J. Biomed. Ther. Sci. 2019, 6(1), 1-7 Article

Journal of Biomedical & Therapeutic Sciences

Antidiabetic effect of *Carissa carandas* in rats and the possible mechanism of its insulin secretagogues activity in isolated pancreatic islets

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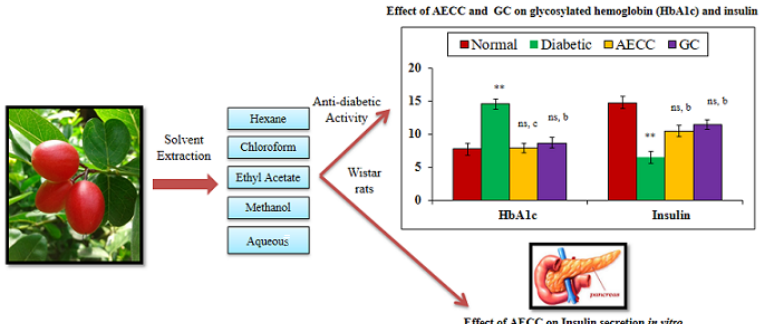
Received on: 10-Jan-2019 Accepted and Published on: 1-Apr-2019

ABSTRACT



Carissa carandas (CC) has been used in folklore medicine for treatment of diabetes. In the present study, hexane, chloroform, ethyl acetate, methanol and aqueous extracts of CC fruit were examined for hypoglycemic activity in healthy Wistar rats. Aqueous Extract of CC (AECC) was most active and showed fall of 67.08% in fasting blood glucose from 0 to 1h in glucose tolerance test (GTT). The ED_{50} of AECC was 300mg/kgbw in streptozotocin induced diabetic rats. Treatment of diabetic rats with ED_{50} of AECC for 28 days significantly reduced post prandial glucose (PPG) by 33.65% ($p<0.01$), glycosylated hemoglobin (HbA1c) by 45.79% ($p<0.01$) and increased insulin level by 69.7% ($p<0.05$). The results indicated that increase in insulin secretion may be partly responsible for antidiabetic effect of AECC. To assess the mechanism of secretagogues activity, AECC was incubated with isolated pancreatic islets of healthy Wistar rats at basal (3.3mM) and high (16.7mM) level of glucose in presence or absence of Diazoxide (K-ATP channel opener), Nimodipine (Ca^{2+} Channel blocker) and Calphostin-C (PKC inhibitor). AECC induced insulin secretion at 16.7mM of glucose was significantly ($p<0.01$) reduced by Diazoxide and Nimodipine but insignificantly ($p>0.05$) by Calphostin-C. The study indicated that the phytochemicals present in AECC may be inducing insulin secretion by closing K-ATP channels in β-cells of pancreatic islets.

Keywords: Carissa carandas, Wistar Rats, Antidiabetic, Pancreatic β-cells, Insulin

INTRODUCTION

Diabetes mellitus (DM) is a major cause of disability and hospitalization and responsible for significant economic burden on societies, worldwide.[1] Its prevalence among adults aged 20-70 years is expected to rise from 285 million in 2010 to 438 million by the year 2030.[2] The developing countries are accounting for more than 80% of reported cases of type 2 DM (T2DM), the prevalent form of DM.[3]

Although a number of therapeutic options are available for management of DM, high cost of treatment, toxicity and loss of efficacy in chronic use, necessitated search for relatively safer and cost effective antidiabetic agents. Recently, the WHO guidelines advocated for use of medicinal plants in management of diabetes. A number of reviews papers about the antidiabetic activity of medicinal plants have been published,[4,5] indicating

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Cite as: *J. Biomed. Ther. Sci., 2019, 6(1), 1-7.*
urn:nbn:sciencein.jbts.2019v6.90

 JBTS ISSN 2394-2274
http://pubs.iscience.in/jbts

the use of around 800 plants in management of DM.[6,7] Indian System of Medicine is having documentation of large number of medicinal plants for the management of DM.[8]

Carissa carandas, commonly known as *Karanda* is wild plant available throughout the Indian subcontinent. The fruits of this plant are available in rainy season and have been used as culinary medicine. It possesses anthelmintic, astringent, appetizer, antipyretic, antidiabetic and aphrodisiac activity.[9] It is useful in treatment of diarrhea, anorexia and intermittent fevers. Fruits have also been studied for its analgesic, anti-inflammatory and lipase inhibitory activities. Itankar et.al, reported the antidiabetic potential of *C. carandas* in Alloxan induced diabetic rats and observed increase in insulin secretion.[10] Increase in insulin secretion by *C. carandas* may be due to repair of pancreatic β- cells as well as insulin secretagogues mechanism of its phytochemicals. This study was planned to evaluate antidiabetic effect of *C. carandas* in diabetic rats as well as the possible mechanism of insulin secretion in isolated pancreatic β- cells.

