Original Research Article

Antidiabetic activity of *Cassia sophera* in STZ induced diabetic rats and its effect on insulin secretion from isolated pancreatic islets

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A b s t r a c t

The aim of the study was to evaluate the antidiabetic effect of *Cassia sophera* seed extracts in streptozotocin (STZ) induced diabetic Wistar rats. Aqueous extract of *Cassia sophera* (AECS) showed maximum fall (60.88%, p<0.01) in 0 to 1 h fasting blood glucose during glucose tolerance test in normal healthy rats. Since AECS exhibited potential hypoglycemic activity, it was further studied for antidiabetic effect in STZ induced diabetic rats. The oral effective dose (ED$_{50}$) of AECS was 200 mg/kg body weight which produced a fall of (70.50%, p<0.01) in diabetic rats. Treatment with AECS significantly reduced the elevated glycosylated haemoglobin (45.67%, p<0.05) and increased insulin level (40.6%, p<0.01). Total cholesterol (19.05%) low density lipoprotein (21.41%), triglyceride (57.87%) levels and very high density lipoprotein (57.85%) were significantly decreased (p<0.05) whereas, high density lipoprotein was significantly increased (38.58%, p>0.05) in diabetic rats. Since AECS treatment resulted in significant increase in serum insulin, its effect on insulin secretion in isolated pancreatic islets was also studied. AECS stimulated insulin secretion from the isolated islets at 3.3 and 16.7mM glucose. The effect of AECS was dose dependent. AECS induced enhancement in insulin secretion was not diminished by nicorandil, suggesting that AECS induced insulin secretion was independent of K-ATP channels of β cells.

Keywords: Cassia sophera, Antidiabetic activity, Pancreatic islets, Nicorandil

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia [1]. Multiple biochemical impairments associated with micro and macrovascular complications are the major causes of morbidity and mortality in DM [2]. The chronic hyperglycemia in DM is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3]. DM is already declared epidemic by WHO and extensive measures are required for its control. A number antidiabetic drugs of synthetic origin are available for management of DM. Loss of efficacy, high cost of treatment and side effects during long treatment regimens associated with current day antidiabetic drugs necessitated the search for novel antidiabetic molecules, especially of herbal origin [4,5]. There are a number of reports of use of medicinal plants in management of diabetes [6,7].

Cassia sophera (CS), commonly known as 'Kasaundi' in India is a medicinally important plant. The seeds of the plant are used in local traditional medicine for the treatment of inflammatory diseases, psoriasis, cough, arthritis and convulsions of children [8]. It is also used as expectorant in asthma, GIT disorder, rheumatic disorders and remedy for various skin aliments [9]. Internally it is used to as febrifuge in rheumatic and inflammatory fever [10]. There are few reports on antidiabetic activity of the genus *Cassia*

[11,12]. CS seeds are used in management of diabetes in Bundelkhand region of central India. Hence the present study was planned to explore the antidiabetic activity of seeds of CS in Wistar rats.

Materials and methods

Plant Material and Extraction

The dried seeds of CS were obtained from the local herbalists and authenticated by experts in Department of Botany, Bundelkhand University, Jhansi. The seed sample was preserved in Department of Biomedical Sciences, Bundelkhand University with accession No. BU/BMS/VS/2010/04. The seeds were pulverized in grinder to obtain coarse powder. Seed powder (100 gm) was extracted separately with hexane, chloroform, ethyl acetate, methanol and water. The respective solvent extracts were filtered and dried in rotary evaporator under vacuum. The aqueous extract was filtered, centrifuged at 5000g for 10 minutes to remove any residual material and then lyophilized. All extracts were stored at -40^{0}C prior to use.

