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Hypoglycemic and Hypolipidemic Effect of *Sida rhombifolia* ssp. *retusa* in Diabetic Induced Animals

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Abstract

In the present study, the anti-diabetic effect of aqueous extract of *Sida rhombifolia* ssp. *retusa* (Malvaceae) leaves was studied in normal and streptozotocin (STZ)-induced (60 mg/kg, single intraperitoneal injection) diabetic rats. Hypoglycemic activity in normal rats was tested after administration of 200 mg/kg of extract. Aqueous extract showed a 15% reduction in plasma glucose level after 1.5 h of extract administration. When tested in STZ-induced diabetic rats the reduction in plasma glucose was 17%. In oral glucose tolerance test in normal rats and STZ-induced rats the decrease in AUC was 15 and 7% respectively. Glibenclamide was used as reference drug and showed significant hypoglycemic effects in normal rats but had marginal activity in STZ-induced diabetic rats. In hypolipidemic study a dose of 200 mg/kg of aqueous extract has shown reduction in triglycerides (TG) (16%), cholesterol (4%), and glucose level (10%). Fenofibrate was used as standard drug for hypolipidemic study.
The results obtained from the experiment provided scientific evidence in favor of the traditional use of *Sida rhombifolia* ssp. *retusa* leaves for the treatment of diabetes mellitus.

Keywords: Diabetes, *Sida rhombifolia* ssp. *Retusa*, hypoglycemic activity and hypolipidemic activity.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms or relative lack of the hormone insulin [1]. This metabolic disorder affecting approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 [2]. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable [3]. Currently available oral anti-diabetic drugs that are used clinically for glycemic control include ulfonylureas, meglitinides, biguanides, α-glucosidase inhibitors, and thiazolidinediones (TZDs) [4]. Each class of drug carries the burden of drug-associated side effects [5]. All oral anti-diabetic drugs therapies have limited efficacy, and mechanism based side

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effects [6]. However, medicinal herbs are expected to have a similar degree of efficacy without the side effects associated with conventional drug treatment.

Presently, there is growing interest in herbal remedies due to the side effects associated with the oral synthetic hypoglycemic agents for the treatment of diabetes mellitus [2]. Herbal medicines have been long used for the treatment of diabetic patients and continue to be accepted as an alternative therapy. There are more than 1000 anti-diabetic plants have been described in the scientific literature [7]. The plant kingdom is a wide field to search for a natural effective oral hypoglycemic or hypolipidemic agent that has slight or no side effects. Natural products with both hypoglycemic and hypolipidemic properties are useful anti-diabetic agents [8]. *Sida rhombifolia* L. ssp. *retusa* (L.) (Malvaceae) is distributed throughout the warmer parts of India, known as Bala in Sanskrit and widely used in Ayurveda in the treatment of fever and as a diuretic [9]. Renowned Ayurvedic physician Charaka has categorized bala as brmhaniya (a bulk- promoting herb), as balya (tonic), and prajasthapana that promote reproduction [10]. Several medicinal plants have been extensively used in Indian system of medicine (Ayurveda) for the management of different types of diabetes. Literature survey finds that *Sida retusa* has been used as an antioxidant and hepatoprotective [11], in rheumatoid arthritis and in neurological complaints including epilepsy [12]. Therefore, the search for safe and more effective agents of herbal origin has continued to be an important area of active research. It is also reported that *Sida retusa* extract is traditionally used by diabetic patients to lower their blood glucose levels [13]. This study was taken to make a conclusive correlation between Ayurvedic claims for this plant and consequent scientific studies.

This study investigated the hypoglycemic and hypolipidemic effects of aqueous extract of *Sida rhombifolia* ssp. *retusa* leaves in normal, and streptozotocin (STZ)-induced diabetic rats.

Material and Methods

Collection of Plant Material

Sida retusa plant was collected from the Academy of Development Sciences, Kashale Post Karjat, Maharashtra during August, 2006. The sample was authentified from Botanical Survey of India, and voucher specimen was deposited in our Department of Pharmacognosy. The leaves were air dried under shade, and powdered to 40 mesh for preparation of aqueous extract.

Preparation of Aqueous Extract

The leaves were crushed in an iron mortar. Crushed material was subjected to extraction by hot maceration at 60–70°C for 6h continuously in distilled water three times. The extract was filtered, combined, and evaporated to dryness. Dried extract was obtained by concentrating filtrate under reduced pressure in rotary evaporator.

