Chapter IV

Antidiabetic Activity of Kalanchoe pinnata Lam.
1. Introduction

Type 2 diabetes mellitus has become a worldwide health problem and an important cause of morbidity and mortality. Through lifelong vascular complications, diabetes leads to excessive rates of myocardial infarction, stroke, renal failure, blindness, and amputations (Holt and Kumar 2010). The prevalence of both type 1 and type 2 diabetes are increasing. Type 2 diabetes is increasing far more rapidly, driven by increasing life expectancy and the epidemic of obesity. It is believed that there will be as many as 300 million people with diabetes worldwide by the year 2025. Type 2 DM results from disorders of insulin action and insulin secretion, either of which may be the predominant feature and both of which are usually present when the disease becomes clinically manifest. Type 2 DM is preceded by insulin resistance and impaired glucose tolerance (IGT). Once insulin resistance is pronounced, the likelihood of type 2 DM development depends on the ability of β-cells to adequately compensate by increasing insulin secretion.

Although no direct evidence is as yet available, both clinical investigation of diabetic patients and experimental studies of animal models suggest that the two pathways, triggering and amplifying, may be impaired in β-cells affected by type 2 diabetes (Ashcroft and Rorsman 2004). Hence there is decreased or unregulated insulin secretion in type II diabetic patients. The people who are in the type 2 prediabetic state already manifest some abnormalities in β-cell function or decrease β-cell mass (Clark et al. 2001). Insulin secretory abnormalities in type 2 diabetes mellitus are decreased glucose sensing, impaired ability to respond to elevations and reductions in glucose during an oscillatory glucose infusion, reduced or absent first-phase insulin secretion in response to intravenous glucose administration, reduced or absent early insulin secretory response to oral glucose, alterations in the rapid oscillations of insulin secretion, reduced effect of gastrointestinal hormones in potentiating glucose-mediated insulin secretion, inadequate insulin secretion for the magnitude of hyperglycemia (Porte and Kahn 1995; Kahn et al. 1997).

Therapy to correct hyperglycemia in type 2 diabetic patients must therefore be directed at decreasing insulin requirements in those who are insulin resistant, thereby bringing endogenous insulin secretion more closely in alignment with insulin need, or at
increasing the insulin available adequately to meet the insulin requirements, whether normal or increased. Numbers of therapeutic approaches such as insulin, insulin secretagogues, alpha-glucosidase inhibitors, insulin sensitizers etc. are used to control diabetes mellitus. Oral antihyperglycemic agents such as thiazolidinediones or metformin decrease insulin resistance; α-glucosidase inhibitors decreases postprandial insulin needs; insulin secretagogues improve and increase endogenous insulin secretion; and insulin and its analogues replace endogenous insulin secretion by exogenous insulin administration.

Understanding that decreased β-cell mass is an important factor in the pathogenesis of type 2 diabetes raises a concern regarding the application of drugs potentially harmful to the remaining β-cells. Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered. The ideal insulin secretagogue should have the following characteristics: it acts rapidly, so that insulin secretion is stimulated soon after meal ingestion; its effect is graded to increase as the plasma glucose increases from 60 to 180 mg/dl; it has little or no effect at plasma glucose levels of less than 60 mg/dl; and its duration of action is short so that is does not continue to stimulate insulin secretion beyond the postprandial period.

1.1 Kalanchoe pinnata Lam.

Kalanchoe pinnata (Lam.) Pers. (Family-Crassulaceae J. St.-Hill.) is cultivated as a popular garden plant. Kalanchoe pinnata (syn. Bryophyllum calycinum, Bryophyllum pinnatum) also known as the air plant, life plant, miracle leaf, Goethe plant. Kalanchoe pinnata is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also vegetatively from leaf bubils. It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers. The plant was native to Masagascar and has become naturalized in temperate regions of Asia, the Pacific and Caribbean.

Kalanchoe pinnata is used as traditional medicine worldwide to treat several ailments such as infections, rheumatism, and inflammation. Juice of the fresh leaves is used very effectively for the treatment of jaundice in folk medicines of Bundelkhand.
region of India. Beside the mentioned traditional use, various researchers shown to possess number of pharmacological activities of the *Kalanchoe pinnata*. The ethanolic extract shows significant wound healing activity (Nayak *et al*., 2010). The juice of the *Kalanchoe pinnata* leaves was studied in rats against CCl₄-induced hepatotoxicity and it was found that plant juice showed effective hepatoprotective action (Yadav and Dixit, 2003). Nephroprotective action *Kalanchoe pinnata* was assessed in gentamicin-induced rats and it was reported that aqueous extract of *Kalanchoe pinnata* shows the nephroprotective action. Antioxidant activity of the extract was postulated to helpful in nephroprotective action of the extract (Harlalka *et al*., 2007). The leishmaniasis are a complex of diseases caused by different species of the protozoan parasite *Leishmania* and are a major public health problem in many developing countries. Flavonoids from *Kalanchoe pinnata* reported with anti-leishmaniasis activity (Da Silva *et al*. 1995; Muzitano *et al*. 2006). *Kalanchoe pinnata* also shows immunosuppressive effects (Bergmann *et al*., 1994), anti-tumor activity (Supratman *et al*., 2001), neuropharmacological activity (Salahdeen and Yemitan, 2006), diuretic and anti-urolithiatic activity (Patil *et al*., 2009).