Animals

Male Wistar rats, weighing about 150-200g obtained from Indian Institute of Toxicology Research, Lucknow, India. Animals were kept for acclimatization for 15 days in the animal house of

Bundelkhand University at around ambient temperature of 25^0C and relative humidity of 45-55%, with 12h each of dark and light cycles. The rats were fed pelleted diet and water *ad-libitum*. Animal experimental protocols were in accordance with the recommendations of the institutional animal ethical committee (BU/ Pharma/ IAEC/10/029).

Hypoglycemic Activity of Various Extracts of CS by Glucose Tolerance Test

Healthy male rats were divided in five groups of six rats each with each group having individual control (C) of six rats. Rats in group I-V were treated (T) with hexane, chloroform, ethyl acetate, methanol and aqueous extracts at a dose of 300 mg/kgbw respectively where as control of each group received the vehicle in which the extract was dissolved for administration. Hexane, chloroform, ethyl acetate and methanol extracts were dissolved in DMSO whereas aqueous extract was dissolved in water. Glucose tolerance test (GTT) was performed in overnight fasted rats as per methods standardized in our laboratory [13]. FBG was recorded from tail end followed by oral administration of extracts. After 90 min, blood was again drawn from tail end and FBG was recorded (termed as 0h value), followed by oral administration of glucose solution (2 g/kg bw). Blood was drawn from tail vein at 1,2,3 h after glucose administration and FBG was measured to assess GTT.

Induction of Diabetes

Diabetes was induced by a single i.p. injection of a freshly prepared solution of STZ (75 mg/kgbw) in citrate-buffered saline (pH 4.5, 0.1 M). FBG was measured at the time of induction of diabetes and postprandial glucose was monitored regularly till stable hyperglycaemia was established. Rats with stabilized diabetes having FBG 250 mg/dl were used in the study.

Determination of ED_{50} of Active Extract in Diabetic Rats

Aqueous extract of CS (AECS) showed better hypoglycaemic activity in GTT, as compared to other extracts, hence ED_{50} of AECS was estimated in diabetic rats. Diabetic rats were divided in six groups of six rats each. Group I received distilled water, group II-V received 100, 200, 300 and 400 mg/kg bw of AECS and group VI was given 10 mg/kg bw glibenclamide. GTT was performed and the dose, which produced maximum effect, was taken as ED_{50}.

Treatment of Diabetic Rats with AECS

Diabetic rats were divided in four groups of six rats each. Group I served as normal healthy control, group II served as diabetic control, group III was treated with 10 mg/kg bw of glibenclamide and group IV was treated with 200 mg/kg bw (ED_{50}) of AECS, once daily for 28 days. FBG and body weight were estimated at 0, 7, 14, 21 and 28 days of the experiment. At the end of experiment, rats were sacrificed under mild ether anaesthesia, blood was collected from ventricle and glycosylated haemoglobin (Hb1Ac), serum insulin, total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were estimated. TC, HDL and VLDL were estimated by span diagnostic reagent kit, India. TG were estimated by Ebra diagnostics kit, India. LDL level was calculated indirectly from the measured levels of TG, HDL, TC using the Friedewald equation (1972) [14]. Hb1Ac was estimated by Euro diagnostic system kit, India and insulin was estimated using Rat specific insulin ELISA kit form DRG diagnostics, Germany.

Isolation of pancreatic islets

Pancreas was excised from healthy Wistar rats of either sex and islets were isolated by the method of Lacy and Kostianovsky [15] with minor modifications. Pancreas was chopped in small pieces in HBBS buffer (pH 7.4) containing 3% glucose and 1 % BSA. The chopped pancreas was added to 2ml centrifuge tubes containing HBBS and was centrifuged at 8000g for 15 min. The pancreatic pellet was transferred to a small beaker containing HBBS and collagenase (3mg/ml). The pellet was incubated for 10 minutes at 37ºC with intermittent magnetic stirring. Small aliquot of incubation medium was diluted with ice cold BSA and examined under microscope to ascertain the extent of digestion and appearance of islets. After optimal period of digestion, magnetic stirring was stooped and the material was subjected to gradient centrifugation for 10 minutes at 800g with different gradients (25, 23, 20 and 11%) of ficol. Islets from 20-11% ficol interface were collected and used.

