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

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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 ED₅₀ of AECC was 300mg/kgbw in streptozotocin induced diabetic rats. Treatment of diabetic rats with ED₅₀ 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 (Ca²⁺ 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
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. 7 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**

Streptozotocin (catalogue no. S0130), dizoxide (catalogue no. D9035), nimodipine (catalogue no. N149) and calphostin-c (catalogue no. C6303) were purchased from Sigma Aldrich. Medisence Optimum 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°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 filter 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°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 ED50

**Long term antidiabetic studies with ED50 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 ED50 of AECC (300 mg/kgbw) and GC (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 AECC 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. Briefly pancreas was excised from healthy Wistar rats of either sex and were isolated by the method of Lacy and Kostianovsky. To evaluate the effect of AECC on insulin secretion and mechanism of insulin induction and release, batches of 4-5 islets 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°C. The leakage of LDH in pancreatic islets (30 nos) 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°C. The results of this study indicated that increase in serum insulin may be partly responsible for antidiabetic effect of AECC in diabetic rats. AECC at doses of 100, 200, 300, and 400mg/kg body weight produced a reduction of 39.40%, 56.16%, 70.98%, and 56.32% respectively in FBG during GTT(Table 2). The dose of 300mg/kg produced highest fall in FBG was termed as ED50 and used for long term antidiabetic studies.

Cell viability assay

Cell viability test was performed on isolated islets by lactate dehydrogenase (LDH) release assay. 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) in presence or absence of AECC (1 mg/ml). After 1h of incubation, the media was centrifuged at 16000g, 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 Dunnet’s comparison tests. Statistical significance was indicated by P-values less than 0.05. GraphPad InStat Software Inc. (v. 3.06, San Diego, 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)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>FBG 0h±SE</th>
<th>FBG 1h±SE</th>
<th>FBG 2h±SE</th>
<th>FBG 3h±SE</th>
<th>% fall between 0h and 1h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.66±5.4</td>
<td>100.6±5.3</td>
<td>151.3±6.3</td>
<td>148.0±5.4</td>
<td>127.3±6.3</td>
</tr>
<tr>
<td>Hexane</td>
<td>99.03±6.32</td>
<td>106.48±8.16</td>
<td>150.48±8.16</td>
<td>141.1±5.45</td>
<td>123.77±6.47</td>
</tr>
<tr>
<td>Chloroform</td>
<td>105.38±5.1</td>
<td>100.14±8.13</td>
<td>142.28±5.47</td>
<td>135.15±9.19</td>
<td>118.44±5.15</td>
</tr>
<tr>
<td>Ethyl-acetate</td>
<td>98.44±5.46</td>
<td>97.03±5.43</td>
<td>134±5.56</td>
<td>128.74±6.66</td>
<td>112.24±6.25</td>
</tr>
<tr>
<td>Methanol</td>
<td>100.05±5.97</td>
<td>98.41±6.36</td>
<td>115.27±4.07</td>
<td>111.53±10.23</td>
<td>99.68±7.09</td>
</tr>
<tr>
<td>Aqueous</td>
<td>101.67±6.87</td>
<td>97.2±5.45</td>
<td>113.87±6.00</td>
<td>99.53±6.57</td>
<td>97.33±5.01</td>
</tr>
</tbody>
</table>