Chemicals

Fenofibrate, Glibenclamide, Streptozotocin, and biochemicals used in this experiment were purchased from Sigma, St. Louis, MO, USA. All other chemicals were of analytical grade.

Experimental Animals

Wistar rats were used at the age of 8 weeks and Swiss albino mice were used at the age of 7 weeks. Rats and mice were assigned in different groups (n = 6). Rats and mice were housed under conventional conditions with controlled temperature, humidity, and lighting (22 ± 2°C, 55 ± 5%, and a 12 h light/dark cycle), and provided with a pellet diet and water ad libitum. All procedures were conducted according to the institutional animal ethics committee.

Induction of Experimental Diabetes

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared streptozotocin (60 mg/kg body weight) in citrate buffer (pH = 7.4). Forty-eight hours after STZ-administration, blood glucose level of each rat was determined. Rats with a blood glucose range of 220–300 mg/100 ml were considered diabetic and included in the study.

Experimental Design

In the experiment, normal rats and STZ-induced diabetic rats were used. The rats were divided into different groups of six rats each.

Group 1: Normal control untreated rats received 0.5% CMC.

Group 2: Normal rats administered aqueous extract of *Sida retusa* orally (200 mg/kg body weight) in distilled water.

Group 3: Normal rats given glibenclamide orally (10 mg/kg body weight) in distilled water.

Group 4: Diabetic Control with STZ-treated.

Groups 5 and 6: STZ-treated diabetic rats administered aqueous extract of *Sida retusa* orally (200 and 300 mg/kg body weight) in distilled water.

Group 7: STZ-treated diabetic rats given glibenclamide orally (10 mg/kg body weight) in distilled water.

For Hypolipidemic study fenofibrate (100 mg/kg) in Swiss albino mice was used as standard drug.

Hypoglycemic Study

Blood was obtained from the retro-orbital puncture of all Wistar rats using capillary tubes. Blood was collected at 0, 30, 60, 90, 120, 180 min in potassium oxalate and sodium fluoride containing tubes for plasma glucose measurement. Blood samples were centrifuged at 4000 rpm for 15 min at 4°C. Plasma was separated for the estimation of plasma glucose level.

Hypolipidemic Study

Swiss albino mice were grouped based on their plasma triglyceride levels and were dosed orally for 14 days. On day 14th animals were bled one hour after dosing and plasma was separated for estimation of cholesterol, triglyceride, and glucose

Oral Glucose Tolerance Test (OGTT)

Animals were fasted for 18 h and were grouped based on their blood glucose levels. The drugs were administered 1 h before glucose load (2 g/kg). Blood samples were collected at 0, 30, 60, 90, 120, and 180 min from the reteroorbital sinus in heparinised tubes. Samples were centrifuged at 4000 rpm for 15 min to separate plasma, for the measurement of plasma glucose.

Biochemical Analysis

Plasma glucose, triglycerides, and cholesterol were measured with the help of commercial point scientific kits (Ranbaxy Laboratories, India).

Statistical Analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Results were expressed as mean ± S.D. from six rats in each group. P-values <0.05 were considered significant.

Results

Hypoglycemic Effect in Normal and STZ Rats

The results of the present studies indicate that *Sida retusa* was found to reduce the glucose level in normal and the animals made diabetic with STZ. Glucose level was assessed in normal rats at various time intervals. In normal rats, extract at dose of 200 mg/kg showed 14.7% reduction in plasma glucose levels after 90 min of oral administration. Glibenclamide used as reference drug has shown significant 36.6% reduction in plasma glucose levels at 180 min (Table 1). In

Table 1. Effect of aqueous extract of *Sida retusa* on plasma glucose concentration in normal and streptozotocin-induced (60 mg/kg) diabetic rats.

Treatment and Dose	Plasma glucose concentration (mg%) Time (min)					
Normal rats	0	30	60	90	120	180
Control (0.5% CMC)	88.4 ± 9.4	94.5 ± 10.4	95.2 ± 9.4	98.7 ± 7.6	100.5 ± 8.3	96.3 ± 7.3
Extract (200 mg/kg)	86.4 ± 1.6	82.8 ± 2.7	75.2 ± 2.7	71.8 ± 2.2	72.2 ± 1.9	76.2 ± 4.2
Glibenclamide (10 mg/kg)	90.3 ± 4.8	77.8 ± 4.8	69.7 ± 4.5	57.3 ± 4.9	55.5 ± 3.7	53.7 ± 4.0
Diabetic rats						
Control	292.6 ± 6.5	317.0 ± 9.5	288.0 ± 17.3	295.0 ± 21.6	276.0 ± 18.5	276.2 ± 20.7
Extract (200 mg/kg)	303.0 ± 4.6	307.0 ± 44.1	270.8 ± 26.4	246.8 ± 23.8	253.6 ± 24.9	252.8 ± 24.7
Extract (300 mg/kg)	297.0 ± 8.5	303.6 ± 22.4	269.3 ± 16.5	243.6 ± 25.5	260.3 ± 19.3	251.9 ± 21.4
Glibenclamide	295.6 ± 4.3	275.4 ± 23.9	255.0 ± 23.6	247.8 ± 23.8	251.6 ± 22.4	259.0 ± 19.5

