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Antidiabetic properties of the alcoholic extract of *Sphaeranthus indicus* in streptozotocin–nicotinamide diabetic rats

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Abstract

We have investigated the possible antihyperglycaemic effects of *Sphaeranthus indicus* extract in rats rendered diabetic by nicotinamide (120 mg kg^{-1} i.p.) and streptozotocin (STZ) (60 mg kg^{-1} i.p). Fasting plasma glucose levels, serum insulin levels, serum lipid profiles, magnesium levels, glycosylated haemoglobin, changes in body weight and liver glycogen levels were evaluated in normal and diabetic rats. Oral administration of *S. indicus* for 15 days resulted in significant decrease in blood glucose levels and increases in hepatic glycogen and plasma insulin levels. Fasting normal rats treated with the alcoholic extract of *S. indicus* showed significant improvement in oral glucose tolerance test. Glibenclamide was used as a reference standard. The findings demonstrate that the alcoholic *S. indicus* extract may be useful in the treatment of diabetes.

Introduction

Many investigations of oral anti-hyperglycaemic agents of plant origin used in traditional medicine have been conducted and many plants have been found to show positive activity (Bailey & Day 1989). Though the active principles of various classes of chemical compounds have been isolated from plants, some remain to be identified (Rahman & Zaman 1989).

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism, caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin, and is characterized by chronic hyperglycaemia (Setter et al 2000). The worldwide prevalence of diabetes has risen in the past two decades. Type 2 diabetes is more common, and its prevalence is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels. Though several new pharmacological agents have been developed for the management of diabetes, the treatment of diabetes with herbal remedies has also been increasing among practitioners. Ancient Indian literature has prescribed various herbs in the treatment of diabetes mellitus. Many indigenous drugs have been used by the practitioners of the Ayurvedic system for the treatment of diabetes mellitus in India (Shirwaikar et al 2005).

Sphaeranthus indicus Linn (Asteraceae), commonly known as Gorakhmundi in Hindi, is an annual spreading herb, distributed throughout the plains and wet-lands in India, Sri Lanka and Australia (Sadaf et al 2006). The plant is known to possess varied medicinal properties and is reportedly used in ayurvedic preparations for treating epileptic convulsions, mental illnesses and hemicranias (Kirtikar & Basu 1987). It is used to treat vitiated conditions of jaundice, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, haemorrhoids, helminthiasis, dyspepsia, skin diseases and as a nerve tonic (Ambavade et al 2006). The oil prepared from the plant root is reported to be useful in treating scrofula and as an aphrodisiac, while the external application of the herb paste is reported to be beneficial in treating pruritus, oedema, arthritis, filariasis, gout and cervical adenopathy (Paranjape 2001).

A large number of constituents have been isolated from extracts of the whole herb, flowers and leaves. The essential oil, obtained by steam distillation of the whole herb, contains ocimene, α-terpinene, methyl-chavicol, α-citral, geraniol, α-ionone, β-ionone, δ-cadinene, p-methoxycinnamaldehyde (Baslas 1959) and an alkaloid sphaeranthine (Basu & Lamsal

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1946). The alcoholic extract of powdered capitulum contains stigmasterol, β-sitosterol, hentriacontane, sesquiterpene lactone (Gogate et al 1986), sesquiterpine glycoside, sphaeranthanolide (Shekhani et al 1990) flavone and isoflavone glycosides (Yadav & Kumar 1998).

Many medicinal properties have been attributed to the extracts, fractions and isolated constituents of *S. indicus*, including hypotensive, peripheral vasodilatory and cathartic activities of the alcoholic extract (Srivastava et al 1971), antimicrobial activity of alkaloidal and non-alkaloidal fractions of alcoholic extract (Sheikh et al 1986) and of sesquiterpene isolated from petroleum ether extract (Singh et al 2001). Essential oil obtained from leaves possesses antifungal properties (Garg & Kasera 1982). Herbal cosmetic creams containing the extract of *S. indicus* have been used for the treatment of dermal wounds (Sadaf et al 2006).

