

Antidiabetic and Antioxidant Effect of *Scoparia dulcis* in Alloxan induced Albino Mice

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Abstract: Herbal drugs are frequently considered to be less toxic and also free from side effects, than synthetic ones. Hence, the present study was designed to evaluate antidiabetic and antioxidant principle of the ethanolic extract of *Scoparia dulcis* (EESD) for its antidiabetic and antioxidant potential against alloxan-induced diabetic mice. Hypoglycemic effect was evaluated in normal and alloxan induced diabetic mice. The oral administration of plant extract at a dose of 100 & 200 mg/kg body weight were given to fasting glucose loaded (200 mg/kg body weight) mice with regard to normal control during 3 hr. study period and in alloxan-induced (150 mg/kg body weight) diabetic mice in comparison with reference drug, Metformin (600 µg/kg) during 2 weeks study period. Considerable fall in elevated blood glucose level was observed in the normoglycemic ($p < 0.05$) and alloxan induced diabetic ($p < 0.001$) mice. The extract, at a dose of 100 & 200 mg/kg body weight showed glucose level reduction of 31.87% & 46.97% respectively in alloxan-induced diabetic mice while 50.74% was found for Metformin after 2 weeks. The antioxidant potential of ethanolic extract of plant was assessed by DPPH (1, 1-Diphenyl, 2- picryl-hydrazyl) free radical scavenging assay at 517 nm and total antioxidant capacity by phosphomolybdenum method at 695 nm. The extract showed petite free radical scavenging capacity (IC_{50} value 243.82 µg/ml for plant extract and 58.92 µg/ml for Ascorbic acid) and moderate total antioxidant capacity compared with Ascorbic acid. These results demonstrate the antidiabetic and antioxidant potential of ethanolic extracts of *Scoparia dulcis* and suggests that the plant may have therapeutic value in diabetes and related complications.

Keywords: *Scoparia dulcis*, Alloxan, Metformin, Phosphomolybdenum, Antioxidative.

Introduction

Medicinal plants, since time immemorial have been in use for treatment of various diseases all over the world. Diabetes mellitus is a metabolic disorder characterized by hyperglycemic, glucoseurea and negative nitrogen balance and it is mainly due to lack of insulin secretion in beta cells of pancreas and desensitization of insulin receptors for insulin. The disease has reached epidemic proportions in the present century. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025 [1]. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycaemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems [2]. Management of diabetes without any side effects is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicine for diabetes as well. Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines/present day drugs for many rural populations in developing countries. In accordance to the recommendations by the WHO expert committee on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important [3].

Scoparia dulcis (Scrophulariaceae), commonly known as sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions. In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach troubles [4], hypertension [4], diabetes, bronchitis [5], and as analgesic and antipyretic agents [6]. A number of different principles such as scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol, and scopadulin [7] have been shown to contribute to the observed medicinal effect of the plant. These compounds were found to possess various biological activities such as inhibition of herpes simplex virus replication, gastric H^+, K^+ -ATPase activation, antitumor activity, etc. Nath [8], in a study of the antidiabetic effect of *S. dulcis*, obtained a glycoside named ammelin from fresh plants which relieved other ailments accompanying diabetes, such as pyorrhea, eye troubles, joint pain, susceptibility to cold, etc., within a very short period of time.

The present study was undertaken to determine the hypoglycaemic as well as antioxidant effects of the ethanolic extract of *S. dulcis* in normal and alloxan induced diabetic mice.

Materials & Methods

Collection and Identification of plants

The investigated plant *Scoparia dulcis* was collected from Manikgong, Bangladesh in July 2008 and was identified initially by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh and later the expert of Bangladesh National Herbarium, Mirpur, Dhaka recognized the plant & deposited the Voucher specimen no: 32766. The collected plant parts (whole herb) were separated from undesirable materials or plant parts. They were dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of Extract

The aerial parts of plant were collected, sun dried for seven days and ground. The dried powder of *Scoparia dulcis* (180gm) was soaked in 500 ml of 95% ethanol for 7 days in cold condition with occasional shaking and stirring. The whole mixture was successively filtered through a piece of clean, white cotton material and No. 1 Whatman filter paper. The filtrate (ethanol extract) obtained was evaporated using rotary evaporator. It rendered a gummy reddish black concentrate which was dissolved in ethanol at different concentrations to carry out the following experiments.

Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. DPPH (Sigma chemical co. USA.), Alloxan (Fluka, Germany), ammonium molybdate (Merck, Germany), sodium phosphate (BDH, England), ascorbic acid and metformin (General Pharmaceutical Bangladesh Ltd), Glucose estimation kit (Human, Germany).

