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Anti-hyperglycemic effect of *Scoparia dulcis* in streptozotocin induced diabetes

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ABSTRACT

Aqueous extract of *Scoparia dulcis* could significantly reduce blood sugar levels in white rats which had been previously induced with diabetes by streptozotocin. Extract was administered at doses of 125 and 250 mg/kg body weight/twice a day respectively for a period of 3 weeks. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 250 mg/kg body wt. /twice a day resulted in a marked decrease in the levels of fasting blood glucose and urine sugar, with a concomitant increase in body weight. The extract also produced a significant decrease in peroxidation products, *viz*., thiobarbituric acid reactive substances. Reduced glutathione and glycogen content, which had shown significant decrease following induction of diabetes, were found to be increased in the hepatic tissue of STZ-diabetic rats treated with the extract. Results suggest that aqueous extract of *Scoparia dulcis* can act as anti-hyperglycemic agent in STZ-diabetic rats.

Key words: *Scoparia dulcis*, diabetic, anti-hyperglycaemic, blood glucose

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INTRODUCTION

Diabetes mellitus is a non communicable disease considered to be one of the five leading causes of death worldwide. About 100 million people around the world have been diagnosed with diabetes and by the year 2010 it is projected that 215 million people will have the disease [1]. Diabetes mellitus is a metabolic disorder and a major cause of disability and hospitalization (Foster, 1994). Diabetes mellitus is generally divided into two different types: insulin-dependent diabetes mellitus (IDDM), and non-insulin-dependent diabetes mellitus (NIDDM). Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [2]. Insulin therapy affords effective glycemic control in IDDM patients, yet its short comings include ineffectiveness on oral administration, short shelf life, need for preservation in refrigeration, fatal hypoglycemia in the event of excess dosage, reluctance to take injection and above all, the resistance due to prolonged administration, limits its usage. Similarly treatment of NIDDM patients with sulfonylurea's and biguanides is always associated with side effects [3]. Hence search for a drug with low cost, more active and without side effect is being pursued in several laboratories around the world.

Scoparia dulcis L. (Scrophulariaceae) is a perennial herb widely distributed in tropical and subtropical regions. In these regions, the fresh or dried plant of *Scoparia dulcis* has traditionally been used as one of the remedies for stomach troubles, hypertension, inflammation, bronchitis, hemorroids and hepatosis and as analgesic and antipyretic. Since no reports on scientific evaluation of this plant in diabetes are available, in the current study, we have evaluated the antidiabetic activity of an aqueous extract of *Scoparia dulcis* plant in rats.

MATERIALS AND METHODS

Scoparia dulcis L (Scrophulariaceae) an annual herbaceous plant was collected from Dakshin Dinajpur district, West Bengal, India during June 2008. It was identified by the Botanical Survey of India, Kolkata and a herbarium specimen was deposited to the North Bengal University herbarium (Acc No- 9491).

Preparation of plant extract

Plant body of *Scoparia dulcis* (each 500g) were extracted with 1500 ml of water by the method of continuous hot extraction at 60^0C for 6 h and evaporated. The residual extract was dissolved in water and used in the study [4].

Drugs and chemicals

All the drugs and biochemical used in this experiment were purchased from the Sisco Research Laboratory Pvt. Ltd. (SRL), Mumbai, India. The chemicals were of analytical grade.

Animals

Male Wister albino rats (180-200g) were obtained from the Ghosh Enterprise, Kol-55. The animals were grouped and housed in polypropylene cages and maintained under slandered laboratory conditions (temperature 25±2^{0}C) with a 12-h/12-h dark and light cycle [5]. All animals maintained on a standard laboratory diet and tap water and had free access to food and water.
All procedures described were reviewed and approved by the University Animals ethical Committee.

Induction of experimental diabetes

A freshly prepared solution of Streptozotocin (55mg/kg, i.p.) in 0.1M citrate buffer, pH 4.5, were injected intraperitoneally in a volume of 1ml/kg [6]. After 48 h of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300mg/dl) were taken for the experiment.

Experimental Procedure

In the experiment, a total of 20 rats (12 diabetic surviving rats, 8 normal rats) will be used. The rats were divided into 9 groups of 4 rats each.