As the tribes of Madhya Pradesh in India advocate the use of *S. indicus* in the management of diabetes, the present study explores the capacity of *S. indicus* extracts to normalize blood glucose, magnesium and lipid levels in the management of diabetes, using the streptozotocin (STZ)–nicotinamide-induced type 2 diabetic rat model.

Materials and Methods

Plant material

Roots and stolons were collected from healthy plants of *S. indicus*, a herb growing approximately 15–30 cm in height, from Manipal, Karnataka, India during the month of June 2006. The plant was identified at Poornaprajna College, Udupi, Karnataka, India by plant taxonomist Dr Gopalkrishna Bhat. A voucher specimen (no. PP 552) has been deposited in the museum of the Department of Pharmacognosy, Manipal. The plant was cleaned, dried in the shade and then coarsely powdered.

Preparation of alcoholic *S. indicus* extract

The alcoholic extract of *S. indicus* was prepared as described by Shirwaikar et al (2004). A total of 1 kg of the shade-dried powdered roots and stolons were exhaustively extracted with 95% ethanol using Soxhlet apparatus. It was then evaporated using a rotary evaporator under vacuum until an approximate 50-fold reduction in solvent was achieved. The extract was stored at 15–20°C in a sealed dessicator.

High-performance TLC

Qualitative densitometric high-performance thin-layer chromatography (TLC) analysis was performed to develop the characteristic fingerprint profile for the alcoholic extract of roots and stolon of *S. indicus*. The extract and β-sitosterol, a biochemical marker, were dissolved in petroleum ether. Ten microlitres of the sample solutions were applied and the plate were developed in chloroform/ethyl acetate (85:15). Developed plates were sprayed with 10% methanolic sulfuric acid, heated at 105°C for 5 min and scanned densitometrically using a Camag TLC scanner 3 (CAMAG, Muttenz, Switzerland) at 600 nm and documented.

Experimental animals

Male Wistar rats (180–200 g) were maintained on a standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions (12 h light–dark cycle; 23–25°C; 35–60% relative humidity). All the experimental protocols for animal care procedures were approved by the Ethical Committee of Manipal University.

Experimental induction of diabetes

Type 2 diabetes mellitus was induced in rats by a single i.p. injection of freshly prepared STZ (60 mg kg^{-1}) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 mL kg^{-1} (Masiello et al 1998) after the i.p. administration of 120 mg kg^{-1} nicotinamide. Two days after STZ administration, rats with blood glucose concentration in the range 200–300 mg dL^{-1} were considered diabetic and were included in the study. Blood was collected by sinocular puncture.

Experimental design

A total of 30 rats (6 normal; 24 diabetic) were used. The rats were divided into five groups of six: Group I were normal untreated rats; Group II were STZ-treated diabetic rats; Groups III and IV were STZ-treated diabetic rats given the extract, 250 and 500 mg kg^{-1}, respectively, orally in 2% gum acacia for 15 days. Group V were STZ-treated diabetic rats given glibenclamide orally (0.25 mg kg^{-1}) in distilled water.

After 15 days' treatment, the rats were decapitated after an overnight fast. Blood was collected into heparinized tubes and serum was separated by centrifugation. Liver tissues were excised immediately and stored in ice-cold containers.

Estimation of blood glucose, hepatic glycogen and serum insulin

Fasting blood glucose was estimated by the o-toluidine method (Sasaki et al 1972). Hepatic glycogen was estimated by the method of Nicholas (1956). Serum insulin was measured by radio immunoassay using a kit from the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BARC), Mumbai, India (Wilson & Miles 1977).

Determination of glycosylated haemoglobin and serum magnesium levels

Glycosylated haemoglobin was estimated by the method of Shirwaikar et al (2005) and serum magnesium levels by the method of Garfinkel (1988).