Instruments

The molecular absorption spectra and absorbance at specific wavelengths were recorded with a HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of 1-cm light path.

Design of experiment

The research work was carried out for 4 weeks. The first 2 weeks were for the induction of diabetic condition in mice and the following 2 weeks were investigational period with crude ethanolic extract *Scoparia dulcis*. 25 mice were alienated into five groups for the hypoglycaemic activity study.

Group-1: Normal saline treated mice

Group-2: Normal saline treated Alloxan induced diabetic mice

Group-3: Metformin 600µg/kg body weight treated Alloxan induced diabetic mice

Group-4: Extract 100 mg/kg body weight treated Alloxan induced diabetic mice

Group-5: Extract 200 mg/kg body weight treated Alloxan induced diabetic mice

Selection, Maintenance and Management of Experimental Animals

For the present study we used Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 gm. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR). They were housed individually in cages at an ambient temperature of $24 \pm 1^{\circ}\text{C}$, relative humidity of 55-65% with 12 h light: 12 h dark cycle and fed ICDDR formulated mice food and water ad libitum. Excreta were removed from the cages on every day. The animals were acclimatized to laboratory condition for one week prior to experimentation. All protocols followed for animal experiment were approved by the institutional animal ethical committee[9].

Preparation of Alloxan solution and Induction of diabetes in mice

The alloxan solution was prepared by dissolving alloxan monohydrate in normal saline. Mice were administered orally with freshly prepared solution at a dose of 150 mg/kg body weight after the fasting blood glucose estimation was done. After giving two weeks alloxan injection, mice with plasma glucose level greater than 10 mmol/L were selected for the research purpose.

Preparation of sample suspension

The sample suspension of *Scoparia dulcis* was prepared at a dose 100 mg/kg body weight by weighing 3.6 gm of crude extract and suspending in 6 ml distilled water using 1% tween-80 as a suspending agent. For the preparation of Metformin suspension at a dose of 600 µg/kg body weights, 7.2 mg of drug was taken and it was suspended in 6 ml of distilled water using 1% tween 80 as a suspending agent. In the same way, *Scoparia dulcis* solution was prepared by dissolving 24 mg of the compound in 6 ml distilled water.

Phytochemical Screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with

chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures[10].

Hypoglycemic Investigations

Glucose tolerance test

A glucose tolerance test is conducted to find out how quickly it is cleared from the blood. The mice were tested in a fasting state (having no food or drink except water for at least 10 hours but not greater than 16 hours). An initial blood sugar is drawn and then the mice are fed glucose. Then blood of mice is tested again 30 minutes, 1 hour, 2 hours and 3 hours after drinking the high glucose drink using glucose estimation kit (Human, Germany).

Blood sugar assessment

For determination of serum glucose level, blood samples of normal and diabetic mice were drawn after an over night fasting (12 hr.) from tail vein at different time intervals (both normal and Alloxan induced mice) at 1st, 7th & 14th day.

Screening for Antioxidant Activity

Antioxidant activities of ethanolic extract of the experimental plant was determined in both qualitative and quantitative assay; their scavenging potential is measured by both stable DPPH free radical, and phosphomolybdenum method.

DPPH radical scavenging activity

i) Qualitative assay

A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted [4].

ii) Quantitative analysis

DPPH method

The ability of extracts to scavenge free radicals was assayed with use of a synthetic free radical compound - 2, 2-diphenyl-1-picrylhydrazyl (DPPH). An ethanolic DPPH solution (0.004%) was mixed with serial dilutions (5 to 500 µg/ml) of crude extract and after 30 minutes, the absorbance was read at 517 nm using a

spectrophotometer. Ascorbic acid was used as reference. The inhibition curve was plotted and IC₅₀ values obtained by Probit analysis [11].

Determination of total antioxidant capacity

Phosphomolybdenum (PMo) assay according to Prieto et al. [12] was used to estimate the capability of the samples to reduce transition metal ions. The reagent solution contained ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulfuric acid (600 mM) mixed with the samples diluted in ethanol. The samples were incubated at 90° C for 90 min, cooled down to room temperature, and the absorbance of the green phosphomolybdenum complex was measured at 695 nm. The reducing capacity of extracts was calculated using the following equation:

$$ABS_{final} = ABS_{sample} - ABS_{blank} - ABS_{extract}$$

Where: ABS_{extract} = absorbance of sample where molybdate solution was replaced by water; ABS_{blank} = absorbance of blank containing ethanol (400µl) instead of extract sample.

