			3+	2+	±		
26	8.8	8.1	7.5			4.37	4.00	3.90			3+	3+	2+		
27	8.2	8.5				3.60	3.50				4+	2+			

[a]Key to insulins other than regular (P) and protamine zinc (Pz) insulin: L = Lente insulin; NPH = natural protamine zinc insulin.

[b]Key: ±, trace; 1+, green with yellow precipitate; 2+, yellow to dark green; 3+, brown; 4+, orange to brick red.

TABLE 2

DATA OF 37 TYPE I DIABETIC PATIENTS ON INSULIN THERAPY ALONE

Patient	Age (years)	Sex	Height (cm)	Weight (kg)	Broca Index (%)	Disease duration (years)	P + P_z Insulin (U/day)		Blood glucose (mg/dl)		Glycosylated haemogolbin (%)		Glycosylated plasma proteins (μg hexose/mg protein)		Glycosuria[a]	
							Initial	10–12 months	Initial	10–12 months	Initial	10–12 months	Initial	10–12 months	Initial	10–12 months
1	20	M	155	50	91	6	40 + 40	40 + 40	250	240	11.8	10.5	3.50	3.15	3+	3+
2	20	M	165	40	62	4	30 + 30	30 + 30	235	220	12.5	13.8	3.21	3.80	3+	3+
3	24	M	150	55	110	5	30 + 30	40 + 40	200	185	10.9	12.6	2.95	3.00	3+	2+
4	8	M	140	25	62	4	30 + 30	30 + 30	240	245	13.4	13.0	3.45	3.01	3+	3+
5	24	M	165	42	65	6	40 + 40	40 + 40	204	225	14.9	14.0	3.00	2.80	3+	3+
6	25	M	165	45	69	1	40 + 40	40 + 40	285	280	16.1	14.8	3.29	2.85	3+	3+
7	26	M	165	48	74	12	20 + 20	30 + 30	190	200	10.8	11.2	4.19	3.83	2+	2+
8	24	M	165	52	80	4	20 + 20	20 + 20	165	150	11.0	9.2	3.85	2.69	2+	1+
9	19	M	150	46	92	5	30 + 30	30 + 30	225	220	15.4	14.5	3.69	3.50	3+	3+
10	19	F	145	40	89	2	10 + 10	20 + 20	200	220	16.0	16.0	3.70	3.52	3+	3+
11	22	M	155	44	80	6	30 + 30	30 + 30	230	198	13.9	12.5	2.85	2.69	2+	2+
12	23	M	156	45	83	4	30 + 30	30 + 30	200	200	14.5	13.5	3.75	3.63	2+	2+
13	26	F	146	55	120	5	20 + 20	20 + 20	185	200	9.8	10.2	2.95	3.00	2+	2+

14	14	M	150	55	70	2	10 + 10	20 + 20	190	198	10.5	12.6	2.82	3.17	2+	2+
15	23	M	160	42	70	4	20 + 20	20 + 20	205	168	11.3	10.0	3.68	3.50	2+	2+
16	28	M	155	40	73	2	30 + 20	30 + 20	210	220	12.5	13.0	3.19	3.20	2+	1+
17	22	M	160	42	70	3	20 + 20	20 + 10	200	185	13.1	10.3	2.65	2.55	2+	2+
18	16	M	161	42	69	6	30 + 30	30 + 30	260	250	13.9	8.9	3.25	3.00	3+	3+
19	24	M	165	52	80	3	20 + 20	20 + 20	205	225	13.8	8.7	2.36	2.54	2+	2+
20	30	M	163	56	89	6	40 + 40	40 + 40	260	190	13.3	13.2	4.26	2.56	3+	2+
21	27	F	143	35	81	1	20 + 20	20 + 20	245	205	9.5	8.4	4.22	3.22	2+	2+
22	22	M	166	53	80	15	30 + 30	30 + 30	180	260	9.1	8.9	4.35	3.60	2+	3+
23	21	M	158	40	69	4	30 + 30	30 + 30	360	240	16.1	12.8	4.73	4.20	4+	2+
24	25	F	156	45	80	3	20 + 20	20 + 20	205	255	13.1	10.8	2.32	4.84	2+	2+
25	22	M	161	49	80	8	30 + 30	30 + 30	295	170	11.4	10.6	3.93	1.39	3+	2+
26	26	M	158	36	62	1	20 + 20	20 + 20	235	245	11.3	12.8	3.30	2.31	2+	3+
27	24	M	163	40	63	3	10 + 10	10 + 10	315	205	16.4	10.4	3.23	3.12	4+	2+
28	26	M	164	43	67	3	40 + 40	40 + 40	238	315	9.2	8.2	2.30	3.31	2+	4+
29	27	F	143	39	91	2	10 + 10	10 + 10	239	266	13.5	7.2	2.54	2.30	2+	3+
30	19	M	168	46	68	4	30 + 30	30 + 30	160	186	13.1	12.4	3.94	2.86	2+	2+
31	20	M	156	40	71	9	30 + 30	40 + 40	300	250	16.0	14.8	2.56	4.22	4+	3+
32	25	M	165	45	69	3	40 + 40	40 + 40	225	275	12.4	8.7	3.04	3.25	2+	3+
33	17	F	155	42	76	2	20 + 20	20 + 20	245	200	8.6	9.6	4.39	2.36	3+	2+
34	13	M	153	47	89	5	30 + 30	40 + 40	335	232	13.0	14.2	3.43	2.20	4+	2+
35	19	M	166	40	61	1	20 + 20	20 + 30	225	226	12.4	14.2	3.45	2.22	2+	2+
36	19	M	164	46	72	1	30 + 30	30 + 30	225	296	12.2	16.8	2.52	2.86	2+	4+
37	14	M	158	42	72	5	40 + 40	40 + 40	260	250	12.3	12.3	3.20	2.28	3+	2+