Oral glucose tolerance test in normal rats

Oral glucose tolerance tests (Bonner-weir 1988) were performed in overnight fasted (18 h) normal rats. Rats divided into three groups (n = 6) were orally administered distilled water or *S. indicus* alcoholic extract (250 or 500 mg kg^{-1}), respectively. Glucose (2 g kg^{-1}) was fed orally 30 min before administration of the extract. Blood was withdrawn by sinocular puncture at

0, 30, 60 and 120 min after administration of the extract and the plasma obtained by centrifugation at 3000 rpm. Fasting plasma glucose levels were measured using a glucose oxidase-peroxidase glucose estimation kit (Ye et al 2002).

Estimation of serum lipid profile

All the rats were bled after 15 days of treatment. Blood was collected into tubes and the serum separated by centrifugation. Lipid levels in serum was estimated using an auto analyser (Hitachi 912) (Roche Diagnostics, Mannheim, Germany). Kits were procured from Roche Diagnostics GmbH, (San Diego, CA, USA (Tietz et al 1995).

Statistical analysis

All data are expressed as mean ± s.d. Statistical data was evaluated by one-way analysis of variance, followed by Dunnett's test for multiple comparisons. $P<0.05$ was considered significant.

Results

High-performance TLC

Co-chromatography of *S. indicus* extract along with β-sitosterol as a biochemical marker revealed the presence of β-sitosterol in the extract, with an Rf value of 0.55 (Figures 1, 2 and 3).

Effect of *S. indicus* on blood glucose levels

The extract at doses of 250 and 500 mg kg^{-1} for 15 days significantly lowered fasting blood glucose ($P<0.01$); the 500 mg kg^{-1} dose showed significantly better activity than the 250 mg kg^{-1} dose throughout the period of analysis ($P<0.01$). However, glibenclamide produced a more significant reduction in fasting blood glucose levels than the test extracts (Table 1).

Effect of *S. indicus* extract on body weight and glycogen levels

Assessment of changes in body weight following 15 days of extract therapy revealed that the experimental group showed no loss in body weight, unlike the diabetic control animals. Rats treated with 250 mg kg^{-1} extract showed significantly higher weight gain ($P<0.01$) than the groups treated with 500 mg kg^{-1} or glibenclamide (Table 2). Analysis of glycogen levels in liver tissue revealed a significant decline in the glycogen levels of diabetic control animals ($P<0.05$) compared with that of normal rats. Treatment with extract at both dose levels remarkably prevented the loss of glycogen, in a similar manner (Table 2).

Effect of *S. indicus* extract on glycosylated haemoglobin, serum insulin and serum magnesium

In comparison with the glibenclamide-treated group, treatment with 250 or 500 mg kg^{-1} of the extract decreased glycosylated haemoglobin in a dose-dependent manner (Table 3). As shown in Table 3, there was a significant decrease in serum insulin in diabetic rats compared with normal rats ($P<0.01$). A comparison of insulin release in various groups showed significant variation ($P<0.0001$). Treatment with 250 or 500 mg kg^{-1} of the extract enhanced insulin release significantly ($P<0.01$) in a dose-dependent manner compared with untreated diabetic rats. Treatment with 500 mg kg^{-1} of the extract showed significantly greater activity ($P<0.01$) than glibenclamide or 250 mg kg^{-1} of the extract.