![Kalanchoe pinnata](image)

The *Kalanchoe pinnata* was also used in diabetes to lower blood glucose (Ojewole, 2005; Ogbonnia *et al*., 2008). Even in Kolhapur and surrounding areas *Kalanchoe* leaves are routinely consumed for control of hyperglycemia, however, its
mechanism of lowering of blood glucose is not known. Hence, the objective of present study was to scientifically evaluate antihyperglycemic activity of *Kalanchoe pinnata* in streptozotocin induced diabetic rats and to determine the probable mechanism of its action.

2. **Material and methods**

2.1 **Plant material**

Plant of *Kalanchoe pinnata* (Lam.) was collected from Shivaji University, Kolhapur, MS-India and identified from Department of Botany, Shivaji University, Kolhapur, MS-India. The voucher specimen (voucher number SUK-5279) is deposited in Department of Botany, Shivaji University, Kolhapur, MS-India.

2.2 **Extraction of fraction from steam distillate**

Fresh, healthy leaves of plant were collected and washed properly with running tap water. About 100 gm of leaves were crushed in mortar & pestle. It was then filtered through several layers of muslin cloth. The filtrate was then subjected to steam distillation at 60 °C. The distillate was collected and subjected to sequential extraction with organic solvents as petroleum ether (pet ether), chloroform followed by dichloromethane (DCM). Each fraction extracted was then evaporated in vacuum rotary evaporator and dried over anhydrous Sodium sulphate. The distillate remaining after the organic extraction was considered as aqueous fraction. The % yield of pet ether, chloroform and DCM fractions with respect to raw plant material was 0.0028%, 0.0066% and 0.0044% w/w respectively. All the dried fractions were stored in airtight screw cap glass vials at 4 °C until further use.

Each fraction was diluted with dimethylsulfoxide (DMSO) just before the use. The final concentration of DMSO not exceeds than 0.1% in each dilution. Vehicle control is used in each *in vivo* and *in vitro* experiment.
2.3 Induction of diabetes

Diabetes was induced in normal male rats by using streptozotocin as per method mentioned in previous chapter.

2.4 Oral Glucose Tolerance Test (OGTT)

Total 42 rats (6 normal and 36 diabetic) were fasted overnight with free access to water. Initial blood glucose of each rat was measured. They were divided into 7 groups (n=6) as normal control, diabetic control, positive control (glibenclamide 2.5 mg/kg body weight) and remaining four groups were as 1.0, 2.5, 5.0 and 10.0 mg DCM fraction/kg body weight. All rats were fed orally with glucose load of 3 mg/g body weight with or without test component. Blood glucose was measured with ACCU-CHECK at 0, 30, 60 and 120 min.

2.5 Experimental design

2.5.1 In vivo prolonged treatment

For prolonged experiment, total 30 male rats (6 normal and 24 diabetic) were divided into 5 groups as below.

Group I - Normal control
Group II - Diabetic control
Group III - Diabetic + glibenclamide (2.5 mg/kg body weight)
Group IV - Diabetic + 5 mg DCM fraction/kg body weight
Group V - Diabetic + 10 mg DCM fraction/kg body weight

The glibenclamide or DCM fraction was fed orally to the animals twice a day while control rats were fed with vehicle (0.1% DMSO).

2.6 Estimation of biochemical parameters

Fasting blood glucose level of all the groups were estimated by ACCU-CHECK on day 0, 8, 16, 24, 32, 40 and 45. Blood was collected from tail tip. At the end of 45
Days, all the rats were fasted overnight and sacrificed by cervical dislocation. Blood was withdrawn immediately and serum collected was used for biochemical assays. Whole blood was used for estimation of glycated haemoglobin. For lipid profile of all the groups; serum total cholesterol and HDL-Cholesterol was estimated by biochemical kit from Crest Biosystems while total triglycerides were estimated by liquid gold kit. Serum LDL and VLDL was calculated by using Friedewald’s formula as:

\[
\text{VLDL} = \text{Triglycerides}/5
\]
\[
\text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{VLDL}
\]

Serum insulin was measured by rat insulin ELISA kit (CalBiotech). The amount of insulin released was quantified by using ELISA microplate reader (Multiskan EX, Thermo Scientific) at 450 nm.

2.7 Estimation of liver glycogen content

Glycogen content of liver was estimated by KOH alkali method (Stetten and Katzen; 1961). Briefly, after sacrifice of experimental animal, one gram of liver was immediately removed, washed properly thrice with chilled saline and homogenized with 5 ml 30% KOH. The homogenate was boiled for 1 hr in boiling water bath. The color of solution turns brown. The homogenate was then cooled and neutralized to pH 7.0 under cold condition by using 1:1 diluted glacial acetic acid. The solution was centrifuged for 10 min at 3000 RPM. Supernatant was collected and used for the glycogen analysis by using the amyloglucosidase treatment. Supernatant was treated with amyloglucosidase in 0.2 M acetate buffer (pH 4.7) for 20 min. at 37 °C. After this enzymatic treatment the glucose released was measured by GOD-POD method. Difference of glucose concentration before and after enzymatic reaction gives the amount of glucose released by glycogen breakdown due to the amyloglucosidase action and it accounts for the glycogen content of tissue.