MATERIALS AND METHODS

Chemicals

Streptozoticin (catalogue no. S0130), dizoxide (catalogue no. D9035), nimodipine (catalogue no. N149) and calphostin-c (catalogue no. C6303) were purchased from Sigma Aldrich. Medisence Optinum Xceed glucometer was used to measure the blood glucose level. All the solvents used in this study were of analytical grade and purchased from different companies with in India. Serum glycosylated haemoglobin (Hb1Ac) was measured using ELISA kits from Euro diagnostics, India. Insulin in serum and culture media during in vitro assay was measured using ELISA kits from Qayee-bio, China)

Animals

Male Wistar rats, weight range 150-200g, obtained from CSIR-Indian Institute of Toxicology Research, Lucknow, India were used in the present study. The animals were kept for acclimatization in the animal house of Bundelkhand University, Jhansi, India for one week at moderate temperature of 25±2^{0}C and relative humidity of 45-55% with light and dark cycles of 12h each. The rats were fed pelleted diet and water *ad-libitum*. The animal experiments were carried out as per the approval of Institutional Animal Ethical Committee (BU/ Pharma/ IAEC/13/20).

Plant Material and Extraction

The fresh unripe fruits of CC were collected from the surrounding hills of Bundelkhand University, Jhansi during August- September. The plant and the fruits were authenticated by experts in Vaidya Ram Narayan Sharma Institute of Ayurveda and Alternative Medical Education and Research, Bundelkhand University, Jhansi and a voucher specimen BU/VRNSI/2013/05-06 was preserved. The fresh fruits (100 g) were crushed using a mechanical blender to obtain a coarse paste. The paste was extracted separately with 500 ml of hexane, chloroform, ethyl acetate, methanol and water. The extracts were filtered twice with Whatman flter paper 1 and the organic solvent filtrates were dried in rotary evaporator under vacuum whereas the aqueous extract was centrifuged at 5000g for 10 minutes to remove any residual material and lyophilized. All extracts were stored at -40^{0}C until use.

Hypoglycemic activity of various extracts of CC by Glucose Tolerance Test

Thirty six healthy rats were randomized in six groups of six animals each. Group 1 served as control and group 2,3,4,5 and 6 were given @ 200 mg/kgbw of hexane, chloroform, ethyl acetate, methanol and water extracts of CC, respectively. All extracts were dissolved in DMSO and the rats in control group were given 1ml DMSO. Glucose tolerance test (GTT) was performed as per methods standardized in our laboratory.[11] Briefly, the rats were fasted overnight and Fasting Blood Glucose (FBG) was measured with the help of one touch glucometer, from a drop of blood from tail vein, followed by oral administration of single dose of CC extracts @ 200 mg/kgbw. FBG was again measured from tail vein after 90 min of administration of the extracts (termed as 0h value), followed by oral administration of glucose solution (2 g/kg bw). A drop of blood was withdrawn from tail vein after 1,2,3h of glucose administration and FBG was measured to assess GTT as well to assess the most active hypoglycemic extract.

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 Post Prandial Glucose (PPG) was monitored regularly till stable hyperglycaemia was established. Rats with stabilized diabetes having FBG of 250±25 mg/dl were used for antidiabetic study.

Determination of ED50 of Active Extract in Diabetic Rats by GTT

Aqueous extract of CC (AECC) showed maximum hypoglycemic activity as compared to other extracts. Hence, antidiabetic activity of AECC was evaluated. Diabetic rats (FBG 250±25 mg/dl) were randomized in 06 groups of 06 animals each. Group 1 served as control and was given 1ml of water. Groups 2-5 were given 100, 200, 300 and 400 mg/kgbw of AECC in 1ml of water, respectively. Group 6 served as positive control and received standard antidiabetic drug glibenclamide (GC) @ 10 mg/kgbw. GTT was performed on diabetic rats and the dose producing maximum effect was considered as $ED_{50.}$

Long term antidiabetic studies with ED_{50} AECC

Diabetic rats were randomized in four groups of six animals each. Group 1 and 2 were served as healthy and diabetic control, respectively. Rats in group 3 and 4 were treated with ED_{50} of AECC (300 mg/kgbw) andGC (10 mg/kgbw), respectively, once daily. PPG was assessed at 0, 7, 14, 21 and 28 days of the experiment. At the end of the experiment, rats were sacrificed under mild ether anaesthesia and blood was collected from ventricle of the heart. Serum glycosylated haemoglobin (Hb1Ac) and insulin were measured using ELISA kits as per manufacturer's details.

Effect of AECC on insulin secretion and mechanism of insulin secretagogue activity in isolated pancreatic islets

The results of this study indicated that increase in serum insulin may be partly responsible for antidiabetic effect of AECC in diabetic rats. The increase in insulin in STZ induced diabetic rats may be either due regeneration of pancreatic β cells by ACCC or by direct action of its phytochemicals on β cells for insulin induction and release. So, we planned to evaluate the direct action of AECC on isolated pancreatic islets of rat, as per previous methods standardized in our laboratory.[12] Briefly pancreas was excised from healthy Wistar rats of either sex and islets were isolated by the method of Lacy and Kostianovsky.[13]To evaluate the effect of AECC on insulin release, batches of 4-5 islets were incubated in HBBS (5% CO_2, pH 7 .4) containing 1% BSA and 1/2/3 mg/ml of AECC with 3.3 or 16.7 mM of glucose. To study the mechanism of AECC induced insulin secretion, islets were incubated with diazoxide (250µM), nimodipin (250 µM) and calphostin-C (1.5 µM) in presence or absence of AECC (1 mg/ml). After 1h of incubation, the media was centrifuged at 16000g, the supernatants were collected and insulin was measured by ELISA kit as per manufacturer's instructions.