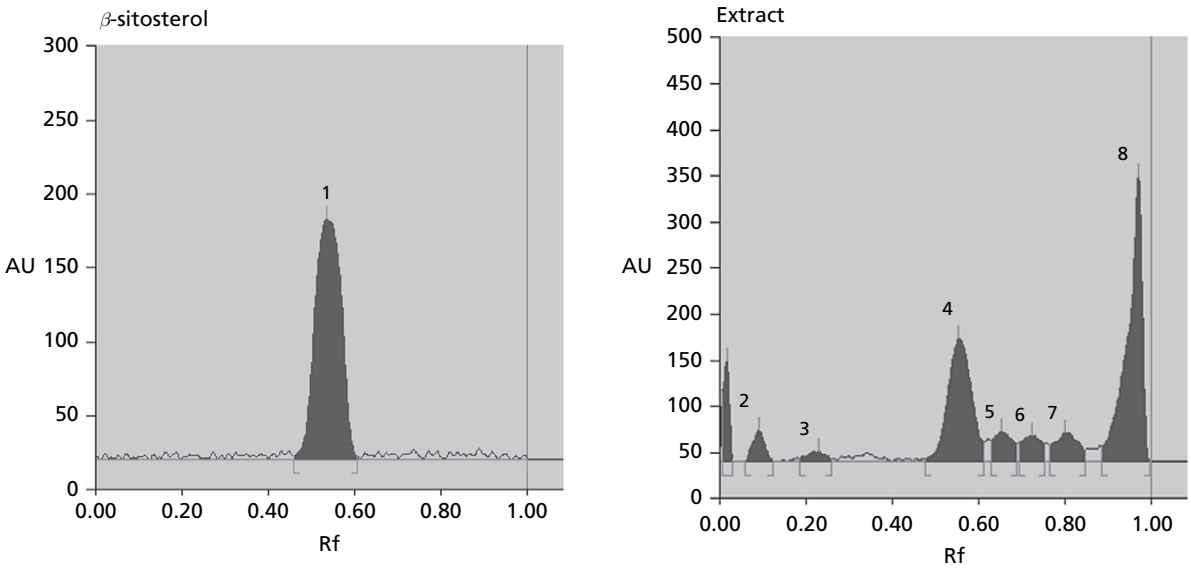

Figure 1 HPTLC chromatograms of β-sitosterol and extract.

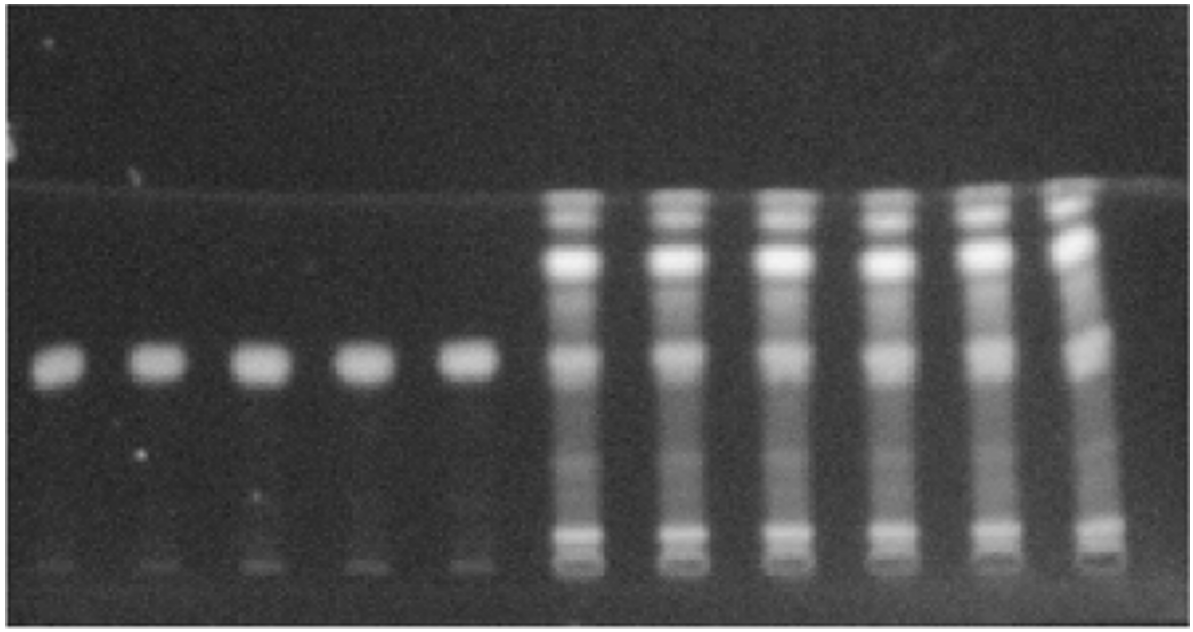

Figure 2 HPTLC chromatogram of β-sitosterol and *S. indicus* extract as viewed under UV at 366 nm.