Effect of AECS on Insulin Secretion

To study the effect of AECS on insulin release, batches of 4-5 islets were incubated in HBBS (5% CO_2, pH 7 .4) containing 1% BSA, 3.3 or 16 .7 mM glucose and 1 and 2 mg/ml of AECS. To investigate the mechanism of insulin release, islets were incubated with nicorandil (1mg/ml) in presence or absence of AECS (1mg/ml). After 1 hr, the incubation mixtures were centrifuged at 16000g, the supernatants were collected and used for insulin measurement by enzyme-linked immune-sorbent assay kit (DRG Instruments GmbH, Germany).
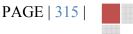
Cell Viability

Cell viability test on isolated islets was performed by trypan blue [16] and lactate dehydrogenase (LDH) release assay [17] with AECS (2mg/ml) and nicorandil (1mg/ml).

Statistical Analysis

Results were expressed as Mean ± SEM for 6 animals in each group. Hypothesis testing method included one-way analysis of various (ANOVA) followed by Dunnette's comparison tests. P-values of less than 0.05 were considered to indicate statistical significance. All the statistics were carried out in GraphPad InStat Software Inc., v. 3.06, San Digeo, USA.

Results

Hypoglycemic Activity of Various Extracts by GTT

Aqueous extract produced a significant (p<0.01) fall of 60.88% in FBG between 0h and 1h during GTT. On the other hand, hexane, chloroform, methanol and ethyl-acetate extracts showed increase in FBG by 117.29%, 259.50%, 214.84% and 296.27% respectively as compared to their individual control (Table 1).

Table 1: Hypoglycemic activity of different solvent extracts of CS by GTT in healthy rats.

Extract treated		FBG	0h	1h	2h	3h	% fall between 0h and 1h
Hexane							
	C	108±1.00	109±2.00	145.66±2.51	138.62±0.68	127.29±1.27	
	T	99.0±0.35	101±4.30	180.66±8.50	169.6±8.50	172.34±2.51	117.29 ↑
Chloroform							
	C	106±1.00	108±3.05	134.00±91.9	127.29±1.61	119.76±1.16	
	T	100.3±2.08	96.6±3.21	189.00±13.1	176.66±10.01	175.67±3.51	259.50 ↑
Ethyl-acetate							
	C	107.0±1.00	109.0±3.0	130.45±3.5	120.33±0.82	113.43±1.78	
	T	105.0±3.46	103±0.78	188.0±19.0	170.6±16.2	174.67±1.52	296.27 ↑
Methanol							
	C	97.67±3.78	96.0±3.60	121.60±4.5	111.74±5.58	104.76±3.97	
	T	98.34±4.04	95.0±4.35	175.60±8.7	172.33±13.3	175.67±2.51	214.84 ↑
Aqueous							
	C	97.00±1.00	101.66±3.5	124.67±3.67	118.85±0.62	105.48±1.51	
	T	101.67±3.05	99.33±3.21	108.33±2.35	96.67±2.08	94.67±2.08	60.88 ↓

The data are expressed as Mean±SD for six rats each.

ED$_{50}$ of AECS in Diabetic Rats

AECS at a dose of 100, 200, 300 and 400 mg/kg produced a fall of 36.69%, 70.50%, 60.23% and 48.68% respectively in FBG during GTT in diabetic rats, indicating 200mg/kgbw as ED$_{50}$ (Table 2).

Table 2: Determination of effective dose of AECS by GTT in diabetic rats.