2.8 In vitro studies

2.8.1 Isolation of rat pancreatic islets
The isolation of islets was carried out using collagenase digestion method (Shewade et al., 1999) with some modifications (Patil et al., 2011). The detailed procedure is mentioned in the previous chapter.

2.8.2 Insulin release assay

Groups of 10 islets were placed in wells each containing 1 ml HBSS (pH 7.4) supplemented with 10 mmol/l HEPES and 2 mg/ml BSA. Cells were then incubated for 1 h with 11.8 mM glucose in presence of 2.5, 5, 10 and 20 µg of DCM fraction/ml of the reaction media. Glibenclamide (10 µg/ml) which is a commercially used sulfonylurea, was used as positive control. To illustrate probable mechanism of insulin secretion by Kalanchoe pinnata DCM fraction, isolated islets were incubated under following conditions as in presence of 2.8 mM glucose with or without DCM fraction, in presence of 11.8 mM glucose and diazoxide (an established opener of K⁺-ATP channel) with or without DCM fraction, in presence of 11.8 mM glucose and nifedipine (an established the L-type Ca²⁺ channel blocker) with or without DCM fraction. After incubation, supernatant from each well was collected and stored at -20 °C until further use. The insulin concentration in all the stored samples was determined by ELISA kit (CalBiotech) and quantified by using ELISA microplate reader (Multiskan EX, Thermo Scientific) at 450 nm.

2.8.3 Viability assessment by MTT conversion

The viability of isolated islets after treatment with Kalanchoe pinnata DCM fraction was assessed by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay (Mosman, 1983; Latha et al., 2009). Briefly, after withdrawing the supernatant medium from each well for estimation of insulin, the islets were allowed to settle at bottom and washed twice with HBSS (pH 7.4) supplemented with 10 mmol/l HEPES and 2 mg/ml BSA. After washing, 20 µl of MTT (5 mg/ml of stock prepared in HBSS, pH 7.4) was added to each well, and the cells were incubated in dark at 37 °C for additional 4 h. After 4 h incubation, 150 µl Dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals and absorbance was measured at 570 nm on ELISA microplate reader (Multiskan EX, Thermo Scientific). The viability of cells was
expressed in percent viability (\% viability) relative to untreated or control group cells which were considered as 100\% viable.

2.9 GC-MS analysis

GC-MS analysis of active fraction was carried out at Common Facility Center (CFC), Shivaji University, Kolhapur using a GCD-1800 A model (Shimadzu) of GC-MS. 0.2 \( \mu l \) of sample was injected on a HP-5 column with a starting temperature of 100 °C and a hold time of 2 min at 150-160 °C.

2.10 FTIR studies

Infrared (IR) spectra of the was carried out at Common Facility Center (CFC), Shivaji University, Kolhapur using a FTIR spectrometer in the range 450–4000 cm\(^{-1}\) by KBr pellet technique.

2.11 Qualitative phytochemical analysis of *Kalanchoe pinnata* DCM fraction

Primary screening and detection of constituents present in bioactive fraction was carried out by qualitative phytochemical investigation. Following screening tests are used for qualitative characterization (Wagner and Bladt 2001).

A) Test for carbohydrates

1) *Molish’s test*- In 2 ml chloroform extract, add 2 drops of alcoholic solution of \( \alpha \)-napthol. Shake well and add 1 ml concentrated sulphuric acid slowly. Violet ring confirms the presence of carbohydrates.

2) *Benedict’s test for reducing sugar*- 2 ml of extract mixed with 2 ml of Benedict’s reagent and boiled on water bath for 1–2 min. Reddish brown precipitate indicates carbohydrate is present.

3) *Fehling’s test*- 1 ml extract boiled in water bath with 1 ml of Fehling solution. Red precipitation confirms the presence of reducing sugar.

B) Test for saponins
5 mg of extract in 20 ml of distilled water. Shaken in graduated cylinder for 15 min. About 2 cm of foam layer confirms the presence of saponines.

C) Detection of phenolic compounds

1) Ferric chloride test- Add few drops of 5% FeCl₃ to extract. Presence of phenolic compound gives deep blue colour.

2) Lead acetate test- Add 10% lead acetate to the extract. Buffy white colour precipitate shows presence of phenolic content.

3) Dilute HNO₃ Test- Add few drops of dil. HNO₃ to extract. Presence of phenols produces reddish colour.

D) Test for flavonoids

1) Shinoda test- An extract was dissolved in 95% ethanol, warmed and filtered. To this sample, add 5 ml of 95% ethanol, few drops of conc. HCl and 0.5 gm of magnesium turnings or chips. Presence of flavonoids gives pink colouration.

2) Sodium hydroxide test for flavonoids- The sample was dissolved in water. About 2 ml of 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute HCl was an indication for the presence of flavonoids.

E) Tests for alkaloids

1) Mayer’s test- Added few drops of Mayer’s reagent to the extract. Alkaloids give cream color precipitate.