Cell viability assay

Cell viability test was performed on isolated islets bylactate dehydrogenase (LDH) releaseassay.[14] Freshly isolated pancreatic islets (30nos) were incubated with AECC (at 1/2/3 mg/ml) and diazoxide (250µM), nimodipin (250 µM) and calphostin-C (1.5 µM) for 1 hr at 37^0C. The leakage of LDH in the supernatant was measured using automated clinical chemistry analyzer VITALAB Selectra E, following kit instructions (Merck Specialist Pvt. Ltd) at 390nm with necessary modifications. Percentage cytotoxicity was calculated by following equation-

$$\text{Cytotoxicity (\%)} = \frac{\text{Experimental value-Low control value}}{\text{High control value-Low control value}} \times 100$$

Whereas, experimental value was LDH activity of islets incubated with AECC and diazoxide, nimodipin &calphostin-C, low control value was islets incubated in HBBS buffer and high control value was islets incubated with 1% (v/v) Triton X-100.

Data and statistical analysis

Results were articulated as Mean ± SEM for six rats in each group. Hypothesis testing method comprised of one-way analysis of variance (ANOVA) and Dunnette's comparison tests. Statistical significance was indicated by P-values less than 0.05. GraphPad InStat Software Inc. (v. 3.06, San Digeo, USA) was used to carry out all the statistics.

RESULTS

Hypoglycemic activity of *C. carandas* by GTT

Hexane, chloroform, ethyl-acetate, methanol and water extract of CC indicated a decrease in FBG between 0 to 1 h by 13.10, 16.77, 25.99, 66.70 and 67.08% respectively as compared to the control (Table 1).

Table 1: Hypoglycemic activity of different solvent extracts of *C. carandas* by GTT in healthy rats by estimating FBG (mg/dl)

Treatment group	FBG	0h	1h	2h	3h	% fall between 0h and 1h
Control	100.66 ±5.4	100.67±5.3	151.3 ±6.3	148.0 ±5.4	127.34±6.3	
Hexane	99.03 ±6.32	106.48 ±6.46	150.48 ±8.16	141.1 ±5.45	123.77 ±6.47	13.10
Chloro-form	105.38 ±5.51	100.14 ±8.13	142.28 ±5.47	135.15 ±9.19	118.44 ±5.15	16.77
Ethyl-acetate	98.44 ±5.46	97.03 ±5.43	134 ±5.56	128.74 ±8.66	112.24 ±6.25	25.99
Methanol	100.05 ±5.97	98.41 ±6.36	115.27 ±4.07	111.53 ±10.23	99.68 ±7.09	66.70
Aqueous	101.67 ±6.87	97.2± 5.45	113.87 ±6.00	99.53 ±6.57	97.33 ±5.01	67.08

Effective dose of AECC by GTT

The hypoglycemic activity of AECC was more pronounced as compared to other extracts. Hence AECC was termed as most active extract and its effective dose (ED_{50}) was estimated in diabetic rats. AECC at doses of 100, 200, 300, and 400mg/kg body weightproduced a reduction of 39.40%, 65.16%, 70.98%, and 56.32% respectively in FBG during GTT(Table 2). The dose of 300mg/kgbw which produced highest fall in FBG was termed as ED_{50} and used for long term antidiabetic studies.

Table 2: Determination of Effective dose of aqueous extract of *C. carandas* by GTT in STZ induced diabetic rats

Group	Dose of AECC (mg/kgbw)	Blood glucose (mg/dl)					% fall between 0h and 1h
		FBG	0h	1h	2h	3h	
1	0	260 ±6.32	258.3 ±6.44	376 ±4.94	369.08 ±7.66	367.2 ±5.54	
2	100	272 ±7.07	268.50 ±6.33	340.02 ±5.97	310.35 ±7.16	287.1 ±6.76	39.40
3	200	258 ±6.32	236 ±5.96	277 ±5.44	266.83 ±7.17	261.2 ±6.96	65.16
4	300	251.41 ±6.76	235.42 ±7.09	270.2 ±6.14	261.24 ±4.76	253.41 ±6.14	70.56
5	400	267.83 ±5.91	257 ±5.44	308.4 ±6.24	307.35 ±5.99	292 ±5.44	56.32
6	GC	275.24 ±5.99	220.3 ±6.17	261.04 ±5.12	248.3 ±11.5	233.42 ±4.37	77.36

Long term antidiabetic studies with AECC

Effect on Postprandial glucose (PPG)

Significant($p<0.01$) decrease in PPG by AECC (33.65%) and GC (34.02%) was observed in diabetic rats on 28 days of treatment (Table 3, Figure 1).