Group-1: Control untreated rats receiving distilled water.

Group-2: Control rats receiving 0.1M citrate buffer (pH 4.5).

Group-3: STZ treated diabetic rats.

Group-4: STZ treated diabetic rats treated with *Scoparia dulcis* plant extract (125mg/kg body weight) in distilled water using intragastric tube twice a day for 20 days.

Group-5: STZ treated diabetic rats treated with *Scoparia dulcis* plant extract (250mg/kg body weight) in distilled water using intragastric tube twice a day for 20 days.

The body weight gain, fasting blood glucose and urine sugar of all the rats were determined at regular intervals during experimental period.

After 20 days, all the rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected in tubes containing sodium fluoride for the estimation of fasting blood glucose. Livers were removed immediately rinsed in ice chilled normal saline and, patted dry and weighed.

Biochemical studies

Qualitative determination of urine sugar

Glucose was detected in the urine by the method of Benedict *et al*. (1908 7). Benedict's reagent (5ml) was taken in a test tube and 8 drops of urine was added to it. Tubes were boiled for 1-2 min and then cooled slowly. The solutions were filled with greenish/yellow/red and no precipitate depending upon the quantity of glucose present. Greenish precipitate would indicate very small amount of glucose. The solution remained clear where no glucose was there.

Estimation of blood glucose

Fasting blood glucose was quantified by the method of Nelson and Somogyi [8]. Absorbances were read at 500 nm and were quantified using a standard curve of glucose.

Quantitative estimation of glycogen

Glycogen was hydrolyzed to glucose by the method of Raghuramula *et al* [9] and the glucose thus formed was estimated by Nelson and Somogyi's method [8]. The liver was taken out rapidly from the animal and the excess blood removed by blotting between folds and filter paper and immediately put into a weight stopper test tube containing 30% KOH and weight again. The amount of alkali was then adjusted to get 2ml per g of liver. The tissue was digested in a boiling water bath for 1 hr. The filtrate was cooled in ice cold water. Two volumes of 95% ethanol were then added and the mixture heated just to boiling. Spurting was avoided. This was left to stand overnight in the cold. The tubes were centrifuged and the precipitate dissolved in 5-10 ml warm water. The glycogen was re-precipitated with 2 volumes of 95% ethanol. The precipitate was centrifuged and washed several times with 60% ethanol. Two ml of 2 N H2SO4 per g of initial liver weights was added and hydrolyzed in a boiling water bath for 3-4 h. The solution was neutralized with NaOH using Phenol red as indicator. Volume was noted and filtered. Glucose was determined in that aliquot. The factor 0.93 used to convert glucose to glycogen.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS in tissues was estimated by the method of Ohkawa *et al*. [10]. After collection of blood samples the rats were killed and livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weight. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. The levels of lipid peroxides were expressed as moles of thiobarbatituric acid reactive substances (TBARS)/mg protein.

Determination of reduced glutathione (GSH)

GSH in tissues was estimated by the method of Ellman *et al*. [11]. After killing the rats livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for estimation of glutathione. The amount of GSH in the sample was calculated using the standard curve generated from known GSH.

Statistical analysis

The collected data were subjected to statistical analysis by standard procedures of Standard Error, CD, Student's't' test and Pearson's correlation.

RESULTS

In all groups prior to streptozotocin administration, the basal levels of blood glucose of the rats were not significantly different. However, after 2 days of STZ administration, blood glucose levels were significantly higher in rats selected for the study.

Table 1: Effect of aqueous extracts of *Scoparia dulcis* on changes in body weight in normal and experimental rats.

Groups	Treatment	Body weight (g)	
		Initial	Final
I	Control	173.5 ± 4.73	202.5 ± 5.86
II	Citrate buffer control	166.0 ± 4.99	191.5 ± 3.50
III	STZ (Diabetic control)	171.0 ± 1.15	147.0 ± 0.57
IV	STZ +SD (125mg/kg)	169.5 ± 4.50	186.5 ± 3.50
V	STZ +SD (250mg/kg)	172.0 ± 1.00	195.5 ± 2.50

Each value represents mean ± SE ; n=4 ; SD= *Scoparia dulcis*
Represents Statistical significance vs. control ($p<0.05$).