N=6	Dose (mg/kg bw)	FBG	0h	1h	2h	3h	% fall between 0h and 1h
Group I	0	266.0±37.3	253.0±33.8	392.0±5.00	350.6±12.6	358.0±12.53	
Group II	100	271.0±28.0	264.0±7.81	352.0±26.1	319.0±28.5	305.0±29.13	36.69 ↓
Group III	200	233.0±15.6	226.0±14.9	267.0±4.61	250.0±13.7	233.6±10.69	70.50
Group IV	300	268.0±16.5	252.56±0.35	307.83±0.39	288.0±12.0	269.3±11.06	60.23
Group V	400	259.0±2.00	247.0±4.58	318.3±4.16	307.0±11.3	272.0±11.35	48.68 ↓

The data are expressed as Mean±SD for six rats each.

Effect of AECS on body weight and FBG of diabetic rats

A highly significant decrease (p<0.01) in body weight on 7th day (7.59%), 14th day (17.18%), 21th day (23.63%), 28th day (26.92%) was observed in diabetic rats as compared to control. Glibenclamide showed progressive increase (p<0.05) in body weight on 7th day (4%), 14th day (9.05%), 21st day (12.54%), 28th day (14.54%). AECS treated rats also showed significant increase (p<0.05) in body weight on 7th day (3.01%), 14th day (8.06%), 21th day (13.16%) and on 28th day (18.83%) from the initial value. There was a significant increase (p<0.01) in FBG of diabetic rats compared to control. There was significant decrease (p<0.01) in FBG on 7th (14.20%), 14th (31.91%), 21th (39.84%) and 28th (45.03%) day in AECS treated rats as compared to control. Significant decrease (p<0.01) in FBG on 7th (15.62%), 14th

(30.15%), 21th (34.21%) and 28th day (40.88%) was observed on glibenclamide treatment.

Effect of AECS on lipid profile, glycosylated haemoglobin and serum insulin of diabetic rats

A highly significant decrease (p<0.01, 28.77%) in the levels of HDL-cholesterol and highly significant increases (p<0.01) in cholesterol, LDL-cholesterol, VLDL and triglycerides levels was observed in diabetic rats (29.07%, 36.95%, 148.35, 148.26% respectively), compared to the normal control. The AECS and glibenclamide treated diabetic rats showed significant increase (p>0.05, p<0.05, respectively) in HDL-cholesterol level with 38.58% and 37.81% respectively compared to diabetic control. Glibenclamide treated rats showed highly significant decrease

(p<0.01) in cholesterol, LDL-cholesterol, VLDL and triglycerides levels (11.43%, 15.40%, 38.71%, 38.70% respectively) as compared to diabetic control. There was a significant decrease (p<0.05) in cholesterol, LDL-cholesterol, VLDL and triglycerides levels (19.05%, 21.41%, 57.87% and 57.85% respectively) on AECS treatment as compared to diabetic control. A significant elevation (p<0.01) in glycosylated haemoglobin (88.88%) and a significant decrease (p<0.01) in insulin (49.74%) was observed in diabetic rats as compared to control. Glibenclamide treated diabetic rats showed 38.13% decrease in glycosylated haemoglobin and 58.16% increase in insulin level by as compared to diabetic control. Administration of AECS showed 45.67% decrease in glycosylated haemoglobin and 40.6% increase in insulin as compared to diabetic control (Table 3).

Table 3: Comparison of lipid profile, glycosylated hemoglobin and insulin of different groups of Wistar rats treated with AECS.

Groups	TC (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)	HbA1c (%)	Insulin (µIU/ml)
I	141.74±01.14	38.61±0.36	12.76±0.08	90.36±1.18	0.30±0.01	7.65±0.05	14.50±0.02
II	182.96±0.60 [c]	27.5±3.97 [c]	31.69±0.72 [c]	123.76±1.23 [c]	1.21±0.017 [c]	14.45±0.22 [c]	7.29±0.028[c]
III	162.03±4.88 [c]	37.90±0.29 [b]	19.42±0.26 c	104.69±5.06 [c]	0.61±0.014 [c]	8.941±0.07 [c]	11.53±0.06 [c]
IV	148.09±4.59 [b]	38.11±0.32 [a]	13.3±0.05 [b]	97.26±5.05 [b]	0.51±0.016 [c]	7.85±0.06 [b]	10.25±0.03 [c]

I-normal control; II-diabetic control; III-glibenclamide; IV-AECS, a=p>0.05, b=p<0.05, c=p<0.01 vs normal control. The data are expressed as Mean±SD for six rats each.