Table 3: Effect of 28 days treatment of AECC and glibenclamide on PPG

Groups	0 days	7 days	14 days	21 days	28 days
Normal	160.36±5.9	163.2±6.07	160.36±5.9	162.59±6.84	161.59±4.93 ns
Diabetic	446.29±6.37	456.1±5.87	457.66±5.10	451.35±6.87	460.2±5.96**
C. carandas	438.16±6.42	389.3±7.32	345.1±5.47	319.65±7.11	305.3±4.05**
GC	435.2±3.79	380.3±5.55	351.43±6.51	316.50±5.84	304.05±4.46**

ns=p>0.05, **=p<0.01 compared 0 day and 28 day. The data are expressed as Mean±SD for six rats each.

Effect on HbA1c and Insulin

Significant (p<0.01, 87.78%) increase in HbA1C in diabetic rats was observed as compared to healthy control. Significant decrease in HbA1C was observed on GC (p<0.05, 40.52%)and AECC (p<0.01, 45.79%) treatment compared to diabetic control (Table 4). Diabetic rats showed significant decrease (p<0.01, 56.00%) in level of insulin as compared to healthy control. Increase in insulin level in diabetic rats was observed on treatment with GC (p<0.05, 76.07%%) and AECC (p<0.05, 61.19%) (Table 4, Figure 2).

Table 4. Effect of AECC on glycosylated hemoglobin (HbA1c) and insulin.

Groups	HbA1c	Insulin
Normal	7.78±0.93	14.82±0.95
Diabetic	14.61±0.82**	6.52±0.89**
AECC	7.92±0.75 ns c	10.51±0.83 ns b
GC	8.69±0.82 ns b	11.48±0.69 ns b

ns=p>0.05, *=p<0.05, **=p<0.01 vs normal control and b=p<0.05, c=p<0.01 vs Diabetic control. The data are expressed as Mean±SD for six rats each.

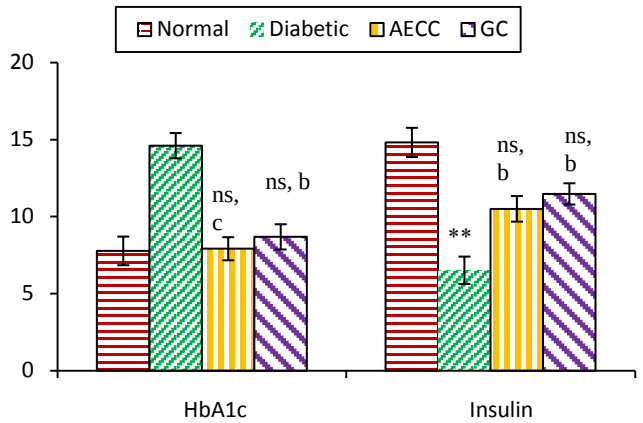

Figure 2. Comparison of glycosylated hemoglobin (HbA1c) and insulin of different groups of Wistar rats treated with AECC

