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Evaluation of comparative antidiabetic effects of ethanolic extracts of *Caesalpinia bouncucella* and *Stevia rebaudiana* in normal and alloxan-induced experimental rats

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Abstract

In the present study, ethanolic extracts of Caesalpinia bonducella seed (ESECB) and Stevia rebaudiana leaf (ELESR) were tested separately to compare their antidiabetic effects in normal and alloxan-induced diabetic rats. To assess the antidiabetic activity, varied concentrations of the ethanolic extracts of both plants were tested in oral glucose tolerance test to determine the blood glucose levels in normal and alloxan-induced (150 mg/kg b.w) diabetic experimental rats for 21 days. The oral administration of ethanolic extracts of both plants at the dosage level of 300 and 400 mg/kg body weight showed significant reduction in blood glucose levels. Comparatively, C. bonducella seed extract caused a more potent antidiabetic effect than S. rebaudiana leaf extract. Significant ($p<0.05$) reduction in fasting blood glucose levels was observed in normal rats as well as in diabetic animals. Changes in body weight assessed in extracts treated diabetic rats were comparable with diabetic control and normal animals. Statistical comparison among all groups was performed by using ANOVA. Significant results were observed in the estimated parameters, thereby justifying the use of these plants in the indigenous system of phytomedicine.

Keywords: Antidiabetic activity, Ethanolic seed and leaf extracts, *Caesalpinia bonducella*, *Stevia rebaudiana*

Introduction

Diabetes mellitus is generally acknowledged to be due to insulin deficiency (Type I) which results from pathologic changes in pancreatic β-cells, or due to insulin insensitivity (Type II). Elevation of glucose blood level and a greatly increased risk of reproductive malfunction are common symptoms in diabetic patients [1]. Diabetes is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action.

As the number of people with diabetes multiplies worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. According to World Health Organization, the diabetic population is likely to increase to 300 million or more by the year 2025 [2]. It is projected to become one of the world's main disablers and killers within few decades. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rate. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, however the therapy with oral hypoglycemic agents is not satisfactory. Several drugs such as biguanides and sulfonylureas are presently used to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and management of diabetes without any side effect is still a

challenge to the medical community, thus searching for a new class of compounds is essential to overcome diabetic problems [3].

The search for a new therapeutical agent devoid of adverse effect originating from plants used in traditional medicine would be of interest. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes [3]. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines/ present day drugs for many rural populations in developing countries. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants. Besides, studies have confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus [4].

Caesalpinia bonducella Roxb. (Caesalpiniaceae) is a large ascendant prickly shrub found throughout the interior parts of India and Sri Lanka. It is common in southern parts of India and is often grown as a hedge plant. *Caesalpinia bonducella* is reported to have multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial [5], anti-anaphylactic and antidiarrheal [6], antiviral [7], antiasthmatic [8], anti-amoebic and anti-estrogenic [9]. This plant has profound medicinal use and is a proved anti-inflammatory [10], anthelminitic and antimalarial drug [11]. Traditionally, the tribes of Andaman and Nicober Island used the aqueous decoction of the seeds of this plant in order to eliminate the symptoms of diabetes mellitus [12].

Stevia rebaudiana Bertoni, belonging to the family Compositae, is a sweet herb native to South America. The plant has also been cultivated in China and Southeast Asia. *Stevia* sweeteners, crude extract from leaves, have been used from decades to sweeten soft drinks, soju, soy sauce, yogurt, and other foods in Japan, Korea and Brazil [13]. The dry extract from the leaves of *S. raubudiana* also contains flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycynnamic acids, neutral water-soluble oligosaccharides, free sugars, amino acids, lipids and essential oils [14]. Stevia sweetener extractives have been suggested to exert beneficial effects on human health, including antihypertensive [15], antihyperglycemic [16] and anti-human rotavirus [17] activities.

So far there has been no comparative study reported on the antidiabetic activities of the ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana*, respectively. Therefore, the present study was undertaken to explore the comparative studies on antidiabetic activities of *C. bonducella* and *S. rebaudiana* in relation to their folklore medicinal properties.