2) Hager’s test- To the extract, add 2-3 drops of Hager’s reagent. Alkaloids give yellow colour precipitate.

3) Dragendorff’s test- Few drops of Dragendorff’s reagent was added to the extract. Alkaloids give reddish brown or orange brown precipitate immediately after spraying.

4) Wagner’s test- To the extract, few drops of Wagner’s reagent was added. Alkaloids give reddish brown precipitate.

F) Test for terpenes
1) **Liebermann-Burchard test**: Extract was spotted on TLC plate and sprayed with freshly prepared reagent. Heat the plate at 100 °C for 5-10 min. Development of violet to blue or bluish-green colour indicates the presence of terpenes.

2) **Vanillin –Sulphuric acid test**: Spry the extract spotted plate with Vanillin-Sulphuric acid reagent. Heat the plate at 100 °C for 5-10 min. Presence of violet blue colour shows presence of terpenes.

3) **Antimony (III) chloride-acetic acid test (for diterpenes)**: Extract was spotted on TLC plate and sprayed with reagent. Heat the plate at 100 °C for 5-10 min. Diterpenes gives red-yellow to blue-violent spots.

**G) Test for proteins and amino acids**

1) **Biuret test**: Extract and drop of 2% copper sulphate, 1ml ethanol and KOH palate shows pink colour.

2) **Ninhydrin test**: Extract with 2 drops of Ninhydrin solution shows purple colour with amino acid.

**H) Cardiac glycosidase**

1) **Antimony (III) chloride reagent (for cardenolides and bufadienolides)**: Extract was spotted on TLC plate and sprayed with reagent and heated for 5-10 min. at 110 °C. Presence of cardenolides and bufadienolides gives grey, violet or brown colour.

2) **Anisaldehyde-Sulphuric acid reagent**: Spraying of TLC plate with Anisaldehyde-Sulphuric acid reagent and heating at 110 °C for 5-10 min bufadienolides shows a prominent blue colour.

**3 Statistical analysis**

All the data obtained was expressed as mean ± SD. Statistical analysis was performed using ANOVA and Unpaired Student t-test. A value with $p < 0.05$ was considered as statistically significant while $p < 0.005$ as extremely significant.
4 Results and discussion

4.1 Evaluation of antihyperglycemic activity of *Kalanchoe pinnata* extracts through oral glucose tolerance test (OGTT)

*Kalanchoe pinnata* is routinely used in traditional medicines in several regions of the world for the treatment of several diseases. *Kalanchoe pinnata* was previously reported for its antihyperglycemic effect. The leaf aqueous extract of *Bryophyllum pinnatum* (400 mg/kg rat body weight) reported with significant hypoglycaemia in streptozotocin induced diabetic rats. The leaf aqueous extract of *Bryophyllum pinnatum* was shown to possess antinociceptive and anti-inflammatory activity in addition to antihyperglycemic activity in the same study. The different flavonoids, polyphenols, triterpenoids and other chemical constituents of the herb are speculated to account for the observed antinociceptive, anti-inflammatory and antidiabetic properties of the plant (Ojewole, 2005).

Another species of *Kalanchoe* viz *Kalanchoe crenata* was reported to possess antihyperglycemic activity. The antihyperglycemic water–ethanol extract of *Kalanchoe crenata* was assessed in diabetic rats which are induced by subjecting Wistar rats to a hypercaloric sucrose diet over 4 months. Six hours after a single oral administration of water–ethanol extract of *Kalanchoe crenata* 135 and 200 mg kg$^{-1}$ body weight extracts significantly ($p<0.01$) reduced the blood glucose levels both in normal and diabetic rats. During the treatment of diabetic rats 200 mg/kg water–ethanol extract of *Kalanchoe crenata* administered daily for 4 weeks significantly reduced blood glucose levels within week 1 ($p<0.05$), with a maximum effect at week 4 (−52%, $p<0.01$), while maintaining glycaemia within the normal range. The authors proposed that *K. crenata* might contain important chemical components that could induce significant improvement in glucose clearance and/or uptake and resistance to body-weight gain and insulin sensitivity (Kamgang *et al.*, 2008).

Though antihyperglycemic activity of the plant was reported previously, the bioactive fraction responsible for the antihyperglycemic activity and mechanism of its action is still unknown. In present study, instead of whole leaves extract, a fractions isolated from steam distillate were used which greatly eliminates non-volatile components of whole extract that may have other activities. The doses used in the present study were much less than the reported non-toxic doses (Torres-Santos *et al.*, 2008).
2003) and did not show any toxicological effects as evidenced in biochemical parameters and no behavioural changes in the experimental animals.

The antihyperglycemic activity of petroleum ether, chloroform, dichloromethane and aqueous fraction extracted from *Kalanchoe pinnata* steam distillate were evaluated through oral glucose tolerance test (OGTT). The results are shown in Table 1. The results were compared with diabetic rats as negative control and glibenclamide (2.5 mg/kg body weight) as a positive control. The petroleum ether, chloroform and dichloromethane extracts were used at a concentration of 10 mg/kg body weight while that of aqueous fraction was used at a concentration of 10 ml/kg body weight.