[a]Key: 1+, green with yellow precipitate; 2+, yellow to dark green; 3+, brown; 4+, orange to brick red.

TABLE 3

FASTING BLOOD GLUCOSE, UREA, HAEMOGLOBIN, GLYCOSYLATED HAEMOGLOBIN (HbA1c), GLYCOSYLATED PLASMA PROTEINS (GPP), SERUM AMYLASE, PLASMA CHOLESTEROL, TRIGLYCERIDES AND FREE FATTY ACIDS (FFA)

Values of normal and Type I patients on insulin therapy alone and under GS_4 supplementation. Tabular values express the mean ± S.E.M.

	N	Insulin (U-day)	Fasting glucose (mg/dl)	HbA1c (%)	GPP (μg hexose/mg protein)	Amylase (*S* units)	Blood urea (mg/dl)	Haemo-globin (g/dl)	Chol-esterol (mg/dl)	Trigly-ceride (mg/dl)	FFA (mg/dl)
Control	100	–	89 ± 1.0	6.0 ± 0.2	1.0 ± 0.03	88 ± 1	24 ± 0.6	13 ± 0.2	179 ± 5	90 ± 1	68 ± 1.0
GS_4 supplement											
Pre GS_4	27	60 ± 4.4	232 ± 12.3	12.8 ± 0.4	3.3 ± 0.15	197 ± 9	25 ± 1.0	15 ± 0.2	206 ± 14	134 ± 4	84 ± 2.5
6–8 Months	27	45 ± 2.9	177 ± 14.4	9.5 ± 0.3	2.8 ± 0.15**	180 ± 9	22 ± 1.0	14 ± 0.4	208 ± 6	121 ± 5	77 ± 2.5
16–18 Months	22	35 ± 2.1	150 ± 11.5	9.0 ± 0.5	2.6 ± 0.20	146 ± 1**	19 ± 1.0	14 ± 0.4	194 ± 6	120 ± 5	76 ± 2.4
20–24 Months	11	25 ± 3.0	152 ± 15.0	8.5 ± 1.0	2.4 ± 0.20	146 ± 11	18 ± 1.5	14 ± 0.3	176 ± 5**	107 ± 6**	72 ± 2.7**
26–30 Months	6	30 ± 6.1	152 ± 16.8	8.2 ± 0.9	2.1 ± 0.20	144 ± 14	22 ± 1.6	14 ± 0.4	169 ± 6	111 ± 11	68 ± 3.3
Insulin alone											
Initial	37	55 ± 3.3	233 ± 7.4	12.7 ± 1.4	3.4 ± 0.10	165 ± 10	22 ± 0.7	14 ± 0.3	225 ± 5	124 ± 2	84 ± 1.3
10–12 Months	37	55 ± 3.3	224 ± 6.1	11.8 ± 0.4	3.0 ± 0.10	160 ± 7	22 ± 0.7	14 ± 0.3	209 ± 4	112 ± 2	80 ± 1.0

TABLE 4

SERUM C-PEPTIDE LEVELS IN FASTING BLOOD IN NORMAL AND IDDM PATIENTS ON INSULIN THERAPY WITH AND WITHOUT GS_4 SUPPLEMENTATION

	N	C-peptide (pmol/ml)	
		Range	Mean ± S.E.M.
Normal	40	0.155–0.340	0.272 ± 0.006
Insulin alone	30	0.065–0.145	0.105 ± 0.005*
GS_4 supplement	22	0.170–0.205	0.185 ± 0.003#

*$P < 0.001$ when compared to normal.
#$P < 0.001$ when compared to diabetic patients without GS_4 supplements.

and patients with IDDM on insulin therapy with and without GS_4. In the 20 cases on GS_4 therapy, the C-peptide levels were higher, suggesting greater availability of endogenous insulin.