Materials and Methods

Collection and identification of plant materials

The seeds of *C. bonducella* and *S. rebaudiana* leaves were collected in March 2006 from Sagar District, Madhya Pradesh, India. Further taxonomic identification was conducted at the Department of Botany, Dr. H. S. Gour University, Sagar, MP, India. The voucher specimens of *C. bonducella* seed and *S. rebaudiana* leaf were deposited in the herbarium at the Laboratory of Department of Botany under the voucher specimen numbers (Bot/H/2692) and (Bot/H/3352), respectively.

Preparation of the ethanolic extracts

The air-dried seeds and leaves of *C. bonducella* and *S. rebaudiana* (50 g each), respectively, were finely powdered and extracted separately with 500 ml of ethanol by using soxhlet apparatus. Each crude extract was filtered, and evaporated under reduced pressure. Extracts obtained were preserved in sterile glass containers separately at 4°C until used for pharmacological assays. A known amount (200 to 400 mg/kg b.wt.) of each residual extract was suspended in 0.9% w/v normal saline and orally administered to the animals by force feeding needle during the experimental period.

Drugs

Alloxan was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and glibenclamide as a standard drug was provided as a gift sample from Khandelwal Laboratories, Mumbai, India. All drugs were dissolved in 0.9% saline water for oral administration.

Experimental animals

Animal use protocol was approved by the Dr. Hari Singh Gour University, Sagar, MP, India (Animal Eths Comm/IE/98/Reg No 379/01/ab/CPCSEA) and was in accordance with international standard on the care and use of experimental animals [18]. Swiss albino rats of either sex weighing between 100 to 125 g were used for the study. Animals were housed under standard conditions of temperature (25°C), 12 h/12 h light/dark cycles and fed with standard pellet diet and tap water.

Toxicity study

To assess the toxicity, healthy Swiss albino rats of either sex, starved overnight, divided in different groups (n = 6) and were orally fed with ethanolic seed and leaf extracts of *C. bonducella* and *Stevia rebaudiana*, respectively in increasing dose levels ranging from 100 to 500 mg/kg body weights. The rats were observed continuously for 2 h for behavioral profile and after 24 and 72 h for any lethality [19].

Collection of blood samples

Blood samples were collected from tail tip and blood glucose levels were estimated using an electronic glucometer (Accu-chek, Roche Diagnostics, USA).

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals [20]. Rats were divided into following 8 groups; group I, II, III, IV, V, VI, VII and VIII. Each group consisted of 6 animals. Rats from Group I were served as control (administered only normal saline). Rats form groups II to IV were treated with different concentrations of ethanolic seed extract of *C. bonducella* (200 - 400 mg/kg, orally), whereas rats from group V to VII were treated with different concentrations of ethanolic leaf extract of *S. rebaudiana* (200 - 400 mg/kg, orally). Rats from group VIII were treated with glibenclamide (0.5 mg/kg b.w per day p.o). The animals from all the groups were loaded with 60% glucose (3 g/kg p.o.) 30 min after extract administration. Blood was withdrawn from the tail prior to drug administration at 0, 30, 60, 90, 120 and 150 min after glucose loading. The blood glucose levels were estimated by using an electronic glucometer (Accu-chek, Roche Diagnostics, USA).

Effect on blood glucose levels in normal rats (single and multi dose study)

To determine the effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana*, respectively, rats were divided into 8 groups of 6 rats per group. Animals from group I were given normal saline water through oral rout, which served as control. Animals from groups II to IV were given ethanolic seed extract of *C. bonducella* at varied concentrations (200 - 400 mg/kg, b.w per orally), whereas the animals from groups V to VII were administered with different concentrations of ethanolic leaf extract of *S. rebaudiana*

(200 - 400 mg/kg, b.w orally). The animals from group VIII were administered with glibenclamide (0.5 mg/kg b.w per day p.o). In a single dose study, after administration of a single dose of each extract, blood samples were collected from the tail vein just prior to 1, 2, 3, 4 and 6 h intervals. The blood glucose levels were estimated by using an electronic glucometer (Accu-chek, Roche Diagnostics, USA).