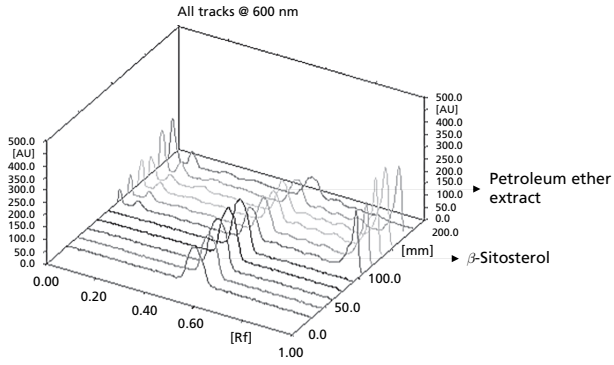

Figure 3 HPTLC chromatogram of β-sitosterol and *S. indicus* extract scanned at 600 nm.

Hypomagnesaemia observed in the diabetic control group was significantly reversed on treatment with 250 or 500 mg kg^{-1} of the extract. Similar results were observed in the glibenclamide-treated group (Table 3).

Effect of *S. indicus* extract on oral glucose tolerance test

Unlike the glibenclamide group, treatment with 250 and 500 mg kg^{-1} alcoholic extract of *S. indicus* did not decrease blood glucose levels following administration of a large oral dose of glucose (3 g kg^{-1}), as shown in Figure 4.

Effect of *S. indicus* extracts on serum lipid levels

As shown in Table 4, serum triglyceride levels were significantly elevated ($P<0.01$) in diabetic animals compared with the normal group. However, treatment with 250 or 500 mg kg^{-1} of the extract significantly ($P<0.01$) reduced serum triglyceride levels, in a dose-dependent manner. The higher dose showed better activity than standard glibenclamide treatment.

A significant elevation was observed in the total cholesterol of the diabetic rats ($P<0.01$) (Table 4). Treatment with the extract at either 250 or 500 mg kg^{-1} resulted in a dose-dependent hypocholesterolaemic effect, which was more significant than that in the glibenclamide-treated group ($P<0.05$).

There was significant lowering of serum high-density lipoprotein (HDL) levels in diabetic compared with normal rats ($P<0.01$). Treatment with 250 or 500 mg kg^{-1} of the extract prevented the decline in HDL levels significantly ($P<0.01$) compared with diabetic control animals. However, the glibenclamide-treated group showed more significant elevations in HDL levels (Table 4).

Discussion

A densitometric high-performance TLC analysis was performed to develop the characteristic fingerprint profile for the alcoholic extract of the underground parts of *S. indicus*. This can be used as a tool for evaluation and standardization of the drug.

Oral administration of *S. indicus* extract to rats with STZ-induced diabetes showed antihyperglycaemic effects.

Effect of *S. indicus* on blood glucose, plasma insulin and body weight

Diabetes mellitus causes a disturbance in the uptake of glucose as well in glucose metabolism. STZ (60 mg kg^{-1}) produces an incomplete destruction of pancreatic β-cells even

Table 1 Effect of *S. indicus* on blood glucose levels in diabetic rats

Group	Blood glucose ($mg\,dL^{-1}$)			
	Day 0	Day 5	Day 10	Day 15
Normal	86.8 ± 7.24	86.11 ± 8.63	91.71 + 6.94	91.81 ± 6.21
Diabetic control	251.1 ± 2.0[a]	259 ± 2.1	268.5 ± 2.0	285.0 ± 1.6
Diabetic + extract (250 $mg\,kg^{-1}$)	265.6 ± 4.1[a]	187.8 ± 4.2[b]	160.8 ± 10[b]	89.3 ± 2.3[b]
Diabetic + extract (500 $mg\,kg^{-1}$)	278.1 ± 2.0[a,c]	170.0 ± 13.0[b,c]	134.5 ± 16.3[b,c]	104.3 ± 9.3[b,c]
Diabetic + glibenclamide (0.25 $mg\,kg^{-1}$)	212.0 ± 1.5[a]	176.3 ± 4.0[b]	99.6 ± 5.0[b]	89.5 ± 2.1[b]

Data are mean ± s.d. for six rats in each group. [a]$P < 0.01$ treatment group vs normal control; [b]$P < 0.01$ experimental groups vs diabetic control; [c]$P < 0.01$, 500 vs 250 $mg\,kg^{-1}$ extract.