For reference, the appropriate solutions of ascorbic acid have been used, and the reducing capacity of the analyzed extract was expressed as the ascorbic acid equivalent (AAE) per gram of sample dry weight.

Acute toxicity study

Acute oral toxicity of the plant extract was conducted in mice according to the Organisation for Economic Co-operation and Development (OECD) guidelines. Different doses of plant extracts up to 2000 mg/kg, p.o. was administered and animals were observed for the first 2 hours of administration and mortality recorded within 48 hours.

Statistical Analysis

All the values in the test are presented as mean ± SEM (Standard Error of the Mean). The data were statistically analyzed using analysis of variance (ANOVA) and post-hoc Dunnett’s tests with the SPSS program (SPSS 16.0, USA). Differences between the means of the various groups were considered significant at p<0.05, 0.001.

Table 1: Result of chemical group test of the ethanolic extracts of *S. dulcis*

Extract	Steroid	Alkaloid	Tannin	Carbohydrate	Gum	Glycoside	Flavonoid	Saponin
EESD	-	++	++	++	-	++	+	-

EESD: Ethanolic extracts of *Scoparia dulcis*; (+): Present; (-): Absent

Table-2: Effect of ethanolic extract of *S. dulcis* on Glucose tolerance test in mice.

Group	Initial (mmol/L)	1 h (mmol/L)	2 h (mmol/L)	3 h (mmol/L)
Normal Control	6.84 ± 0.13	4.44 ± 0.36	3.10 ± 0.21	2.38 ± 0.11
Diabetic-Extract (100 mg/kg)	8.46 ± 0.35**	6.920 ± 0.72*	5.38 ± 0.71	4.30 ± 0.42
Diabetic-Extract (200 mg/kg)	8.08 ± 0.14*	5.56 ± 0.28	5.46 ± 0.43*	4.77 ± 0.58**

Values are mean ±SEM, (n = 6); *: p<0.05, **: p<0.001 Dunnet test as compared to control.

Table- 3: The effect of 2 weeks treatment of ethanolic extract of *S. dulcis* on blood sugar of alloxan induced diabetic mice.

Group	1 st day (mmol/L)	7 th day (mmol/L)	14 th day (mmol/L)
Group-1	4.94±0.35	5.36±0.41	4.69±0.31
Group-2	12.23±0.33	12.22±0.54	12.53±0.47
Group-3	8.17±0.39**	7.22±0.34**	6.17±0.32**
Group-4	9.24±0.46**	8.87±0.38**	8.53±0.47**
Group-5	8.11±0.33**	7.31 ± 0.49**	6.64 ± 0.50**

Values are given as mean ± SEM, (n = 6); *: p<0.05, **: p<0.001, Dunnet test as compared to diabetic control (Group-2).

Table 4: Scavenging of free radical by crude ethanol extract of *S. dulcis* and ascorbic acid in DPPH method.

Sample	DPPH method IC ₅₀ (µg/ml)
EESD	243.82
Ascorbic acid	58.92

Table 5: Total antioxidant activity of *S. dulcis* against Ascorbic Acid. Values are given for two successive experiments and expressed as Mean ± Standard deviation.

Extract Conc. (µg/ml)	Absorbance of Plant Extract	Absorbance of Ascorbic Acid	Number of equivalents Ascorbic Acid (mg/g)
5	0.0295 ± 0.006	0.0635 ± 0.002	464.56
10	0.025 ± 0.001	0.0565 ± 0.001	442.47
20	0.0425 ± 0.001	0.1165 ± 0.003	364.80
50	0.1125 ± 0.0007	0.39 ± 0.070	288.46
100	0.218 ± 0.003	0.7625 ± 0.003	285.90
500	0.846 ± 0.032	3.95 ± 0.070	214.17

Table 6: Data on mortality in mice after acute treatment of the plant extract

Dose (mg/kg bodyweight)	No. of mice	Mortality
Control	6	0
100	6	0
500	6	0
1000	6	0
1500	6	0
2000	6	0

Results

Phytochemical Screening

The results of phytochemical screening are given in Table 1. Phytochemical analyses of the plant extracts revealed the presence of alkaloids, carbohydrates, glycoside, flavonoid & tannins.

Hypoglycemic Investigation

The blood Glucose level is measured in case of both normal and experimental mice and the result indicated that the antidiabetic effect of EESD was promising which are given in Table 2 and Table 3 (long term study). The results in Table 2 showed that after a single dose load of 200 mg/kg glucose in mice, there was a significant drop of fasting blood glucose level ($p < 0.05$) during the 3 hr. study period, where as in case of chronic administration (Table 3), highly significant variation ($p < 0.001$) was observed between investigational and diabetic control mice in lowering fasting blood glucose level. The extract significantly lowered blood glucose level at both doses (100 mg/kg & 200 mg/kg body weight) and showed maximum reduction; at 100 mg/kg it was found 31.87% and 200 mg/kg it was 46.97% on 14th day, whereas reduction of 50.74% was found for metformin on day 14 as a peak.