For multi dose study, the same groups of normal animals were continued with the same dose level once daily, up to 21 days. The glucose levels of all the animals were measured on 3, 7, 9, 14 and 21 day.

Effect on blood glucose levels and body weights in alloxan-induced diabetic rats (single and multi dose study)

To determine the effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana*, respectively on blood glucose levels, diabetes was induced in Swiss albino rats by single intraperitoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg b.w) in physiological saline after overnight fasting for 12 h [21]. Rats treated with single intraperitoneal injection of alloxan monohydrate (150 mg/kg b.w), after overnight fast for 12 h were assessed by measuring the blood glucose concentration 3 days after alloxan treatment. The rats with blood glucose level above 250 mg/dl were considered diabetic and were used for experimental study.

In this assay, the diabetic rats were divided into 8 groups of 6 rats per group. Control diabetic rats from group I were given only distilled water. Rats from groups II to IV were given ethanolic seed extract of *C. bonducella* (200 to 400 mg/kg, b.w, p.o, orally), whereas the rats from groups V to VII and VIII were treated with different concentrations of ethanolic leaf extract of *S. rebaudiana* (200 - 400 mg/kg, b.w, orally) and standard glibenclamide (0.5 mg/kg b.w per day p.o), respectively. In a single dose study, after administration of a single dose, blood samples were collected from the tail vein just prior to 1, 2, 3, 4 and 6 h intervals and blood glucose levels were estimated. For multi dose study, the same groups of diabetic animals were continued with the same dose level once daily, up to 21 days. The blood glucose levels of all the animals were measured on 3, 7, 9, 14 and 21 day, and changes in body weights were assessed in the diabetic animals treated with extracts and compared with diabetic control and normal animals

Data and statistical analysis

Data were expressed as mean ± standard deviation of mean (SD). Statistical comparison among all groups was performed by using ANOVA.

Results

Toxicity

Acute toxicity study revealed non-toxic nature of the ethanolic extracts of *C. bounducella* seeds and *S. rebaudiana* leaves in the tested experimental animals. There were no lethality or toxic reactions found at any of the doses selected until the end of study.

Oral glucose tolerance test

The ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* exhibited remarkable blood glucose lowering effect in the glucose tolerance test, respectively (Table 1). These results indicate that the ethanolic seed extract of *C. bonducella* has profound capacity to block the glucose absorption than *S. rebaudiana* ethanolic leaf extract. The ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* (200 to 400 mg/kg) showed a significant reduction in blood glucose levels from 60 min onwards in oral glucose tolerance test (Table 1). The ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* at various concentrations (200, 300, 400 mg/kg) showed antidiabetic activities on blood glucose levels (78.8 to 71.4 and 88.2 to 81.4 mg/dl) in dose dependent manner, respectively.

Table 1. Effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* on oral glucose tolerance test.