Table 2 Effect of *S. indicus* on body weight and glycogen levels in diabetic rats

	Body weight (g)		Glycogen ($g\,100\,g^{-1}$ tissue)
	Initial	Final	
Normal	180.9 ± 7.1	205.7 ± 0.3	3.2 ± 0.11
Diabetic control	206.7 ± 6.2	168.7 ± 7.9[a]	1.1 ± 0.03[a]
Diabetic + extract (250 $mg\,kg^{-1}$)	185.6 ± 6.2	205.3 ± 7.4[b]	2.42 ± 0.1
Diabetic + extract (500 $mg\,kg^{-1}$)	179.4 ± 9.2	190.4 ± 3.5[b,c]	1.59 ± 2.4
Diabetic + glibenclamide (0.25 $mg\,kg^{-1}$)	183.4 ± 1.2	195.4 ± 8.4[b]	2.79 ± 1.5

Data are mean ± s.d. for six rats in each group. [a]$P < 0.01$ treatment group vs normal control; [b]$P < 0.01$ experimental groups vs diabetic control; [c]$P < 0.01$, 500 vs 250 $mg\,kg^{-1}$ extract.

Table 3 Effect of *S. indicus* on glycosylated haemoglobin (HbA1c), serum insulin levels and magnesium levels in diabetic rats after 15 days

	HbA1c ($mg\,100\,mL^{-1}$)	Serum insulin ($\mu U\,mL^{-1}$)	Magnesiun ($mEq\,L^{-1}$)
Normal	0.55 ± 0.4	15.6 ± 1.5[b]	3.1 ± 1.2
Diabetic control	0.74 ± 0.4	6.65 ± 1.1[a]	1.1 ± 1.4
Diabetic + extract (250 $mg\,kg^{-1}$)	0.52 ± 2.5	9.2 ± 0.6[a, b]	2.71 ± 4.2
Diabetic + extract (500 $mg\,kg^{-1}$)	0.56 ± 1.7	14.2 ± 0.3[b,c,d]	2.6 ± 1.4
Diabetic + glibenclamide (0.25 $mg\,kg^{-1}$)	0.62 ± 1.3	12.1 ± 1.8[a, b]	2.9 ± 0.9

Data are mean ± s.d. for six rats in each group. [a]$P < 0.01$ treatment group vs normal control; [b]$P < 0.01$ experimental groups vs diabetic control; [c]$P < 0.01$, 500 vs 250 $mg\,kg^{-1}$ extract; [d]$P < 0.01$ 500 $mg\,kg^{-1}$ extract vs glibenclamide group.

though the rats became permanently diabetic (Aybar et al 2001). After treatment with a low dose of STZ there should be many surviving β-cells, and regeneration is also possible (Gomes et al 1995). The alcoholic extract of *S. indicus* had the capacity to significantly lower the elevated glucose levels in rats with STZ-induced diabetes. However, glibenclamide had more significant activity. The antihyperglycaemic action of *S. indicus* results from the potentiation of insulin from existing β-cells of the islets of Langerhans. This is evident from the significant increase in plasma insulin concentration in STZ-diabetic rats. An increase in insulin secretion may lead to inhibition of lipid peroxidation due to a decrease in blood glucose levels. Like glibenclamide, *S. indicus* extract, at both doses tested, was able to increase the insulin levels in diabetic animals.