**Table 1- Effect of solvent fractions isolated from *Kalanchoe pinnata* on blood glucose levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dl)</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Treatment and dose/kg body wt)</td>
<td></td>
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</tr>
<tr>
<td>Normal control</td>
<td>75.5±6.6</td>
<td>103.7±4.7</td>
<td>93.5±4.5</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>245.3±7.6</td>
<td>546.2±9.7</td>
<td>462.8±8.3</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (2.5 mg/kg)</td>
<td>243.3±6.6</td>
<td>368.7±6.4*</td>
<td>282.0±7.3**</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Pet ether fraction (10 mg/kg)</td>
<td>254.3±6.9</td>
<td>527.7±7.4*</td>
<td>431.7±6.7**</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Chloroform fraction (10 mg/kg)</td>
<td>240.7±5.8</td>
<td>453.5±5.8**</td>
<td>377.3±7.8**</td>
<td></td>
</tr>
<tr>
<td>Diabetic + DCM fraction (10 mg/kg)</td>
<td>254.5±7.2</td>
<td>332.0±8.6**</td>
<td>268.2±6.0**</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Aqueous fraction (10 ml/kg)</td>
<td>234.7±5.9</td>
<td>541.3±5.1</td>
<td>448.7±6.0*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6. *p*<0.01 and **p**<0.001 with respect to diabetic control.

In diabetic control rats, blood glucose levels progressively increases at 60 min and remained high even after a period of 120 min as compared to normal control.
Glibenclamide is a commercially used sulfonylurea drug in treatment of diabetes mellitus. Glibenclamide at a concentration of 2.5 mg/kg rat body weight showed very good antihyperglycemic action. Among the four fractions, DCM fraction showed most prominent antihyperglycemic action. The antihyperglycemic potency and glucose level lowering pattern of DCM fraction resembles with that of glibenclamide control; however it requires higher concentration of 10 mg/kg body weight as compared to glibenclamide (2.5 mg/kg body weight). The chloroform fraction also showed antihyperglycemic action but is not as effective as compared to the DCM fraction. The remaining two fractions viz. pet ether fraction and aqueous fractions were not able to reduce blood glucose levels as compared to glibenclamide control and DCM fraction group. Hence, the antihyperglycemic activity of Kalanchoe pinnata was supposed due to DCM fraction extracted from Kalanchoe pinnata steam distillate.

All four extracts were further evaluated for insulin secretagogue effect on isolated islets.

4.2 Insulin secretagogue effect of extracts isolated from Kalanchoe pinnata

The insulin secretagogue effect of pet ether, chloroform, DCM and aqueous fractions were assessed and results are shown in Figure 1.

Insulin secretion in presence of 11.8 mM glucose was considered as control. Glibenclamide, a commercially used sulfonylurea, at a concentration 10 µg/ml was used as positive control. The pet ether, chloroform and DCM extracts were used at a concentration of 10 µg/ml while that of aqueous fraction was used at a concentration of 100 µl/ml. Insulin secretion in each test was expressed in terms of μIU/10 islets/60 min.

Among all the four extracts, the DCM extract showed maximum insulin secretion (132.56 IU) as compared to other fractions. The insulin stimulation due to DCM extract was almost comparably to insulin secretion in presence of glibenclamide (128.34 IU). Among the other extracts, chloroform fraction showed moderate while pet ether and aqueous fractions showed slight insulin stimulatory action.
Figure 1. Effect of pet ether, chloroform, DCM and aqueous fractions on insulin secretion

![Insulin secretion graph]

Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from 11.8 mM glucose control.

DCM extract showing maximum activity in both *in vivo* and *in vitro* experiment was carried forward for further studies.

### 4.3 Dose dependent antihyperglycemic activity of DCM extract

The dose dependent effect of DCM extract on blood glucose level was evaluated by using four different concentrations as 1, 2.5, 5 and 10 mg/kg body weight. The results are tabulated in Table 2. In OGTT, *Kalanchoe pinnata* DCM fraction showed very good antihyperglycemic activity in a dose dependent manner.

At lowest concentration (i.e. 1.0 mg/kg body weight) DCM fraction slightly able to reduce the blood glucose levels as compared diabetic control. The antihyperglycemic activity of fraction at 2.5 mg/kg body weight is nearly comparable to the activity of glibenclamide (at the same dose of 2.5 mg/kg body weight). Increase in the concentration...
of DCM extract further reduces the blood glucose levels in a dose dependent manner. Among four concentrations, the maximum concentration used (10 mg/kg body weight) showed most prominent antihyperglycemic activity.

Table 2. Dose dependent antihyperglycemic activity of DCM extract through OGTT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Normal control</td>
<td>78.7±3.6</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>244.5±8.1</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (2.5 mg/kg)</td>
<td>243.7±7.4</td>
</tr>
<tr>
<td>Diabetic + 1.0 mg DCM fraction</td>
<td>252.0±8.9</td>
</tr>
<tr>
<td>Diabetic + 2.5 mg DCM fraction</td>
<td>2870.7±9.2</td>
</tr>
<tr>
<td>Diabetic + 5 mg DCM fraction</td>
<td>255.0±8.2</td>
</tr>
<tr>
<td>Diabetic + 10 mg DCM fraction</td>
<td>261.2±8.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6. *p<0.05 and **p<0.005 with respect to diabetic control.