Group	Treatment	Blood glucose concentration (mg/dl)					
		0 min	30 min	60 min	90 min	120 min	150 min
I	Control (10 ml/kg vehicle control)	90.2 ± 5.6	122.9 ± 2.3	100.8 ± 5.2	98.7 ± 1.6	95.9 ± 2.1	92.3 ± 3.5
II	ESECB (200 mg/kg)	86.4 ± 4.2	110.9 ± 1.3	98.5 ± 6.8	95.8 ± 2.5	88.4 ± 5.2	78.8 ± 2.6
III	ESECB (300 mg/kg)	87.9 ± 3.5	109.4 ± 4.6	95.4 ± 5.3*	88.5 ± 2.2*	81.0 ± 4.1*	76.5 ± 2.2*
IV	ESECB (400 mg/kg)	89.3 ± 2.6	106.8 ± 4.0	92.4 ± 4.6*	86.4 ± 4.6*	79.3 ± 3.8*	71.4 ± 3.8*
V	ELESR (200 mg/kg)	88.5 ± 2.1	114.6 ± 2.7	100.6 ± 5.5	95.2 ± 2.8	90.3 ± 3.0	88.2 ± 4.2
VI	ELESR (300 mg/kg	86.6 ± 3.8	110.2 ± 3.3	99.8 ± 3.5*	91.4 ± 3.9*	88.6 ± 4.1*	85.5 ± 2.1*
VII	ELESR (400 mg/kg	87.4 ± 6.1	109.4 ± 2.6	98.2 ± 6.1*	88.8 ± 3.3*	82.1 ± 1.9*	81.4 ± 1.6*
VIII	Glibenclamide (0.5 mg/kg b.w per day p.o)	85.2 ± 5.2	94.5 ± 4.2	80.5 ± 5.6	77.3 ± 2.4	74.1 ± 2.4	69.2 ± 4.2

ESECB: Ethnaolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*.

Each value represents mean ± S.D., n = 6, $p<0.05$ significant; * Represents statistical significance vs control.

Effect on blood glucose levels in normal rats

Single dose study

In normal rat animals, significant reduction in the blood glucose levels was observed as compared to the control group (Table 2). Administration of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* was found to reduce blood glucose levels in normal rats in a single dose study at the used concentrations. As shown in Table 2, in a single dose study, the maximum reduction in blood glucose levels was noted to be 52.0 and 43.0% at 3 h by the administration of ethanolic seed and leaf extracts (400 mg/kg) of *C. bonducella* and *S. rebaudiana*, respectively as compared to glibenclamide (62.0%).

Table 2. Effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* on blood glucose levels in fasted normal rats (single dose).

Group	Treatment	Blood glucose concentration (mg/dl)					
		Fasting	1 h	2 h	3 h	4 h	6 h
I	Control (10 ml/kg vehicle control)	74.2 ± 3.3	75.6 ± 2.1	74.8 ± 1.4	73.9 ± 3.2	75.0 ± 2.6	74.4 ± 2.3
II	ESECB (200 mg/kg)	76.8 ± 3.1	75.6 ± 5.6	74.7 ± 2.5	60.6 ± 2.4	72.8 ± 3.3	69.7 ± 2.0
III	ESECB (300 mg/kg)	72.8 ± 2.5	72.2 ± 2.6	70.6 ± 2.6*	53.5 ± 5.1*	70.4 ± 2.6	66.5 ± 1.6
IV	ESECB (400 mg/kg)	79.4 ± 2.9	72.0 ± 4.1	71.3 ± 3.1*	38.1 ± 1.9*	69.6 ± 2.9	60.4 ± 2.2
V	ELESR (200 mg/kg)	80.6 ± 3.4	79.2 ± 2.3	77.4 ± 4.5	65.1 ± 2.1	78.6 ± 3.1	77.3 ± 3.4
VI	ELESR (300 mg/kg	78.4 ± 2.9	77.2 ± 2.2	76.7 ± 2.8*	57.9 ± 4.6*	76.9 ± 3.0	76.8 ± 2.9
VII	ELESR (400 mg/kg	76.9 ± 2.0	75.8 ± 1.5	75.4 ± 2.4*	43.8 ± 2.2*	74.4 ± 2.4	74.1 ± 4.2
VIII	Glibenclamide (0.5 mg/kg b.w per day p.o)	79.8 ± 2.4	69.4 ± 2.4	66.9 ± 2.0	30.3 ± 3.1	62.2 ± 1.8	59.8 ± 1.8

ESECB: Ethnaolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*.

Each value represents mean ± S.D., n = 6, $p<0.05$ significant; * Represents statistical significance vs control.

Multi dose study

In multi dose study from day 7 to 11, both seed and leaf ethanolic extracts significantly ($p<0.05$) reduced the blood glucose levels at the used concentrations. Ethanolic seed extract of *C. bonducella* showed more potent results than *S. rebaudiana* leaf extract. However, in case of ethanolic seed and leaf extracts, 400 mg/kg dose was found more effective to reduce the blood glucose levels in a multi dose study as compared to low range of doses (Table 3).