Decrease in the body weight of the diabetic rat is possibly due to dehydration and the catabolism of fats and proteins (Hakim et al 1997). Rajkumar et al (1991) have also reported that increased catabolic reactions, leading to muscle wasting, might also contribute to the reduced weight gain by diabetic rats. Moreover, the increased secretion of insulin may also result in increased protein synthesis due to its anabolic effect. Oral administration of the extract or glibenclamide improved the body weight in the diabetic rats.

Oral administration of *S. indicus* extract showed significant lowering of blood glucose following oral administration of glucose in normal rats without inducing the hypoglycaemic state. The glucose load was well tolerated in rats treated with *S. indicus*.

Effect of *S. indicus* extract on liver glycogen and glycosylated haemoglobin

Impairment of liver glycogen synthesis in diabetic rats has been reported by Huang et al (2000) and by Whitton & Hems (1975). The lack of insulin in the diabetic state causes a decrease in the hepatic glycogen content, which results in inactivation of the glycogen synthase systems (Singh et al 2001). Oral administration of the extract significantly improved hepatic glycogen synthase systems as a result of increased insulin secretion. Both doses of the extract, as well as glibenclamide, increased the level of glycogen in diabetic rats.

Glycosylated haemoglobin increases in patients with diabetes mellitus (Koenig et al 1976). In diabetes, protein synthesis is decreased in all tissues because of the relative insulin deficiency, and thus the synthesis of haemoglobin is also suppressed (Chatterjee & Shinde 1994). A number of proteins, including haemoglobin, are glycated to a greater degree

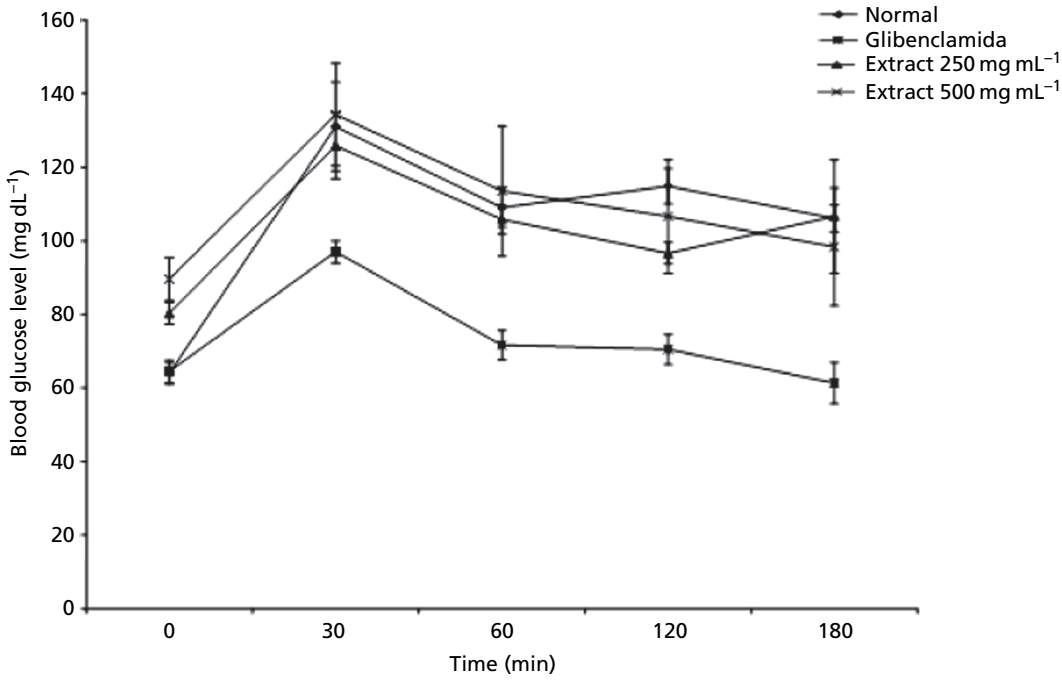

Figure 4 Average blood glucose concentrations during an oral glucose tolerance test. Data are mean ± s.d. (n = 6 rats). $P < 0.05$ treatment vs normal rats.