Effect of AECS on Insulin Release

Exposure of isolated islets to 16.7mM glucose stimulated insulin release as compared to basal insulin level at 3.3mM glucose concentration, confirming the sensitivity of the assay system. AECS (1mg/ml) stimulated insulin secretion from 3.31±0.3 µU/islet/1hr to 14.78±0.9 at 3.3 mM glucose and 12.3±0.6 to 33.32±0.4 to at 16.7 mM glucose. At 2mg/ml concentration of AECS, insulin level was 18.21 ±0.4 and 48.43±0.6 at 3.3 and 16.7mM glucose respectively. The results indicated that insulin secretagogues effect of AECS was dose dependent (Table 4).

Table 4: Dose dependant effect of AECS on insulin release at 3.3 and 16.7mM glucose.

Sr.No.	Addition to the medium	Glucose	
		3.3 mM	16.7 mM
1.	None (Control)	3.31±0.3	14.78±0.9
2.	1mg AECS	12.3±0.6[c]	33.32±0.4[c]
3.	2mg AECS	18.21±0.4[c]	48.43±0.6[c]

Values are represented as Mean ± SD for 6 observations. ** = P<0.01 compared with control.

Effects of AECS and nicorandil on insulin release from isolated islets

Nicorandil significantly (p<0.01) decreased glucose stimulated insulin release at 16.7mM of glucose, however no significant (p>0.05) change was observed at basal insulin level at 3.3mM of glucose. Nicorandil reduced AECS (1mg/ml) induced insulin secretion at 16.7mM by 18% where as only 8.8 % reduction was observed at 3.3mM glucose (Table 5).

Table 5. Effect of AECS and nicorandil on insulin release from isolated islets.

Sr.No.	Addition to the medium	Glucose	
		3.3 mM	16.7 mM
1.	None	3.31±0.3	14.78±1.1
2.	1mg AECS	12.32±0.6[c]	32.4±0.5[c]
3.	1mg Nicorandil	3.1±0.2[a]	4.61±0.9[c]
4.	1mg AECS and 1mg Nicorandil	11.23±0.4[c]	26.55±0.4c

a=p>0.05, c=p<0.01 vs normal control, % calculated on comparison with ACES (1mg/ml).

Cell Viability Assay

Trypan blue gained access to 6.8±1.3% and 8.4±1.9% islet cells respectively, at 1 and 2 mg/ml concentration of AECS and 3.6±1.3% cells on nicorandil (1mg/ml) exposure for 1 hr. In LDH

release assay, the percentage of dead islet cells after 1 hr exposure was 6.5± 1.1% and 6.9± 1.7% respectively at 1 and 2 mg/ml concentration of AECS and 3.2±1.3% at 1mg/ml nicorandil. The study indicated that AECS and nicorandil were non toxic at tested concentrations.

Discussion

DM is projected as epidemic in Indian population mainly due to change in lifestyle patterns, and food habits and genetic predisposition. DM is a metabolic disorder characterized by disturbances in carbohydrate, protein, and lipid metabolism and by complications like retinopathy, microangiopathy, and nephropathy [18]. Currently available synthetic antidiabetic agents produce serious side effects like hepatorenal disturbances [19]. Moreover they are not safe for use during pregnancy [20]. Ayurvedic system of medicine relies on the administration of crude or partially purified plant extracts for the treatment of DM. Preliminary studies demand further research for possibilities of isolating novel hypoglycemic agents [21, 22]. STZ-induced experimental diabetes is a valuable model antidiabetic drug discovery as the STZ diabetic animals exhibits most of the diabetic complications, particularly cardiovascular, gastrointestinal, nervous, kidney and urinary bladder dysfunctions [23].