Table 3. Effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* on blood glucose levels in fasted normal rats (multy dose).

Group	Treatment	Blood glucose concentration (mg/dl)				
		3rd day	7th day	9th day	14th day	21st day
I	Control (10 ml/kg vehicle control)	76.6 ± 4.2	76.4 ± 2.5	74.8 ± 6.8	73.9 ± 3.5	74.8 ± 5.4
II	ESECB (200 mg/kg)	80.5 ± 4.4	79.0 ± 3.9	78.6 ± 5.2	77.7 ± 5.2	76.2 ± 2.6
III	ESECB (300 mg/kg)	83.5 ± 2.2	79.5 ± 2.6*	76.4 ± 5.4	75.1 ± 2.6	72.1 ± 3.8*
IV	ESECB (400 mg/kg)	88.6 ± 3.2	76.8 ± 2.2*	73.4 ± 3.8	72.6 ± 2.4	71.8 ± 2.2*
V	ELESR (200 mg/kg)	82.6 ± 3.5	82.0 ± 2.2	81.4 ± 4.6	80.7 ± 3.1	79.2 ± 4.1
VI	ELESR (300 mg/kg	83.1 ± 1.9	82.8 ± 1.0*	81.3 ± 2.2	80.4 ± 4.5	78.6 ± 4.0*
VII	ELESR (400 mg/kg	79.4 ± 2.2	78.1 ± 3.4*	77.6 ± 2.9	76.0 ± 3.7	76.4 ± 3.9*
VIII	Glibenclamide (0.5 mg/kg b.w per day p.o)	81.8 ± 2.9	73.1 ± 3.8	72.0 ± 3.7	70.8 ± 2.4	69.9 ± 3.0

ESECB: Ethanolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*.

Each value represents mean ± S.D., n = 6, $p<0.05$ significant; * Represents statistical significance vs control.

Effect on blood glucose levels in alloxan-induced diabetic rats

Single dose study

The effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* in alloxan-induced diabetic rats is shown in Table 4 and Table 5, respectively. The fasting blood glucose levels in alloxan-induced diabetic rats were ranged from 269 to 350 mg/dl. During the single dose study, the results were found to reduce blood glucose levels in concentration dependent manner. Reducing effect was found maximum within 3 - 4 h. After 6 h, blood glucose concentration was increased in all groups of animals. Administration of the ethanolic seed and leaf extracts (400 mg/kg) of *C. bonducella* and *S. rebaudiana* significantly lowered the blood glucose levels in the rats after 4 h by 13.8 and 11.6%, respectively. However, glibenclamide reduced the blood glucose level by 20.2% in a single dose study.

Table 4. Effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* on blood glucose levels in fasted diabetic rats (single dose).

		Blood glucose concentration (mg/dl)					
Group	Treatment	Fasting	1 h	2 h	3 h	4 h	6 h
I	Diabetic control (10 ml/kg vehicle control)	299.9 ± 2.9	290.6 ± 2.5	295.4 ± 5.6	294.6 ± 4.8	295.3 ± 2.9	299.8 ± 4.0
II	ESECB (200 mg/kg)	290.8 ± 3.6	289.4 ± 4.3	288.3 ± 4.3	280.4 ± 4.1	279.6 ± 6.1	284.3 ± 2.6
III	ESECB (300 mg/kg)	269.1 ± 3.4	260.6 ± 2.6	260.1 ± 2.9	250.4 ± 6.2*	248.5 ± 5.1*	256.4 ± 3.8
IV	ESECB (400 mg/kg)	285.8 ± 2.8	278.1 ± 2.7	262.6 ± 3.5	251.1± 2.1*	246.1 ± 2.4*	250.6 ± 3.3
V	ELESR (200 mg/kg)	298.9 ± 2.2	298.4 ± 3.6	295.4 ± 4.1	291.5 ± 3.7	290.1 ± 4.5	290.6 ± 2.2
VI	ELESR (300 mg/kg	290.8 ± 3.4	285.4 ± 2.9	285.2 ± 3.0	280.4 ± 2.8*	277.6 ± 3.3*	280.1 ± 3.1
VII	ELESR (400 mg/kg	288.9 ± 2.7	277.4 ± 3.4	265.4 ± 2.8	258.8 ± 3.9*	255.1 ± 2.7*	254.5 ± 4.5
VIII	Glibenclamide (0.5 mg/kg b.w per day p.o)	350.2 ± 2.0	300.5 ± 5.1	292.1 ± 2.0	280.3 ± 4.5	283.3 ± 3.4	280.6 ± 2.2