Table 1 shows the effect of treatment with extract on changes of body weight. There was a significant decrease ($p < 0.01$) in the body weight of the diabetic controls compared with the normal controls. Administration of *Scoparia dulcis* plant extract to diabetic rats (Group-4 and Group-5) increased body weight significantly ($p < 0.01$) which was comparable to the increase in the body weight of normal controls.

Evaluation of the effect of *Scoparia dulcis* plant extract on urine sugar of normal and experimental rats revealed that, results were positive (+++) in the diabetic rats while no sugar could be deceted in diabetic rats treated with *Scoparia dulcis* plant extract (250 mg/kg) treated groups. Little amount (+) of sugar was found in the *Scoparia dulcis* plant extract (125mg/kg) treated groups (Table 2).

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Table 2: Effect of different extracts on Urine sugar in control and diabetic rats

Groups	Treatment	Urine sugar (STZ-2day)	Urine sugar (STZ-20day)
I	Normal	Nil	Nil
II	Citrate buffer	Nil	Nil
III	Diabetic Control	++	+++
IV	SD (125mg/kg)	++	+
V	SD (250mg/kg)	++	Nil

SD= *Scoparia dulcis*; +++ = Reddish thick precipitation; ++ = Yellowish lighter precipitation; + = greenish colour; Nil= no colouration.

Table 3: Effect of different extracts on changes in Fasting Blood glucose in normal and experimental rats (Initial-final)

Group	Treatments	Initial	Final
I	Normal (only vehicle distilled water)	76.10 ± 1.04	77.75 ± 2.88
II	Citrate buffer treated	76.85 ± 0.68	76.85 ± 1.13
III	Diabetic Control	76.40 ± 0.81	337.07 ± 6.36
IV	SD (125mg/kg)	78.07 ± 0.29	117.19 ± 1.18
V	SD (250mg/kg)	74.49 ± 0.49	79.66 ± 0.97

Each value represents mean ± SE ; n=4 ; SD= *Scoparia dulcis*
Represents Statistical significance vs. control ($p<0.05$).

Fasting blood glucose levels in the normal controls rats remained unchanged during the course of the experiment. There was a significant ($p< 0.05$) increase in blood glucose in diabetic rats after two days of STZ administration. Administration of *Scoparia dulcis* plant extract (250mg/kg) decreased significantly ($p< 0.05$) the level of fasting blood glucose and brought the sugar level near to normal (Table 3).

Table 4: Effect of different extracts on glycogen in control and diabetic rats

Groups	Treatments	Glycogen (mg/100g)
I	Control	40.64 ± 2.58
II	Citrate buffer Control	41.40 ± 2.76
III	Diabetic Control	21.17 ± 2.66
IV	SD-125	30.53 ± 1.46
V	SD-250	41.33 ± 0.72

Each value represents mean ± SE, n=4; SD= *Scoparia dulcis*.
Represents Statistical significance vs. control ($p<0.05$).

The effects of *Scoparia dulcis* plant extract on glycogen content are depicted in Table 4. The level of glycogen content decreased significantly ($p< 0.001$) in the STZ-diabetic rats as compared to control. Treatment with *Scoparia dulcis* plant extract (250mg/kg and 125mg/kg) significantly ($p< 0.001$) increased the glycogen and brought them near to normal level.

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Table 5: Effect of different extracts on TBARS and Reduced glutathione level in control and diabetic rats

Groups	Treatment	TBARS (mM/100g)	Reduced glutathione (GSH) (mM/100g tissue)
I	Control	0.81 ± 0.03	45.33 ± 1.76
II	Citrate buffer control	0.79 ± 0.05	46.00 ± 1.52
III	Diabetic Control	1.84 ± 0.11	25.66 ± 1.85
IV	SD (125mg/kg)	1.22 ± 0.09	33.33 ± 1.45
V	SD (250mg/kg)	0.84 ± 0.06	44.00 ± 1.15

Each value represents mean± SE, n=4; SD= *Scoparia dulcis*
Represents Statistical significance vs. control ($p< 0.001$ in case of TBARS and $p< 0.01$ in case of GSH).