4.4 Prolonged treatment effect of DCM extract on experimental animals

In a prolonged treatment, two concentrations of the DCM fractions viz. 5 mg and 10 mg/kg body weight are used in treatment groups. Diabetic rats administrated with vehicle (0.1% DMSO) are considered as negative control while those treated with glibenclamide (2.5 mg/kg body weight) are considered as positive control.
4.4.1 Changes in blood glucose, glycated hemoglobin, serum insulin and liver glycogen

The changes in blood glucose, glycated hemoglobin, serum insulin and liver glycogen of long term treated rats are shown in Table 3. After 45 days, there is increase in fasting blood glucose level of diabetic control comparing the glucose value at the day 1<sup>st</sup>. Glibenclamide (2.5 mg/kg body weight) showed effective control on fasting blood glucose levels. It was observed that DCM fraction significantly reduced the fasting blood glucose levels at both the concentration 5 mg and 10 mg/kg body weight as compared to diabetic control. The glycemic control was more pronounced at dose of 10 mg/kg body weight and it was even better than glibenclamide treated group. Thus, the DCM fraction of <i>K. pinnata</i> demonstrates good antihyperglycemic action even in prolonged treatment.

Table 3- <i>In vivo</i> long term effect of <i>Kalanchoe pinnata</i> DCM fraction on blood glucose, glycosylated haemoglobin, serum insulin.

<table>
<thead>
<tr>
<th>Group (Treatment and dose)</th>
<th>Blood glucose (mg/dl)</th>
<th>GHb (%)</th>
<th>Serum insulin (µIU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 45</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>78.3±6.5</td>
<td>81.7±5.7</td>
<td>5.06±0.67</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>218.7±14.8</td>
<td>359.0±11.8</td>
<td>12.94±0.92</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>228.3±17.6</td>
<td>116.3±15.8**</td>
<td>7.3±1.1**</td>
</tr>
<tr>
<td>(2.5 mg/kg body wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic + 5 mg DCM</td>
<td>262.7±15.0</td>
<td>154.7±7.6**</td>
<td>10.93±1.15</td>
</tr>
<tr>
<td>fraction/kg body wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic + 10 mg DCM</td>
<td>245.3±15.5</td>
<td>109.3±10.4**</td>
<td>8.47±1.39*</td>
</tr>
<tr>
<td>fraction/kg body wt</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are mean ± SD; n=6. *p<0.05 and **p<0.005 with respect to diabetic control

In diabetic control group there is very high percentage of glycated haemoglobin (GHb) value (12.94%) as compared to the normal control (5.06%). There is a controlled
glycated Hb level in glibenclamide treated rats. An effective and dose dependent control on GHb levels in DCM extract treated rats was observed. The treatment also improved the serum insulin levels both at 5 mg and 10 mg/kg body weight dose as compared to diabetic control. The improvement in serum insulin level at dose of 10 mg/kg body weight is equal to that of glibenclamide treated rats. The effect on liver glycogen was shown in Table 4. There is improvement in liver glycogen content in glibenclamide and DCM treated rats as compared to diabetic control rats.

Table 4. Effect of long term treatment on liver glycogen content

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver glycogen (mg/gm of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.43±1.34</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>2.28±0.66</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (2.5 mg/kg body wt)</td>
<td>4.06±0.85*</td>
</tr>
<tr>
<td>Diabetic + DCM fraction 5 mg/kg body wt</td>
<td>3.96±0.38*</td>
</tr>
<tr>
<td>Diabetic + DCM fraction 10 mg/kg body wt</td>
<td>4.91±0.86*</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6. *p<0.05 and **p<0.005 with respect to diabetic control

4.4.2 Lipid profile

The antilipidemic action of aqueous ethanol (80%) extracts of T. africana leaves and K. pinnata plants and their mixture, in an equal proportion, were evaluated on postprandial glycaemic status and lipid profile in prolonged treatment for 21 days was evaluated previously (Ogbonnia et al., 2008). The dose of 500 mg/kg (1:1) mixture of extracts was reported to reduce postprandial blood glucose levels significantly. The treated rats also showed significant reduction in triglyceride levels, low density lipoprotein (LDL) level, and increase in high density lipoprotein (HDL) levels (Ogbonnia
et al., 2008). However, the reported effect due to the mixture of *T. africana* and *K. pinnata* aqueous ethanol (80%) extracts. The prolonged study of *K. pinnata* alone or its active fraction was not reported. Hence, the effect of prolonged treatment of bioactive *K. pinnata* DCM fraction was further evaluated.

The effect of DCM fraction on lipid profile was summarized in Table 5. There was improvement in lipid profile of rats treated with 5 mg DCM fraction/kg body weight but significant improvement was observed in rats treated with 10 mg DCM fraction/kg body weight as compared to diabetic control rats. The levels of triglycerides, total cholesterol, LDL and VLDL were significantly reduced whereas HDL was significantly increased in treated rats. The improvement in HDL and decrease in LDL which is very pronounced as compared to diabetic control would have important significance in prevention of cardio-vascular diseases (CVD).