ESECB: Ethanolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*.

Each value represents mean ± S.D., n = 6, $p<0.05$ significant; * Represents statistical significance vs control.

Multi dose study

Similar results could be seen upon continuous administration of *C. bonducella* and *S. rebaudiana* mediated ethanolic seed and leaf extracts. The blood glucose levels of only alloxan-treated group were significantly increased from 76.4 to 302 mg/dl on day 7 after the alloxan injection. In extracts treated groups, blood glucose levels were significantly decreased from day 9 to day 21. All the extract treated groups showed significant reduction effect ($p<0.05$) on blood glucose levels when compared with alloxan-treated control group. As shown in Table 5, the highest dose of ethanolic seed extract (400 mg/kg) of *C. bounducella* was found more effective to reduce the blood glucose levels than ethanolic leaf extract (400 mg/kg) of *S. rebaudiana*. Blood glucose reduction capacity of *C. bounducella* mediated ethanolic seed extract was well matched with standard glibenclamide drug. Comparison by ANOVA test on day 21 revealed that *C. bounducella* ethanolic seed extract (400 mg/kg) showed highly significant action. However, among the tested groups, both seed and leaf extracts showed antidiabetic activity as they were homogeneously significant (Table 5).

Table 5. Effect of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* on blood glucose levels in fasted diabetic rats (multi dose).

Group	Treatment	Blood glucose concentration (mg/dl)				
		3rd day	7th day	9th day	14th day	21st day
I	Control (10 ml/kg vehicle control)	298.4 ± 3.1	302.6 ± 2.9	309.8 ± 2.8	312.2 ± 4.6	321.8 ± 2.4
II	ESECB (200 mg/kg)	274.5 ± 5.2	240.3 ± 3.6	218.9 ± 3.6	200.3 ± 5.1	189.2 ± 3.8
III	ESECB (300 mg/kg)	244.6 ± 6.1	220.1 ± 3.3*	219.6 ± 2.4*	198.5 ± 2.6*	160.8 ± 2.7*
IV	ESECB (400 mg/kg)	242.8 ± 4.3	222.1 ± 4.1*	211.6 ± 2.9*	193.4 ± 3.1*	152.8 ± 1.9*
V	ELESR (200 mg/kg)	272.1 ± 3.3	271.4 ± 4.0	268.5 ± 2.2	251.4 ± 2.4	222.8 ± 4.8
VI	ELESR (300 mg/kg	271.2 ± 4.6	268.5 ± 3.5*	242.4 ± 3.6*	221.8 ± 3.7*	197.8 ± 4.1*
VII	ELESR (400 mg/kg	240.3 ± 1.9	232.6 ± 2.9*	229.5 ± 3.1*	218.4 ± 2.8*	189.9 ± 2.8*
VIII	Glibenclamide (0.5 mg/kg b.w per day p.o)	279.6 ± 2.5	251.4 ± 2.0	232.1 ± 3.3	198.5 ± 1.9	160.2 ± 3.0

ESECB: Ethanolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*.

Each value represents mean ± S.D., n = 6, $p<0.05$ significant; * Represents statistical significance vs control.