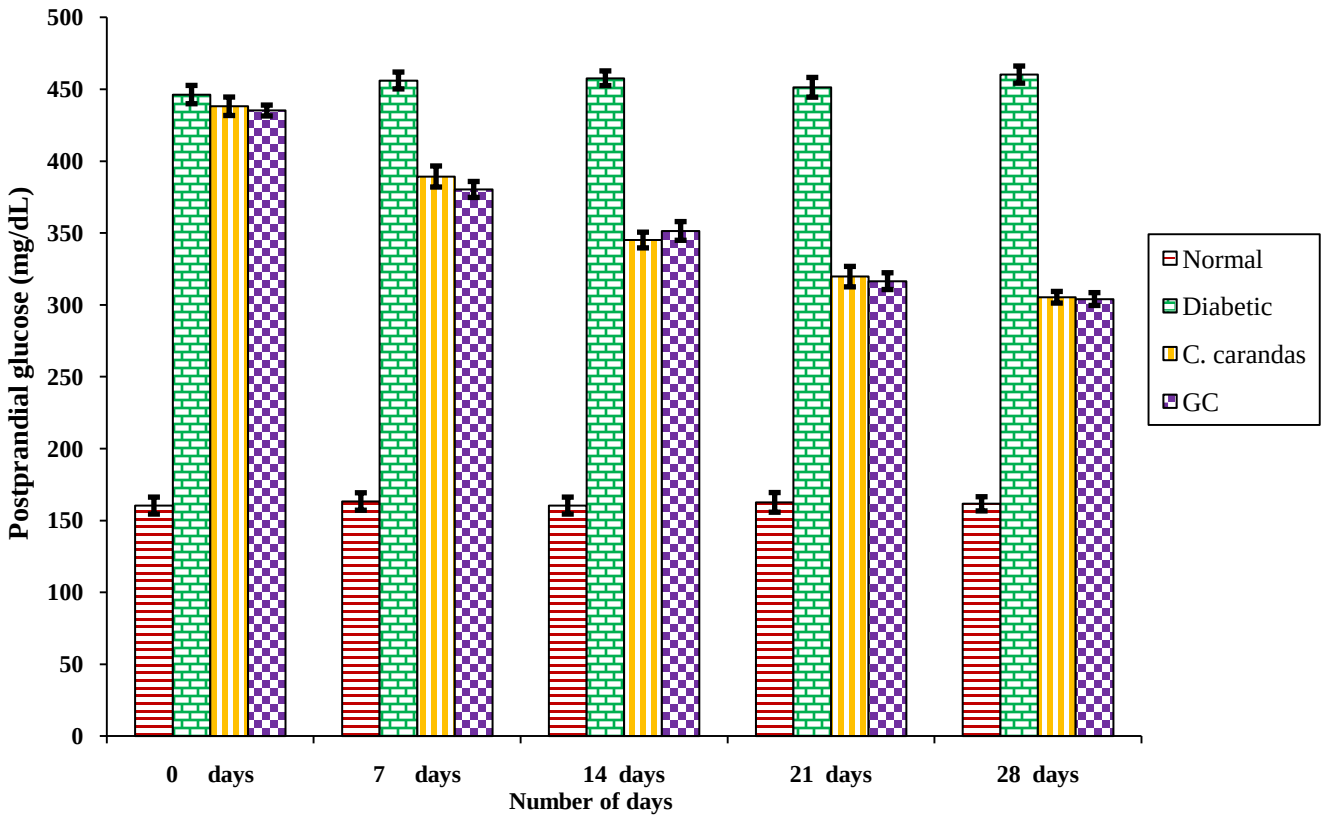

Figure 1. Effect of 28 days treatment of AECC and glibenclamide on PPG.

Effect of AECC on *in vitro* insulin release

The 3.92 fold increase in insulin release was observed at 16.7 mM glucose concentration as compared to 3mM. The result indicated that the islets were healthy and responding to glucose, the inherent inducer of insulin. Co-incubation of the islets with 3.3mM of glucose and 1/2/3mg/ml concentration of AECC resulted in 1.20, 3.20 and 4.84 fold increase in insulin release. Whereas co-incubation of the islets with 16.7mM of glucose and 1/2/3mg/ml concentration of AECC resulted in 2.78, 4.22 and 4.66 fold increase in insulin release (Table 5).

Table 5. Effects different concentration of AECC on *in vitro* insulin release.

HBBS, BSA, Islet and addition to the medium	Insulin release (µU/islet/1hr) at different concentrations of glucose and AECC	
	3.3mM Glucose	16.7mM Glucose
None	3.9±0.032	15.3±0.131
AECC (1mg/ml)	12.5±0.095	42.6±0.265
AECC (2mg/ml)	18.9±0.107	64.7±0.189
AECC (3mg/ml)	19.4±0.128	71.3±0.171

Effect of Diazoxide, Nimodipin and Calphostin-C on AECC induced insulin secretion in isolated islets

Insulin release was significantly ($p<0.01$) decreased by dizoxide at 3.3mM (62.84%) and 16.7mM (57.60%) of glucose stimulation. AECC (1mg/ml) induced insulin secretion was non-significantly ($p>0.05$, 21.19%) decreased by dizoxide at 3.3mM, whereas significantly ($p<0.01$, 55.37%) at 16.7mM glucose. Nimodipin showed a decrease in insulin release at 3.3 mM and 16.7mM of glucose concentration by 41.39% and 61.15% respectively. Nimodipin non-significantly ($p>0.05$, 36.15%) reduced the AECC (1mg/ml) induced insulin secretion at 3.3mM whereas significantly ($p<0.01$, 57.02%) at 16.7mMglucose.The glucose stimulated insulin release was reduced non-significantly ($p>0.05$, 2.7%) at 3.3mM and significantly ($p>0.05$, 12.56%) at 16.7mM by calphostin-C. However the combination of calphostin-c and AECC significantly increased insulin secretion at 3.3 mM($p<0.01$, 131.92%) and 16.7mM ($p<0.01$, 151.81%) as compared to control and decreased at 3.3mM ($p<0.05$,29.81%) and 16.7mM ($p<0.05$,34.51%) as compared AECC induced group (Table 6).

Table 6: Effect of AECC, dizoxide, nimodipin and calphostin-c on insulin release from isolated islets

Addition to the median	Glucose	
	3.3mM	**16.7mM**
None (control)	4.01±2.28	12.1±2.85
AECC (1mg/ml)	13.25±1.92**	46.53±3.125*
Dizoxide	1.49±0.35**	5.13±2.47**
AECC+Dizoxide	3.16±1.60^{ns}	5.4±2.49**
Nimodipin	2.35±0.67^{ns}	4.7±2.35**
AECC+Nimodipin	2.56±0.73^{ns}	5.2±3.21**
Calphostin-C	3.9±0.94^{ns}	10.58±1.18**
AECC+Calphostin-C	9.3±2.44**,^{b}	30.47±2.69 **,^{b}

ns=p>0.05, *=p<0.05, **=p<0.01 vs control where as a=p>0.05, b=p<0.05, c=p<0.01 represents comparison with AECC (1mg/ml). The data are expressed as Mean±SD for six rats each.