Table 5 - *In vivo* long term effect of *Kalanchoe pinnata* DCM fraction on serum lipid profile.

<table>
<thead>
<tr>
<th>Group (Treatment and doses)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>73.3±3.8</td>
<td>70.9±4.9</td>
<td>44.1±4.8</td>
<td>15.0±4.2</td>
<td>14.2±1.0</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>131.5±5.5</td>
<td>127.1±5.9</td>
<td>26.3±4.0</td>
<td>79.8±1.7</td>
<td>25.4±1.2</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (2.5 mg/kg body wt)</td>
<td>94.4±5.3**</td>
<td>89.7±4.1**</td>
<td>37.7±4.0*</td>
<td>38.8±1.2**</td>
<td>17.9±0.8**</td>
</tr>
<tr>
<td>Diabetic + 5 mg DCM fraction/kg body wt</td>
<td>115.3±4.2*</td>
<td>113.4±3.5*</td>
<td>30.0±2.5</td>
<td>62.6±3.9**</td>
<td>22.7±0.7*</td>
</tr>
<tr>
<td>Diabetic + 10 mg DCM fraction/kg body wt</td>
<td>91.5±4.5**</td>
<td>99.0±5.9**</td>
<td>36.2±4.0*</td>
<td>35.5±1.9**</td>
<td>19.8±1.2**</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n=6. *p<0.05 and **p<0.005 with respect to diabetic control
5 Isolation and characterization of bioactive component from DCM extract of *K. Pinnata*

5.1 Qualitative phytochemical analysis of *Kalanchoe pinnata* DCM fraction

Primary qualitative phytochemical analysis of *Kalanchoe pinnata* DCM fraction was shown in Table 4. The extract was tested for presence of carbohydrates, saponins, phenolics, flavonoids, alkaloids, terpenes, proteins, amino acids, and cardiac glycosidases. The carbohydrates, saponins, alkaloids, proteins, amino acids, and cardiac glycosidase compounds are absent in the fraction. The major compounds present in the DCM fraction of *Kalanchoe pinnata* are phenols, flavonoids and terpenes.

Table 6. Qualitative phytochemical analysis of *Kalanchoe pinnata* DCM fraction

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Test For carbohydrates</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test For carbohydrates</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Test for saponins</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Test for phenolic compounds</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Test for flavonoids</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tests for alkaloids</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Test</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fehling’s test</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Benedict’s test</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Barfoed’s test</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Molish’s test</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Test for saponins</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Test for phenolic compounds</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ferric chloride test</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>Lead acetate test</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>Dilute HNO₃ Test</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Test for flavonoids</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Shinoda test</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>Sodium hydroxide test for flavonoids</td>
<td>Positive</td>
</tr>
</tbody>
</table>
In primary qualitative phytochemical analysis it was observed that carbohydrates, saponins, alkaloids, proteins, amino acids are absent in the fraction. The major compounds present in the DCM fraction of *Kalanchoe pinnata* are phenols, flavonoids and terpenes. Cardiac glycosides are major compound present in the *Kalanchoe* species. These cardiac glycosides are toxic to animals. In South Africa and Australia, where these plants are found in the wild, cattle and sheep poisonings are common. *Kalanchoe* species toxicity is primarily due to a group of bufadienolide compounds, including bryotoxins, bryophyllins, and bersalgenins. Bufadienolides are cardiac glycosides that are similar to digitalis compounds. Inhibition of the cellular membrane sodium-potassium pump (Na⁺, K⁺- ATPase enzyme system) produces the cardiotoxic effects (Geof 2004; Krenn and Kopp 1998). In present study, DCM fraction extracted from *Kalanchoe pinnata* distillate does not showed presence of any cardiac glycosidase compounds. The process of

<table>
<thead>
<tr>
<th>Test for terpenes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Liebermann-Burchard test</td>
<td>Positive</td>
</tr>
<tr>
<td>Vanillin –Sulphuric acid test</td>
<td>Positive</td>
</tr>
<tr>
<td>Antimony (III) chloride-acetic acid test</td>
<td>Positive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test for proteins and amino acids</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biuret test</td>
<td>Negative</td>
</tr>
<tr>
<td>Ninhydrin test</td>
<td>Negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test for Cardiac glycosidase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony (III) chloride reagent</td>
<td>Negative</td>
</tr>
<tr>
<td>Anisaldehyde-Sulphuric acid reagent</td>
<td>Negative</td>
</tr>
</tbody>
</table>
distillation eliminates number of compounds including cardiac glycosides and hence did not show any toxicity in experimental animals.

5.2 GC-MS analysis

The bioactive DCM fraction was subjected to GC-MS analysis. The main chromatogram of GC-MS analysis is shown in Figure 2. In GC-MS, only a single major peak was observed at a retention time (RT) of 15.478.

Figure 2. GC-MS spectrum of bioactive DCM extract

The mass fragmentation of the compound was shown in Figure 3. The fragmentation pattern does not match any compound present in a NIST library. The mass of this unknown compound was postulated to be 496 with a base peak of 150.

Figure 3. Mass fragmentation pattern of compound at RT 15.478.