Effect on body weights

Diabetes is characterized by weight loss and it was also seen in this study. Changes in body weight of each animal in different groups were noted on day 1, 7, 14 and 21. Alloxan caused body weight reduction, which was reversed by ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* after 7 days of treatment. Significant (p<0.05) differences were observed for the changes in body weights of extract treated diabetic animals when compared with the diabetic control animals (Fig. 1). Vehicle control animals were found to be stable in their body weights, however, diabetic rats showed significant reduction in body weights during 21 days (Fig. 1). Changes of body weights in control group were remarkable. A significant increase in body weights ranging from 162.4 to 190.4 and 154.4 to 196.4 g, among the tested groups was observed on day 21 with ethanolic seed and leaf extracts (200 to 400 mg/kg) of *C. bonducella* and *S. rebaudiana*, respectively. Body weights of the animals in standard drug glibenclamide treated group were also increased (196.4 g) significantly on day 21 (Fig. 1).

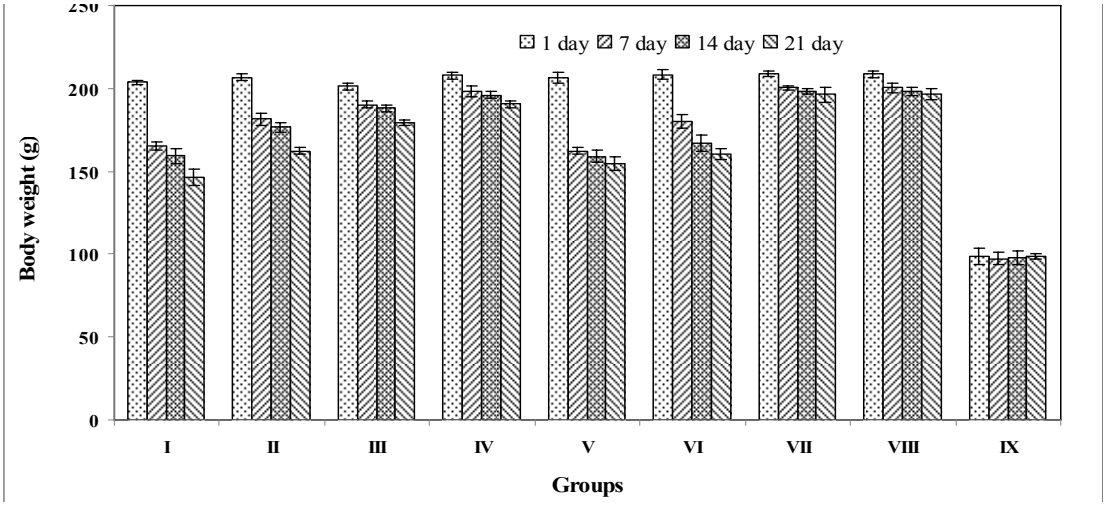

Fig. 1. Effect of three weeks treatment with various concentrations of ethanolic seed (ESECB) and leaf (ELESR) extracts of *C. bonducella* and *S. rebaudiana* on body weight (g) after alloxan-induced (160 mg/kg, i.p.) diabetes in rats.

Group I: Diabetic control (no treatment); Group II: Diabetic rats treated with ESECB (200 mg/kg); Group III: Diabetic rats treated with ESECB (300 mg/kg); Group IV Diabetic rats treated with ESECB (400 mg/kg); Group V: Diabetic rats treated with ELESR (200 mg/kg); Group VI: Diabetic rats treated with ELESR (300 mg/kg); Group VII: Diabetic rats treated with ELESR (400 mg/kg); Group VIII: Glibenclamide (0.5 mg/kg b.w per day p.o) treated rats; Group IX: Normal control (no treatment).

ESECB: Ethanolic seed extract of *C. bonducella*; ELESR: Ethanolic leaf extract of *S. rebaudiana*. Values are mean ± S.D., n = 6.