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 (ED50) was estimated in diabetic rats. AECC at doses of 100, 200, 300, and 400mg/kg body weight produced a reduction of 39.40%, 56.16%, 70.98%, and 56.32% respectively in FBG during GTT(Table 2). The dose of 300mg/kg which produced highest fall in FBG was termed as ED50 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of AECC (mg/kg bw)</th>
<th>Blood glucose (mg/dl)</th>
<th>% fall between 0h and 1h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>260±6.32</td>
<td>258.3±6.44</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>272±7.07</td>
<td>268.50±6.33</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>258±6.32</td>
<td>236±5.96</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>251.41±6.76</td>
<td>235.42±7.09</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>267.83±5.91</td>
<td>257±5.44</td>
</tr>
<tr>
<td>6</td>
<td>GC</td>
<td>275.24±5.99</td>
<td>220.3±6.17</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>160.3±6±5.9</td>
<td>163.2±6.07</td>
<td>160.36±5.9</td>
<td>162.59±6.84</td>
<td>161.59±4.93ns</td>
</tr>
<tr>
<td>Diabetic</td>
<td>446.29±6.37</td>
<td>456.11±5.87</td>
<td>457.66±5.10</td>
<td>451.35±6.87</td>
<td>460.2±5.96**</td>
</tr>
<tr>
<td>C. carandas</td>
<td>438.16±6.42</td>
<td>389.3±7.32</td>
<td>345.1±5.47</td>
<td>319.65±7.11</td>
<td>304.05±4.46**</td>
</tr>
<tr>
<td>GC</td>
<td>435.2±3.79</td>
<td>380.3±5.55</td>
<td>351.43±6.51</td>
<td>316.50±5.84</td>
<td>304.05±4.46**</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1c</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.78±0.93</td>
<td>14.82±0.95</td>
</tr>
<tr>
<td>Diabetic</td>
<td>14.61±0.82**</td>
<td>6.52±0.89**</td>
</tr>
<tr>
<td>AECC</td>
<td>7.92±0.75mc</td>
<td>10.51±0.83mb</td>
</tr>
<tr>
<td>GC</td>
<td>8.69±0.82**</td>
<td>11.48±0.69**</td>
</tr>
</tbody>
</table>

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

Figure 2. Comparison of glycosylated hemoglobin (HbA1c) and insulin of different groups of Wistar rats treated with AECC.
**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 3.5mM 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.**

<table>
<thead>
<tr>
<th>HBBS, BSA, Islet and addition to the medium</th>
<th>Insulin release (µU/islet/1hr) at different concentrations of glucose and AECC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3M Glucose</td>
<td>16.7M Glucose</td>
</tr>
<tr>
<td>None</td>
<td>3.9±0.032</td>
</tr>
<tr>
<td>AECC (1mg/ml)</td>
<td>12.5±0.095</td>
</tr>
<tr>
<td>AECC (2mg/ml)</td>
<td>18.9±0.107</td>
</tr>
<tr>
<td>AECC (3mg/ml)</td>
<td>19.4±0.128</td>
</tr>
</tbody>
</table>

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

Insulin release was significantly (p<0.01) decreased by dioxizone 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 dioxizone 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 16.7mM glucose. 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, dixoizone, nimodipin and calphostin-c on insulin release from isolated islets**

<table>
<thead>
<tr>
<th>Addition to the medium</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.3M</td>
</tr>
<tr>
<td>None (control)</td>
<td>4.0±2.28</td>
</tr>
<tr>
<td>AECC (1mg/ml)</td>
<td>13.25±1.92**</td>
</tr>
<tr>
<td>Dizoxide</td>
<td>14.9±0.35**</td>
</tr>
<tr>
<td>AECC+Dizoxide</td>
<td>3.16±1.60**</td>
</tr>
<tr>
<td>Nimodipin</td>
<td>2.35±0.67**</td>
</tr>
<tr>
<td>AECC+Nimodipin</td>
<td>2.56±0.73**</td>
</tr>
<tr>
<td>Calphostin-C</td>
<td>3.9±0.94**</td>
</tr>
<tr>
<td>AECC+Calphostin-C</td>
<td>9.3±2.44**</td>
</tr>
</tbody>
</table>

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 1 hour. The study indicated that AECC, dixoizone, nimodipin and 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. Hence, in search for antidiabetic principle(s) from medicinal plants,4,18,19 the anti diabetic 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 chebula20,21 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. HbA1c has been used as indicator of diabetic state for long period studies. 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. HbA1c has been used as indicator of diabetic state for long period studies. The decrease in PPG level during long term study indicated better glucose management by AECC treatment.
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 pancreatic β-cells are widely used for management of type-1 as well as type-2 diabetes.\textsuperscript{29} ATP-sensitive K\(^+\) (K-ATP) dependent\textsuperscript{25} and K-ATP channel-independent\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 exocytosis 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.\textsuperscript{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 insulinoitropic 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 diazoxide 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.\textsuperscript{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 insulinoitropic action via DAG-PKC mediated pathway.

\section*{CONCLUSION}

The results of the study indicated that aqueous extract of \textit{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 \textit{in-vitro} study indicated that insulin secretagogues activity was dose dependent. The experiment related to mechanism study showed the insulinoitropic activity of AECC may be dependent on K\(^{-}\)ATP and Ca\(^{2+}\) channels and independent on DAG-PKC pathway.

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