Cell viability assay

In LDH release assay, the percentage of dead islet cells after 1 hr exposure was 7.2± 0.9, 7.1± 1.7, 7.4±1.3% and respectively at 1, 2 and 3 mg/ml concentration of AECC. Whereas the percentage of cell death was 4.3±1.1, 10.2±0.9 and 3.3±1.8% on exposure to diazoxide (250µM), nimodipin (250 µM) and calphostin-C (1.5 µM)for 1hour. The study indicated that AECC, diazoxide, nimodipinand calphostin-C were non-toxic at tested concentrations.

DISCUSSION

Presently available drug for the management of diabetes mellitus have certain limitations, so, there is a necessity for cost effective, safer and newer anti-diabetic molecules. Medicinal plants are potential sources of safer and effective drugs.[15–17] Hence, in search for antidiabetic principle(s) from medicinal plants,[4,18,19] the antidiabetic effect of fresh fruits of *C. carandas* fruit in diabetic Wistar rats was evaluated. Insulin secretagogues activity of the active extract of *C. carandas* was also studied in isolated pancreatic islets to explore the mechanism of action.

Decrease in BG was observed on administration of hexane, chloroform, ethyl-acetate extracts, methanol and water extracts of *C. carandas* fruit at 300 mg/kg body weight in healthy rats. The maximum hypoglycemic activity was observed in aqueous extract of *C. carandas* (AECC), which produced 67.08% fall in BG during GTT and showed better glucose tolerance. The increase in glucose tolerance may be better utilization of glucose by the rats, hence further antidiabetic studies were aimed using AECC.

Different dosages (100-400 mg/kg body weight) of AECC were used for estimation of effective dose in STZ-induced diabetic rats. GTT was performed as per earlier experiment and fall (0 to 1hr) in glucose level was 39.40% (100mg/kg/bw), 65.16% (200mg/kg/bw), 70.56% (300mg/kg/bw) and 56.32% (400mg/kg/bw). The results of this experiment showed that the effective dose was 300mg/kg/bw. The study showed that at lower concentration, dose dependant effect of AECC on glucose tolerance was observed. However at higher concentrations, a decrease in glucose tolerance was observed. Such a phenomenon of less hypoglycemic effect by plant extracts at higher concentrations have been reported previously in *Terminalia chebula*[20,21] and *Annona squamosal*.[22] GC at a dose of 10mg/kgbw produced a fall of 77.36 % during GTT.

In long term (28 days) study, AECC effectively decreased PPG (33.65%) in diabetic rats as compared to GC (34.02%). PPG refers to the plasma glucose concentration after meal. The decrease in PPG level during long term study indicated better glucose management by AECC treatment. Glycosylated hemoglobin (HbA1c) has been used as the most consistent marker in standard diagnostic practices for estimating the degree of protein glycation.[23] HbA1c has been used as indicator of diabetic state for longer period studies. The decrease in HbA1c has been considered as indicator of better glycemic control by the test drug. The induction of diabetes in Wistar rats in this study resulted in 87.78% increase in serum HbA1c level. Administration of AECC significantly ($p<0.01$, 45.79%) reduced HbA1c by virtue of its normo-glycemic activity. Thus

the treatment with AECC declined glycation of proteins in blood, mainly because of management of glucose level in blood for longer period.

Increase in insulin secretion has been targeted for management of DM. Drugs enhancing insulin release from the pancreatric β-cells are widely used for management of type-1 as well as type-2 diabetes.[24] ATP-sensitive K^+ (K-ATP) dependent[25] and K-ATP channel-independent[26]pathways have been proposed for insulin release form β cells. In the K-ATP channel-dependent pathway, enhanced glucose metabolism increases the cellular ATP/ADP ratio leading to closure of K-ATP channels, depolarization of cell membrane and activation of the voltage-dependent L-type Ca^{2+} channels. The activation of Ca^{2+} channels increases Ca^{2+} entry in the cell which stimulates insulin release.[26] The K-ATP channel-independent mechanisms include Acetyle choline and incretin mediated pathways involving IP_3, PKC, Epac2 and PKA as secondary messengers.

The results of the study showed that treatment with AECC treatment (28 days) enhanced serum insulin level by 61.19% in diabetic rats. This rise in serum insulin level may be partly responsible for the antidiabetic activity of AECC. AECC induced increase in serum insulin may be either by regenerating the partially destroyed pancreatic β-cells in diabetic rats due to STZ exposure and/or by stimulating the release of insulin stored in the vesicles in β-cells.[27] We have explored the mechanism of action of AECC on insulin release using isolated pancreatic β-cells from healthy adult rats. Isolated pancreatic β- cells can be used for developing fast and cost effective insulin secretagogue assays.

K^+-ATP and voltage gated Ca^{2+} channels are well-known physiological mechanism for insulin release. A number of secondary messengers are also involved in insulin secretion from β-cells. The secondary messenger IP_3 is responsible for release of Ca^{2+} from intracellular storage organelle endoplasmic reticulum, raising intracellular Ca^{2+} concentration and thus, insulin biosynthesis increases. Also, increase in concentrations of secondary messengers such as PKA, PKC and Epac2 augment exocyostis of insulin. Hence this research was focused on the identification of probable mechanism of insulin secretion by AECC.