Discussion

The salient features of our clinical observations are that prolonged oral administration of GS_4 produces (i) a reduction in the insulin requirement, possibly by enhancing endogenous insulin availability, (ii) an improved blood glucose homeostasis as seen by lowered HbA1c and glycosylated plasma protein levels, (iii) better control of the hyperlipaemia associated with diabetes mellitus; (iv) a reduction in serum amylase activity; and (v) apparently increased *beta* cell function as seen by higher levels of serum C-peptide.

Abnormal blood glucose levels are the cause for the development and progression of diabetic microangiopathy in humans (Siperstein et al., 1973) and West (1981) has observed that good control of diabetes has a retarding effect on the development of such complications. Diabetic microangiopathy develops due to poor blood glucose control over a 24-h period with accumulation of glycoproteins and glycosylated proteins producing a thickening of the basement membrane in the kidney (Serrano et al., 1983). Glycoprotein and glycosylated proteins are elevated in uncontrolled diabetes, and glycosylated haemoglobin (HbA1c) can be used as an excellent marker of overall glycaemic control (Bunn et al., 1976). Glycosylation of proteins depends on the glucose in the cell. Once formed, HbA1c is stable and accumulates throughout the life span of red cells. Kennedy et al. (1981) and several others have observed that the measurement reflects the blood glucose equilibrium state of the 6–8 weeks prior to sampling. The significant reduction of both HbA1c and glycosylated plasma proteins in diabetics with GS_4 supplementation (Table 3) confirms that overall blood glucose control is improved by the her-

bal therapy. It also suggests that the onset of secondary complications (microangiopathy or diabetic kidney disease and retinopathy) may be delayed by the herbal therapy. Unlike the animal models (alloxan- and streptozotocin-induced), IDDM in humans is reported to be associated with the appearance of islet cell antibodies in plasma (Bottozzo et al., 1980). This could render the newly formed *beta* cells and the islets inactive in the course of time. Continuous GS_4 therapy could lead to the eventual replacement of insulin injection in the long run.

At the present time, insulin administration remains the only therapy for the treatment of insulin-dependent diabetes mellitus (IDDM) and any method to increase endogenous insulin stores should provide relief to these patients.

In the *beta* cells, proinsulin is synthesized as a single polypeptide, coded by a single gene. This polypeptide undergoes cleavage in the middle, giving rise to an inactive breakdown product C-peptide, while the remaining fragments of proinsulin combine together to form the insulin molecule. Insulin and C-peptides are released in equimolar amounts and in diabetic patients on insulin therapy serum C-peptide levels provide the true measure of endogenous insulin biosynthesis.

The appearance of greater *beta* cell function is inferred from the higher serum C-peptide levels observed in the 20 cases of Type I diabetes on GS_4 therapy compared to those on insulin alone (Table 4). Residual *beta* cell function in insulin treated diabetes has been evaluated by the serum C-peptide levels by several workers (Ludwigson and Heding, 1976) and insulin secretory capacity has been reported to be variable in patients classified as IDDM on clinical grounds (Klaff et al., 1986). Kloppel et al. (1984) have obtained morphometric evidence for a striking *beta* cell reduction at the clinical onset of diabetes. Volume, density and absolute volume of *beta* cells were reduced to less than one third that of healthy controls within 7 days. They have concluded that in the majority of Type I diabetics, *beta* cell destruction proceeds slowly, starting years before the clinical onset of the disease, and that the disease is manifested only when the residual *beta* cells are less than 20%.

In the Diabetes Control and Complications Trial (DCCT Research Group, 1987), serum C-peptide levels measured in 610 cases of IDDM in the fasting condition (basal) were found to have significant direct co-relation to C-peptides after 'Sustacal' stimulation, confirming the earlier reports of Garcia-Webb et al. (1984). Further, the DCCT Research Group also noted that in the cases of IDDM with higher *beta* cell secreting capacity, each of the parameters of metabolic control was significantly lesser than in the others and that their daily insulin requirement was also lower. Since a reduction in insulin requirement is observed with GS_4 therapy, it may be concluded that there is increased *beta* cell function possibly by repair/regeneration of the *beta* cells. From Table 2, it may be seen that the metabolic changes in insufficiently controlled diabetics with IDDM are elevated levels of HbA1c, glycosylated plasma proteins, cholesterol and triglycerides, and that these are brought down during GS_4 therapy.

Atrophy of the acinar tissue surrounding insulin deficient islets has been reported in autopsy studies made in IDDM (Foulis and Stewart, 1984). Acinar damage in streptozotocin-diabetic rats leads to reduced pancreatic amylase and increased serum amylase activities (unpublished observations). It would appear that enhanced *beta* cell formation in GS_4-treated IDDM may reverse acinar atrophy and the resultant pancreatitis leads to the lowered serum amylase activities observed in Table 3. After GS_4 therapy, insulin requirements go down with lowered HbA1c levels suggesting better 24-h control of blood glucose. Longer test periods and continuous monitoring may be able to confirm the *beta* cell repairing/regenerating capacity of this herbal extract.

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