A number of pharcamological activities have been attributed to the genus *Cassia* [24] with few spp. exhibiting antidiabetic activity. *Cassia* spp. exhibiting antidiabetic activity are *C. auriculata* [11], *C. kleinii* [25] and *C. tora* [26]. Literature survey indicated that there is no published report on the antidiabetic activity of *C. sophera,* however the seeds of this plant has been used for management of diabetes by local people. Therefore the present study was undertaken to investigate antidiabetic activity of *C. sophera.*

The results of present study showed that the oral administration of the different solvent (hexane, chloroform, ethyl-acetate, methanol, aqueous) extracts (300 mg/kg bw) of *C. sophera* seeds resulted in a decrease in blood glucose level. The oral glucose tolerance test showed that *C. sophera* extracts lowered blood glucose level at the end of 1h in GTT. The *C. sophera* enhanced glucose utilization, so the blood glucose level was significantly decreased in glucose-loaded rats. The maximum hypoglycemic activity of the plant was observed with aqueous extract that produced a significant fall at 1h of treatment during GTT in normoglycemic rats. Studies with different doses (100-400 mg/kg bw) of the AECS in STZ-induced diabetic rats indicated that 200 mg/kgbw was most effective dose producing 70.50% fall in glucose during GTT. The next higher dose of 300mg/kgbw also showed good activity producing 60.23% fall. Such a phenomenon of less hypoglycemic effect at higher dose is not uncommon with medicinal plants and has been observed in *Momordica dioica* [13], *Brassica nigra* [27] and *Murraya koenigii* [28].

A reduction in body weight observed in diabetic rats may be due to excessive breakdown of tissue proteins [29]. Treatment with AECS, improved body weight, indicating prevention of muscle wasting due to hyperglycemic condition. Improvement in body weight of diabetic

animals on treatment with *Casearia esculenta* root extract [30] and *Costus pictus* leaves [31] have been reported earlier. Treatment of diabetic rats with AECS for 28 days brought down the elevated blood glucose levels from more than 250 mg/dl to nearly normal range. The study shows that AECS is able to bring down FBG level considerably, hence it may be very useful in management of diabetes. This glucose lowering effect may be by activation of β-cells and granulation returns to normal giving insulinogenic effect [32]. Perhaps AECS bring about its hypoglycemic action through stimulation of surviving pancreatic β-cells to release more insulin. This is clearly evidenced by the increased level of serum insulin in diabetic rats treated with AECS. There are reports that hyperglycaemia increases the generation of free radicals by glucose auto-oxidation. Some plants extracts have shown hypoglycemic activity by protecting β-cell from STZ induced damage through scavenging free radicals [33], hence this protective action of AECS is also not ruled out. Further studies are required to study the effect of AECS on lipid peroxidation and antioxidant enzyme level to confirm this hypothesis.

Cholesterol is a structural component of cell membrane and an important form in which lipoprotein are transported in the body. In our study, there was a significant increased level of serum total cholesterol, triglycerides and LDL-cholesterol but markedly decreased level of serum HDL- cholesterol in STZ- induced diabetic rats. Increase in TC, LDL, and VLDL cholesterol and TG while decrease in HDL cholesterol have been reported in diabetes and all these factors contribute to the coronary artery disease [34,35]. Several studies propose that most of the drugs that decrease total cholesterol also decrease HDL cholesterol. The present study AECS favourably modified serum lipid profile of diabetic rats with significant decreases in TC, LDL, VLDL and TG and significant increase in HDL.From this point of view, it is encouraging that the AECS brought down the elevated TC levels after treatment of 28 days (Table 3) and also increased the HDL levels, which is commonly considered good cholesterol. An increase in triglyceride may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase and hydrolyses TG under normal condition. In the present study, AECS effectively reduced TG possibly by decreasing the non-esterified fatty acids (NEFA) in diabetic rats. The significant control on serum lipids of diabetic animals by plant extracts may prevent simultaneous coexistence of hypercholesterolemia and hypertriglyceridemia, reducing cardiovascular risk factors [36,37].