Glucose is the natural insulinotropic agent to pancreatic β-cells. The post parandial increase in glucose level leads to accelerated entry of glucose in β- cells via GLUT2 transporters. The increase in intracellular glucose concentration, up regulates glucose oxidation shifting the ATP/ADP ratio towards ATP. The increase in cellular level of ATP, triggers closure of K^+ ATP channels, leading to depolarization β-cell membrane followed by opening of voltage gated Ca^{2+} channels, increasing Ca^{2+} influx and thus enhancing intracellular Ca^{2+}. This results in exocytosis of insulin from stored vesicles as well as upregulation of biosynthesis of insulin.[28,29] Dizoxide (a K^+ATP channel opener), nimodipin (Ca^{2+} channels blocker) were used in the study to check the interaction of phytochemicals with K^+ATP and Ca^{2+} channels. Exposure of isolated islets to higher concentration of D-glucose (16.7mM) stimulated insulin release as compared to basal D-glucose (3.3mM) concentration. The results indicated the integrity of β- cells in the islets and sensitivity of the assay. The co-incubation of islets with 1, 2 and 3 mg/ml of AECC resulted in 1.20 3.20 and 4.84 fold increase in insulin level at 3.3mM of glucose and 2.78, 4.22 and 4.66 fold increase at 16.7mM of glucose. The results indicated that induction of insulin secretion by AECC was dose dependant, although only 03 concentrations of AECC were used. The dose dependent induction in insulin level by AECC was more pronounced at basal concentration of glucose (3mM) as compared to higher concentration (16.7mM). The relatively low induction at higher concentration of AECC (3mg/ml) and glucose (16.7mM) may be due to saturating effect of AECC at higher glucose concentration.

The other aim of the reach was to explore the mechanism of insulinotropic action of AECC. For this part of experiment only one concentration (1mg/ml) of AECC was used. The incubation of isolated islets with diazoxide considerably reduced glucose stimulated insulin secretion at 3.3mM as well as 16.7 mM. The co-incubation of AECC with daizoxide and nimodipin completely abolished AECC stimulated insulin secretion at 3.3mM as well as 16.7 mM. The results indicated that the phytochemicals present in AECC might be interacting with K^+ATP and/or Ca^{2+}channels on the β-cell membrane. To further explore the target sites, calphostin-C (PKC inhibitor) was used. PKC induces exocytosis of insulin from the stored vesicles in β-cells.[30] Incubation of islets with calphostin-C, non-significantly decreased insulin level both at 3.3mM and 16.7mM of glucose. During co-incubation with AECC, calphostin-C reduced insulin secretion by 29.81% (3mM glucose) and 34.51% (16.7mM glucose). The finding showed that although calphostin-C decreased AECC mediated induction in insulin level, but it was not reduced to a larger extent. The results indicated that phytochemicals of AECC are not exerting their insulinotropic action via DAG-PKC mediated pathway.

CONCLUSION

The results of the study indicated that aqueous extract of *Carissa carandas* possess blood glucose lowering activity in diabetic rats. During 28 days study, AECC significantly decreased PPG, HbA1c and increased serum insulin. The antidiabetic activity of AECC was comparable with that of Glibenclamide. The *in-vitro* study indicated that insulin secretagogues activity was dose dependent. The experiment related to mechanism study showed the insulinotropic activity of AECC may be dependent on K^+ATP and Ca^{2+} channels and independent on DAG-PKC pathway.

ACKNOWLEDGMENTS

The authors are thankful to Innovation Center, Bundelkhand University, Jhansi for extending equipment facility for this research.

REFERENCES

1. S. Nag, P.H. Bennett, H. King, et al. The Burden of Mortality Attributable to Diabetes: Realistic estimates for the year 2000. *Diabetes Care* **2007**, 28 (9), 2130–2135.
2. S.H. Wild, G. Roglic, A. Green, R. Sicree, H. King. Global prevalence