The STZ-diabetic rats showed a significant increase in TBARS when compared with normal. There was a significant ($p< 0.001$) reduction in TBARS in the liver of rats with the treatment of *Scoparia dulcis* plant extract (250mg/kg and 125mg/kg) when compared with normal rats (Table 5). Besides, it was observed that while the reduced glutathione level in the liver of diabetic rats had significantly ($p< 0.01$) decreased, administration of *Scoparia dulcis* plant extract (250mg/kg and 125mg/kg) increased the levels of GSH in the liver ($p< 0.01$).

DISCUSSION

In the present study the body weight of the diabetic rats decreased significantly after the treatment with STZ. The body weights of *S.dulcis* treated groups were increased significantly after the 20th day compared with control and diabetic control. Decrease in bodyweight due to derangement of metabolic pathways is a common feature in diabetes [12]. Administration of *Coccinia indica* leaves extract [13] to diabetic rats significantly reversed the loss in body weight, apparently due to its ability to reduce hyperglycemia. Similar results were also reported after oral administration of the methanol fraction of *Salacia reticulata* twice daily to diabetic animals which gained body weight [14]. Results of the present study also support the findings of Chakraborty and Das [15, 16].

Increased urea production in diabetes may be accounted due to enhanced catabolism of both liver and plasma proteins [17]. Qualitative estimation of urine is a conventional method for the determination of sugar level in urine. The results of the test were positive (+++) to the diabetic rats and amount of sugar were nil to the *S.dulcis* (250mg/kg) treated groups. Fasting blood glucose levels in the normal controls rats remained unchanged during the course of the experiment. The combination of *Catharanthus roseus* (0.5ml/kg) and fenugreek (50ml/kg) produced significant ($p<0.001$) reduction in blood glucose 35.41% at 10h and increased the duration of action compared to either one [18]. The butanolic fraction of *Helicteres isora* root at a dose of 250mg/kg produced maximum fall (48.86%) in the blood glucose of diabetic rats [19]. Venkateswaran and Pari [13] also found that the diabetic rats showed a significant increase in blood glucose. In their experiment it was observed that the administration of *Coccinia indica* leaves extract in diabetic rats restored the level of blood glucose to near normal levels. Results of the present study are also in conformity with previous studies. On the other hand, diabetes

mellitus is associated with a marked decrease in the level of liver glycogen [20]. In the present study, results revealed that the levels of glycogen, which had fallen due to induction of diabetes, could be elevated by treatment with aqueous extract of *S.dulcis*. Pari and Latha [21] have shown that the hepatic and skeletal muscle glycogen content was reduced significantly in diabetic control.

Aqueous extract of *S.dulcis* could bring back to normal, levels of TBARS and reduced glutathione, which had been enhanced and decreased, respectively, following induction of diabetes. Kamalakkannan and Stanely [22] investigated on the antidiabetic and antioxidant activity of *Aegle marmelos* in streptozotocin-induced diabetic rats. The diabetic rats showed a significant increase in TBARS and hydroperoxides in liver and kidney. Oral administration of *Aegle marmelos* fruit extract maintained the tissue TBARS and hydroperoxides to near normal status. The effect of treatment with *Anacardium occidentale* on lipid peroxidation index (MDA) in normal and fructose-fed rats was investigated [23]. The MDA levels were significantly higher in fructose-fed rats compared with those in normal rats. Treatment with *Anacardium occidentale* produced significant reduction in MDA. Under *in vivo* condition glutathione (GSH) acts as an antioxidant and its decrease is reported in diabetes mellitus [24]. The decrease in GSH levels represents increased utilization due to oxidative stress [25].

The results of the current investigation of *Scoparia dulcis* plant showed significant antidiabetic activity. It is not known which of the recorded groups of biologically active compounds are responsible for this observed hypoglycemic effect. Neither is the mechanism of action clearly understood. However, this work has clearly brought out the importance of common plants in medicine, which if used properly, can be as good as any other forms of medicine.

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