HbA1c increases in DM and this increase is directly proportional to FBG level [38]. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form HbA1c [39]. HbA1c is used as a marker for estimating the degree of protein glycation in diabetes. HbA1c alters the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals, exacerbating oxidative stress in diabetes [40]. A fall in HbA1c is considered as indicator of antidiabetic activity of test drug and it is more reliable diagnostic tool. Administration of AECS considerably reduced HbA1c by 45.67% (Table 3) by virtue of its normoglycaemic activity. This normalization of glycosylated

hemoglobin indicates decreased glycation of proteins. These finding also suggests ameliorating role of AECS in reducing hyperglycaemia induced oxidative stress. Lack of insulin is critical factor in the development of diabetes [41]. Pancreatic toxins such as STZ and alloxans induces experimental diabetes by damaging β-cells and reducing insulin output. AECS increased serum insulin levels by 40.6%, indicating that it enhances insulin release from stressed pancreatic β-cells, either by regenerating the partially destroyed cells or by the stimulating release of insulin stored in the granules, which in turn improves glucose tolerance.

Isolated pancreatic islets serve as a fast and cost effective *in vitro* model for studying insulin release [42]. In present study, AECS stimulated insulin release form isolated islets in dose dependant manner with 2mg/ml concentration showing high release as compared to 1mg/ml. The effect at higher dose was not cumulative possibly due to other limiting factors like concentration of Ca^+ or exocytosis of insulin to near maximal. Paesuad *et al.* reported that loss of islet cells membrane integrity was responsible for increase in insulin release by saponins isolated from *Gymnema sylvestre* [43]. Alteration in islets cell membrane permeability by AECS may be a possible mechanism of insulin release in present study also. AECS treatment of islets for 1 hr did not increase trypan blue uptake as compared to untreated islets, indicating that membrane integrity was not alerted. The findings were further supported by LDH release assay where only $6.9 \pm 1.7\%$ dead islets cells were reported on AECS (2mg/ml) treatment. Hence the possibility of alteration of membrane integrity by AECS as possible mechanism of insulin release is ruled out by this study. The other well known physiological mechanism for insulin release involves K-ATP and voltagegated Ca^{2+} channels present on β-cells. The common physiological stimulant, glucose exerts its insulinotropic action by increasing cellular level of ATP, causing closure of K-ATP channels, leading to depolarization of β-cell membrane and increase in Ca^{2+} influx via the Ca^{2+} channels. This Ca^{2+} influx stimulates release of insulin by exocytosis. To explore this possibility of insulin release, nicorandil (a K-ATP channel opener) was used along with AECS. Addition of nicorandil completely abolished glucose stimulated insulin secretion at 16.7mM glucose. When islets were incubated with AECS and nicorandil, AECS induced insulin release at 16.7mM was decreased but not abolished completely. However little decrease in insulin secretion was observed at 3.3mM of glucose. The results indicated that AECS induced insulin secretion is independent of K-ATP channels of β-cells. The possible targets of AECS may be posterior to the K-ATP channels and further work is required to explore these targets. Hence, present study showed that aqueous extract of *Cassia sophera* exhibited significant antidiabetic activity in diabetic Wistar rats. The antidiabetic activity was partly by inducing insulin secretion. Incubation of isolated pancreatic islets with ACES confirmed its in vivo insulin secretagogues activity. The target site of active insulin secretagogues molecules of ACES may be posterior to the K-ATP channels of pancreatic β-cells.

Acknowledgement

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