of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* **2004**, 27 (5), 1047–1053.
3. N.H. Cho, J.E. Shaw, S. Karuranga, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* **2018**, 138, 271–281.
4. R. Singh, T. Arif, I. Khan, P. Sharma. Phytochemicals in antidiabetic drug discovery. *J. Biomed. Ther. Sci.* **2014**, 1 (1), 1–33.
5. R. Dabur, B. Sharma, A. Mittal. Mechanistic approach of anti-diabetic compounds identified from natural sources. *Chem. Biol. Lett.* **2018**, 5 (2), 63–99.
6. P.K. Prabhakar, M. Doble. Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chin. J. Integr. Med.* **2011**, 17 (8), 563–574.
7. S. Perez-Gutierrez, A. Aguilar-Contreras, J.. Flores-Saenz, et al. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J. Ethnopharmacol.* **1998**, 61 (2), 101–110.
8. J.K. Grover, V. Vats. Shifting Paradigm: From Conventional to Alternative Medicines — An Introduction on Traditional Indian Medicines. *Asia. Pac. Biotech News* **2001**, 05 (01), 28–32.
9. J.K. Grover, S. Yadav, V. Vats. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.* **2002**, 81 (1), 81–100.
10. P.R. Itankar, S.J. Lokhande, P.R. Verma, et al. Antidiabetic potential of unripe Carissa carandas Linn. fruit extract. *J. Ethnopharmacol.* **2011**, 135 (2), 430–433.
11. P. Sharma, P. Sharma, R. Singh, P. Bhardwaj. Antidiabetic activity of Cassia sophera in STZ induced diabetic rats and its effect on insulin secretion from isolated pancreatic islets. *Int. J. Phytomedicine* **2013**, 5 (3), 314–321.
12. R. Singh, P. Sharma. Momordica dioica stimulated insulin secretion from rat pancreatic islets is independent of ATP-sensitive K+ channel. *Int. J. Pharmacogn. Phytochem. Res.* **2016**, 8 (2), 248–251.
13. P.E. Lacy, M. Kostianovsky. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **1967**, 16 (1), 35–39.
14. P. Sharma, M. Singh, S. Arora, R. Singh. The evaluation of in vitro toxicity of the protein (LL 4218) isolated from leaves of Argemone mexicana in human hepatoma-derived cell line (HepG2) using multiplexing approach. *J. Nat. Pharm.* **2011**, 2 (4), 179–179.
15. I. Singh. Antimicrobials in Higher Plants: classification, mode of action and bioactivities. *Chem. Biol. Lett.* **2017**, 4 (1), 48–62.
16. J.J. Kaushik, Y.T. Keleta, D.K. Emha, et al. Antiquorum sensing activity of selected Eritrean traditional medicinal plants against Pseudomonas aeruginosa. *J. Biomed. Ther. Sci.* **2017**, 4 (1), 21–26.
17. B. Thorat. Chemical extraction and biomedical importance of secondary organic metabolites from plants – A review. *J. Biomed. Ther. Sci.* **2018**, 5 (1), 9–42.
18. S. Sekar, K.V. Pugalendi, B. Kishnan, K.K. Ganesan. Evaluation of anti-hyperglycemic effect of Gracilaria corticata extract in Normal and Streptozotocin-induced Diabetic rats. *Chem. Biol. Lett.* **2015**, 2 (1), 6–11.
19. V. Mehra, M. Khatri, S. Mishra, et al. Assessment of Anti-diabetic and Anti-oxidant activity of Murraya Koenigii extracts using in-vitro assays. *J. Biomed. Ther. Sci.* **2018**, 5 (1), 1–8.
20. Y. Murali, P. Anand, V. Tandon, et al. Long-term Effects of *Terminalia chebula* Retz. on Hyperglycemia and Associated Hyperlipidemia, Tissue Glycogen Content and in Vitro Release of Insulin in Streptozotocin Induced Diabetic Rats. *Exp. Clin. Endocrinol. Diabetes* **2007**, 115 (10), 641–646.
21. J. Singh, S. Kumar, B. Rathi, K. Bhrara, B.S. Chhikara. Therapeutic analysis of *Terminalia arjuna* plant extracts in combinations with different metal nanoparticles. *J. Mater. Nanosci.* **2015**, 2 (1), 1–7.
22. R.K. Gupta, A.N. Kesari, P.S. Murthy, et al. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annona squamosa L. in experimental animals. *J. Ethnopharmacol.* **2005**, 99 (1), 75–81.
23. D.B. Goldstein, D.D. Pollock. Least squares estimation of molecular distance—noise abatement in phylogenetic reconstruction. *Theor. Popul. Biol.* **1994**, 45 (3), 219–226.
24. D.E. Moller. New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*. December 2001, pp 821–827.
25. R.H. Chow, P.E. Lund, S. Löser, U. Panten, E. Gylfe. Coincidence of early glucose-induced depolarization with lowering of cytoplasmic Ca2+ in mouse pancreatic beta-cells. *J. Physiol.* **1995**, 485 (Pt 3), 607–617.
26. B. Hellman, E. Gylfe, P. Bergsten, et al. The role of Ca2+ in the release of pancreatic islet hormones. *Diabete Metab.* 20 (2), 123–131.
27. R. Singh, A. Seherawat, P. Sharma. Hypoglycemic, Antidiabetic and Toxicological Evaluation of Momordica dioica Fruit Extracts in Alloxan Induced Diabetic Rats. *J. Pharmacol. Toxicol.* **2011**, 6 (5), 454–467.
28. S.G. Straub, S. Daniel, G.W.G. Sharp. Hyposmotic shock stimulates insulin secretion by two distinct mechanisms. Studies with the βHC9 cell. *Am. J. Physiol. Metab.* **2015**, 282 (5), E1070–E1076.
29. P.E. MacDonald, M.B. Wheeler. Voltage-dependent K+ channels in pancreatic beta cells: Role, regulation and potential as therapeutic targets. *Diabetologia* **2003**, 46 (8), 1046–1062.
30. W.S. Zawalich, G.J. Tesz, K.C. Zawalich. Are 5-Hydroxytryptamine-preloaded β-Cells an Appropriate Physiologic Model System for Establishing that Insulin Stimulates Insulin Secretion? *J. Biol. Chem.* **2001**, 276 (40), 37120–37123.