The short-term and long-term experiment was done to find out the effect of EESD on blood glucose level in alloxan induced diabetic mice. Experimental data (Table 2 and Table 3) showed that the plant extract was found to be highly effective in blood sugar lowering activity in alloxan-induced hyperglycemic mice.

Antioxidant Activity

DPPH radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method and Total antioxidant capacity method were used to determine the antioxidant property. The DPPH radical scavenging activity of *Scoparia dulcis* was compared with ascorbic acid at different concentrations of 5, 10, 50, 100 and 500 $\mu\text{g/ml}$ and from the graph the IC_{50} values of EESD (243.82 $\mu\text{g/ml}$) and Ascorbic Acid (58.92 $\mu\text{g/ml}$) were calculated and the results are given in Table 4.

Total antioxidant activity

Although the antioxidant activity of EESD in the DPPH method was not worth mentioning but in the total antioxidant method the crude extract showed modest antioxidant activity when compared to standards as Ascorbic acid. The results are shown in the Table 5.

Acute toxicity

The treated mice were found normal and no toxic reaction or mortality caused by the doses of EESD was observed during this investigation.

Discussion

In the present study alloxan was chosen to create diabetic condition in mice because alloxan is a specific toxin that destroys the pancreatic β cells, provoking a state of primary deficiency of insulin without affecting other islet types [13, 14]. In other words, Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of Langerhans and thus induces hyperglycaemia [15]. As there is a growing trend towards using natural remedies adjunct to conventional therapy, traditionally used plants might provide a useful source of new hypoglycemic compounds [16]. Still now many authors have been reported about the antidiabetic potential of *S. dulcis* [15, 17, 20]. A number of plants were found to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through the increased insulin secretion from β cells of islets of Langerhans or its release from bound insulin. That is to say such hypoglycemic effects of plant extracts could also be due to their insulin like actions [18, 19]. It is assumed that EESD exerts its hypoglycemic activity in the same manner. So the possible mechanism of action of EESD could be correlated with the reminiscent effect of the hypoglycaemic sulphonylureas, which promote insulin-secreting channels, membrane depolarisation, and stimulation of Ca^{2+} influx, an initial key step in insulin secretion [20]. The plant extract may also act by stimulating glucose utilization by peripheral tissues since alloxan treatment causes permanent destruction of β cells [15].

Preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids & tannis in the plant extracts. On the basis of the above results, we speculate that EESD may also have brought about anihyperglycemic action through stimulation of β -cells of islets of langerhans to release more insulin and this effect may be due to its constituents like saponins, flavonoids and glycosides [21]. Besides flavonoid and terpenes isolated from the other antidiabetic medicinal plants has been found to stimulate secretion or possess an insulin like-effect [22]. Moreover Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [23, 24]. Many plants contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids, tannins and thus can be utilized to scavenge the excess free

radicals from the human body [25]. So it is assumed that flavonoids and phenolic compounds might be responsible for the observed antioxidant activity.

The involvement of free radicals in diabetes and the antioxidant defense system have been studied. The oxidative stress and resultant tissue damage are important component in the pathogenesis of diabetic complications [26]. The augmented oxygen free radicals in diabetes could be due to increase in blood glucose levels, which upon auto oxidation generate free radicals [15]. Administration of the plant extract increased the activity of enzymes and may help to control free radical, as *Scoparia dulcis* has been reported to be rich in flavonoids and diterpenoids, well-known antioxidants [27,28,29], which scavenge the free radicals produced during diabetes. Any compound, natural or synthetic, with antioxidant

properties, might contribute towards the partial or total alleviation of this damage [20]. Though the crude extract of the investigated plant exhibited potent antidiabetic and moderate antioxidant activities but we still don't know which of the components have the above properties and some principles may have toxic effects. As no mortality was observed during 48 hours acute toxicity study period, LD₅₀ was not calculated. The results found in this investigation showed that EESD possess significant blood glucose lowering effect in alloxan-induced hyperglycemic mice and fair antioxidant activity by free radical scavenging system and total antioxidant activity test. So the non-toxic effects of EESD (Table 6) provide support to the widespread use of the plant. However, further studies are required before it can be recommended for use as a nutritional supplement, health food and adjuvant in the management of diabetes and patho-oxidative disorders.

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