Table 4 Effect of *S. indicus* on lipid levels in diabetic rats after 15 days

	Triglycerides ($mg\,dL^{-1}$)	Total cholesterol ($mg\,dL^{-1}$)	HDL cholesterol ($mg\,dL^{-1}$)
Normal	92.4 ± 1.7	77.5 ± 2.6	71 ± 3.2
Diabetic control	186.1 ± 2.3[a]	129.9 ± 4.3[a]	33.4 ± 2.4[a]
Diabetic + extract ($250\,mg\,kg^{-1}$)	105.4 ± 1.4[b]	87.5 ± 2.7[b,d]	71.5 ± 1.7[b]
Diabetic + extract ($500\,mg\,kg^{-1}$)	82.6 ± 4.7[b,c]	78.1 ± 0.9[b,d]	73.6 ± 3.4[b]
Diabetic + glibenclamide ($0.25\,mg\,kg^{-1}$)	98.6 ± 2.1[b]	92.5 ± 1.4[b]	84.3 ± 0.7[b]

Data are mean ± s.d. for six rats in each group. [a]$P < 0.01$ treatment group vs normal control; [b]$P < 0.01$ experimental groups vs diabetic control; [c]$P < 0.01$, 500 vs $250\,mg\,kg^{-1}$ extract; [d]$P < 0.01$ $500\,mg\,kg^{-1}$ extract vs glibenclamide group.

in diabetes (Subash Babu & Prince 2004). The significant reduction in glycosylated haemoglobin in diabetic rats treated with the extract indicates the efficiency of the extract in glycaemic control. The extract at $250\,mg\,kg^{-1}$ showed better activity than glibenclamide.

Effect of *S. indicus* extract on lipid levels and magnesium

The present study showed a marked increase in serum triglycerides and cholesterol levels in control diabetic rats, which is in agreement with the findings of Nikkila & Kekki (1973) and Chase & Glasgow (1976). Under normal circumstances insulin activates the enzyme lipoprotein lipase, which hydrolyses the triglycerides. Insulin deficiency results in failure to activate the enzymes, thereby resulting in hypertriglyceridaemia. The significant improvement in the levels of serum lipids in the extract-treated diabetic rats and glibenclamide-treated diabetic rats may therefore be because of the improvement in insulin levels.

Magnesium is an important component of many unprocessed foods, such as whole grains, nuts and green leafy vegetables, and it is largely lost during the processing of some foods. Hypomagnesaemia is common in patients with type 2 diabetes. Although diabetes can induce hypomagnesaemia, magnesium deficiency has also been proposed as a risk factor for type 2 diabetes. Magnesium is a necessary cofactor for several enzymes that play important roles in glucose metabolism. Animal studies have shown that magnesium deficiency has a negative effect on the post-receptor signalling of insulin. Some short-term metabolic studies suggest that magnesium supplementation has a beneficial effect on insulin action and glucose metabolism (Lopez-Ridaura et al 2004). Animals treated with the test extracts and glibenclamide showed a significant improvement in serum magnesium levels.

In our study, we observed that the oral administration of *S. indicus* restored plasma glucose levels to near normal in rats with STZ-induced diabetes. Phytochemical studies carried out on the alcoholic extract of *S. indicus* revealed the presence of sterols, phenols and flavonoids (Shirwaikar et al 2006). Flavonoids are reported to have a major role in reducing oxidative stress associated with diabetes, which in turn helps the regulation of plasma glucose concentration (Shirwaikar et al 2004). Flavonoids isolated from different sources have been documented to show antihyperglycaemic activity (Vertommen et al 1994).

Conclusion

Our finding shows that the alcoholic extract of *S. indicus* possesses significant antihyperglycaemic activity. The phytoconstituents, viz. flavonoids, probably scavenge free radicals and prevent the depletion of endogenous antioxidants. An increase in insulin levels improves blood glucose levels and thus validates the antidiabetic claims for *S. indicus* made by the tribes of Madhya Pradesh.

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