Discussion

Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, neuropathy and nephropathy. These complications have long been assumed to be related to chronically elevated glucose levels and subsequent oxidative stress. Previous studies in our laboratory showed that the ethanolic seed and leaf extracts of *C. bounducella* and *S. rebaudiana*, respectively, were able to exert potent *in vivo* antioxidant activities [22], [23]. Some other researchers also evaluated some plants for their antidiabetic activities and their relation with antioxidant activity [24]. In the present study, higher doses (300 and 400 mg/kg) of ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* could produce a significant fall in blood glucose levels in diabetic rats, after 2 h of

treatment without any significant changes in body weights. Similar findings were observed by other researchers [25]. Hence it may be considered that ethanolic seed and leaf extracts form *C. bonducella* and *S. rebaudiana* contained good antidiabetic principles without any adverse effect, unlike insulin and other synthetic drugs. Previous phytochemical screening of ethanolic extracts of both plants has shown the presence of various phytochemical constituents, mainly phenolics, alkaloids, saponins and flavonoids which may be responsible for their antidiabetic properties. Thus, antidiabetic plant extracts may involve one or more compounds to decrease the blood glucose levels suggesting that the natural constituents could act separately or synergistically to induce hypoglycemic effect as described by others [26].

Different mechanisms of action to reduce blood glucose levels utilizing plant extracts have been well established. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide; they reduce blood glucose levels in normoglycaemic animals [27]. Some other plants act like biguanides such as metformin which is an antihyperglycaemic compound; they do not affect blood glucose levels in normal state [28].

Glycogen metabolism in the liver regulates the blood glucose levels. The control of the synthesis and breakdown of glycogen in the liver is central to the regulation of blood glucose levels. Previously, liver glycogen levels were estimated to check whether test drug could increase liver glycogen content, thereby exerting its antidiabetic action. In diabetic models, the ethanolic extracts of *C. bonducella* and *S. rebaudiana* increased liver glycogen levels significantly [29]. In the present study, the decrease in the blood glucose levels of the diabetic rats treated with ethanolic seed extract of *C. bounducella* (400 mg/kg b.w.) was significantly higher ($p<0.05$) than the other groups (Table 1). These results were similar to the reports of some medicinal plants that have been proven effective in lowering blood glucose levels [30], [31].

Alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release [32]. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose levels in alloxan-induced diabetic animals [33]. In the present study, the ethanolic seed and leaf extracts of *C. bounducella* and *S. rebaudiana* also effectively decreased the blood glucose levels in alloxan-induced diabetic rats similar to the standard drug glibenclamide. Kim et al. (2003) reported the antidiabetic activity of *M. alba* proves to aid for the recovery from the central nervous system complications of diabetes mellitus and in controlling the desire for food under diabetic conditions [34]. However, the mechanism of these plants used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent alloxan [35]. In our previous study, both extracts showed strong free radical scavenging and antioxidant activities and also provoked a protective effect on DNA damage caused by hydroxyl radicals [22], [23]. These plant materials produced significant inhibition of alloxan induced diabetes in experimental rats, where *C. bounducella*, as an active antioxidant [22], was found to be the most active antidiabetic agent to reduce blood glucose levels, followed by *S. rebaudiana*. However, the mechanism of action of these drugs in reducing the diabetes is not known. Infact, it has been found that traditional medicines used in human diabetes also have a significant antioxidant activity. Therefore, it may be possible that these extracts may reduce the effect of inflammatory cytokine release during diabetes which may be one of the causative agents for the tissue distraction and insulin resistance [36].

Conclusion

In this study, the ethanolic seed and leaf extracts of *C. bonducella* and *S. rebaudiana* possessed potential antidiabetic activities in normoglycaemic and alloxan-induced diabetic rats. *C. bouncucella* was found to be a more potent antidiabetic agent than *S. rebaudiana* to reduce the blood glucose levels in diabetic rats. In conclusion, these results seem to confirm the alleged antidiabetic activity by the traditional phytomedicine without any toxic effect. Further studies will be focused on herbal formulations of *C. bonducella* and *S. rebaudiana* extracts to determine their mechanism(s) of action.

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