STZ-induced rats, the decrease in plasma glucose was 16.9 and 16.1% at 90 min with extract at a concentration of 200 and 300 mg/kg respectively and with glibenclamide it was 16.44% at same 90 min time point (Table 1).

Oral Glucose Tolerance Test (OGTT) in Normal and STZ Rats

In normal rats, extract (200 mg/kg) has shown 14.21% reduction in total AUC when compared with normal control (0.5% CMC) treated animals. The peak increase in mean plasma glucose at 30 min was 190 mg/dl with normal control group animals and it was only 151 mg/dl with extract treated animals at a dose of 200 mg/kg. Glibenclamide treated animals has shown only 100 mg/dl plasma glucose at peak and reduction in total AUC was 40.86% (Table 2).

Table 2. Effect of aqueous extract of *Sida retusa* in normal and diabetic induced rats on oral glucose tolerance.

Treatment and Dose	Blood glucose level (mg/dl) Time (min)					
Normal rats	0	30	60	90	120	180
Control (0.5% CMC)	88.4 ± 9.4	190.5 ± 11.4	174.5 ± 3.5	142.6 ± 6.3	140.2 ± 10.1	136.5 ± 5.7
Extract (200 mg/kg)	83.7 ± 2.7	151.9 ± 6.6	161.1 ± 3.0	130.5 ± 6.0	112.4 ± 3.9	116.9 ± 3.1
Glibenclamide (10 mg/kg)	65.6 ± 2.2	100.7 ± 5.3	94.2 ± 2.5	97.2 ± 9.4	92.1 ± 5.7	68.3 ± 4.3
Diabetic rat						
Control	254.5 ± 12.5	418.0 ± 20.4	434.8 ± 23.8	379.0 ± 12.4	355.3 ± 16.2	295.0 ± 18.9
Extract (200 mg/kg)	254.5 ± 12.5	375.3 ± 5.3	401.3 ± 14.0	341.0 ± 16.0	328.8 ± 22.1	275.3 ± 23.9
Extract (300 mg/kg)	297.0 ± 18.5	303.6 ± 22.4	280.4 ± 16.5	256.6 ± 25.5	260.3 ± 19.3	251.9 ± 21.4
Glibenclamide (10 mg/kg)	250.0 ± 15.4	341.2 ± 3.9	359.2 ± 12.8	315.0 ± 11.5	312.4 ± 10.8	270.2 ± 17.8

In STZ-induced rats, extract (200 mg/kg) has shown 7.95% reduction in total AUC (60393 ± 2686) when compared with diabetic control (0.5% CMC) treated animals. The peak increase in mean plasma glucose at 30 min was 435 mg/dl with control group animals and it was 401 mg/dl with extract treated animals. Glibenclamide treated animals has shown only 359 mg/dl plasma glucose at peak and reduction in total AUC (56376 ± 1465) was 14.07%. The normal (citrate buffer injected) rats have 71% (19008 ± 727) vs. 65606 ± 2571) less total AUC compared to STZ animals (Table 2).

Hypolipidemic Activity in Swiss Albino Mice

Extract has shown 14.4% reduction in plasma tryglyceride compared to control (84.20 mg/dl, control and 70.40 mg/dl, extract). Fenofibrate showed significant ($p<0.05$) reduction in plasma tryglyceride level. There was no effect on the plasma cholesterol levels in both extract as well as in fenofibrate. Extract has also 13% reduction in plasma glucose levels. Fenofibrate has no effect on plasma glucose levels (Table 3).

Table 3. Effect of aqueous extract of *Sida retusa* on tryglyceride, cholesterol, and glucose level.

Treatment	Tryglyceride (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
Control (CMC 0.5%)	84.20 ± 14.28	122.97 ± 3.36	127.10 ± 11.20
Extract (200 mg/kg)	70.40 ± 4.28	106.80 ± 6.35	114.00 ± 2.43
Fenofibrate (100mg/kg)	38.20 ± 2.44	130.00 ± 10.89	123.92 ± 9.28

Discussion

It is reported that *Sida rhombifolia* ssp. *retusa* extract have been used traditionally by diabetic patients to lower their blood glucose levels. Here, we screened leaves extract of this plant in diabetic induced and normal rats for its hypoglycemic activity. Streptozotocin-induced hyperglycaemic has been described as a useful experimental model to study the activity of hypoglycaemic agents [14].

Streptozotocin selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state [15]. We found small reduction in plasma glucose in normal rats even after 2 h of dosing, but glibenclamide significantly reduced plasma glucose levels. When tested in STZ rat model extract has shown nearly same reduction in plasma glucose levels as in normal rats. Glibenclamide showed only little decrease in plasma glucose levels. In oral glucose tolerance test (OGTT) in normal rats, we found both decrease in peak plasma glucose and in total AUC but it was not significant. In STZ-induced diabetic rats, we performed the OGTT after 5 days of injection. There was significant ($p<0.001$) difference in total AUC between the normal (citrate buffer), and STZ injected rats. This is due to destruction of pancreas; hence STZ rats were not able to release insulin in response to glucose. Extract has reduced both peak plasma glucose and total AUC. Glibenclamide, a potent hypoglycemic agent has showed significant decrease in AUC in OGTT in normal rats but has only marginal effect in OGTT in STZ rats.

As diabetes is also associated with hypertriglycerides, dyslipidemia, and high levels of free fatty acids. A higher level of triglycerides, and free fatty acids also causes insulin resistance by inhibiting insulin signaling which further worsen the situation. Here we tested hypolipidemic activity in Swiss albino mice, as this is reported to have mild hypertriglyceridemia and had been used by many authors for testing hypolipidemiac activity. Here we found decrease in plasma triglycerides after 14 days of treatment. But extract has no effect on plasma cholesterol levels. We found some reduction in plasma glucose levels also. Fenofibrate used as a reference drug has significantly reduced plasma triglyceride levels but has no effect on plasma cholesterol and glucose levels. The decrease in triglyceride level proves mild hypolipidemic activity of the extract and decrease in plasma glucose confirms its hypoglycemic activity.

Glibenclamide treatment was not as effective in reducing blood glucose in STZ-diabetic rats as in normoglycaemic rats. It has been reported that glibenclamide was not effective when destruction of β-cells has occurred and hence more effective in moderate diabetic rats than in severe diabetic animals [16]. The acute hypoglycaemic effect of glibenclamide results has been shown from the stimulation of insulin release from the residual β-cells and inhibition of glucagon secretion. As reported glibenclamide do not have effect in STZ rats but metformin has effect in this model. This effect is due to inhibition of hepatic gluconeogenesis. We have also seen the same thing here. Glibenclamide showed significant hypoglycemic effect per se and also reduced glucose excursion in OGTT in normal rats. But in STZ-induced diabetic rats glibenclamide has very marginal activity. This might be due to insufficient pancreas left because of toxicity caused by STZ, so there is no sufficient release of insulin to cause further reduction in plasma glucose.

Extract has shown mild hypoglycemic activity. The mechanism of glucose reduction is either due to increased insulin release in response to glucose load, peripheral glucose consumption, and inhibition of intestinal or renal glucose absorption or inhibition of endogenous glucose production. Inhibition of hepatic gluconeogenesis could be a factor as in OGTT both normal and STZ-induced rats were on fasting and fasting causes more hepatic gluconeogenesis. As extract has shown similar activity in both normal and STZ rats so stimulation of peripheral blood glucose utilization and inhibition of endogenous glucose production appears to be most probable mechanism for its action. Due to its effect on triglyceride levels this extract could be more effective in type II diabetes, where diabetes is associated with abnormal lipid profile. We have tested acute dosing of the extract in normal and STZ rats. Chronic dosing of this in another type II diabetic model could be more useful as in Swiss albino mice we got more than 10% reduction in plasma glucose after 2 weeks of dosing.

In conclusion, *Sida rhombifolia* ssp. *retusa* leaves extract has shown mild hypoglycemic and hypolipidemic activity. Mechanism for hypoglycemic activity could be due to peripheral

blood glucose utilization, and inhibition of endogenous glucose production. The investigation provides biochemical evidence to validate the use of *Sida retusa* as anti-diabetic by Ayuvedic physicians.

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