5.3 FTIR analysis

The FTIR spectrum of the unknown compound is shown in Figure 4.
Figure 4. FTIR spectrum of unknown compound from the DCM fraction

The peak observed and probable functional group in the fraction

<table>
<thead>
<tr>
<th>Peak observed in FTIR</th>
<th>Probable function group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3054</td>
<td>C-H stretch (aromatic)</td>
</tr>
<tr>
<td>2987</td>
<td>C-H stretch (alkyl)</td>
</tr>
<tr>
<td>1598 and 1422</td>
<td>C-C stretch (in aromatic ring)</td>
</tr>
<tr>
<td>1265</td>
<td>C-O stretch in</td>
</tr>
<tr>
<td>1033</td>
<td>C-O stretch (cyclic ether) or C-H bending in plane</td>
</tr>
<tr>
<td>746 and 705</td>
<td>C-H &quot;oop&quot; or out-of-plane bands</td>
</tr>
</tbody>
</table>

The FTIR spectrum of the unknown compound was shown in Figure 4. In FTIR spectrum, peak at 3054 cm$^{-1}$ represents aromatic C-H stretch, peak at 2987 cm$^{-1}$ represents alkyl C-H stretch while peaks at 1598 and 1422 shows C-C stretch in aromatic
ring. The two bands present in 1300-1000 cm\(^{-1}\) region viz. at 1265 and 1033 cm\(^{-1}\) indicates the presence of phenyl alkyl ether while strong peak at 746 represents C-H "oop" or out-of-plane bands.

Hence, from the GC-MS and FTIR analysis it can be predicted that single unknown major compound present in DCM fraction of *Kalanchoe pinnata* may be a phenyl alkyl ether derivative with probable molecular weight of 496. From the structural data it is observed that DCM contains a single unknown compound with a probable molecular weight to be 496.

6 *In vitro* studies of isolated compound on isolated pancreatic islets

The dose dependent effect DCM fraction on *in vitro* insulin secretion is shown in Figure 5.

Figure 5. Dose dependent effect of DCM fraction on insulin secretion

Results are mean ± S.D.; n=6. *p*<0.05 and **p*<0.005 significant from 11.8 mM glucose control.
Insulin secretion in presence of 11.8 mM glucose was considered as negative control. Glibenclamide, a commercially used sulfonylurea was used as positive control. The insulin released in presence of DCM fraction (2.5, 5, 10 and 20 µg/ml reaction mixture) and glibenclamide (10 µg/ml) was expressed in terms of µIU/10 islets/60 min. Glibenclamide (10 µg/ml) showed 3.07 fold insulin secretion than 11.8 mM glucose control. The DCM fraction of *Kalanchoe pinnata* showed dose dependent *in vitro* insulin secretion up to a concentration of 10 µIU/10 islets/60 min. The insulin secretion at 10 µg/ml concentration was equal to that of glibenclamide control. No significant increase was observed at higher concentration of 20 µg/ml of the plant extract. The viability of islets after experiment was assessed by MTT assay (Figure 6).

![Figure 6. Viability assessment of islets by MTT conversion](image)

The viability of islets incubated at 11.8 mM glucose was considered as 100%. Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from glibenclamide control.

MTT (3-[4, 5-dimethylthiazol-2-y1]-2, 5-diphenyltetrazolium bromide) is a yellow water soluble tetrazolium salt. The dye is converted to water-insoluble purple formazan on the reductive cleavage of its tetrazolium ring by the succinate dehydrogenase system of the active mitochondria (Slater *et al*. 1963). Thus, the amount
of formazan formed can be determined spectrophotometrically and serves as an estimate of the number of mitochondria and hence the number of living cells in the sample (Denizot and Lang 1986). The viability of cells was expressed in percent viability (\% viability) relative to 11.8 mM glucose control group cells which were considered as 100% viable.

The viability of glibenclamide treated islets was 94.56\%. The viability of islets was not much affected by 2.5, 5 and 10 \(\mu\)g DCM fraction/ml (i.e. 95.02, 96.07 and 92.06 \% respectively) but there was slight decrease in viability (i.e. 82.59\% viability) at a 20 \(\mu\)g DCM fraction/ml. Hence, the concentration 10 \(\mu\)g DCM fraction/ml reaction mixture showing optimum secretion and viability of cells was used for further in vitro studies.

7 Evaluation of mechanism of insulin secretagogue activity of unknown compound from DCM fraction

Insulin secretagogues can stimulate insulin secretion in number of ways. To illustrate probable pathway for insulin stimulatory action of *Kalanchoe pinnata*, its effect in absence of extracellular glucose, its dependency on \(K^+\)-ATP channel and its dependency on \(Ca^{2+}\) channel was assessed.

7.1 Effect of basal glucose (2.8 mM)

Effect of basal glucose (2.8 mM) on insulin stimulatory activity of *Kalanchoe pinnata* DCM fraction was evaluated (Figure 7). At a concentration of 10 \(\mu\)g/ml in presence of 2.8 mM glucose, *Kalanchoe pinnata* DCM extract showed 2.70 folds increase in insulin secretion.

The finding that the DCM fraction stimulates 2.70 folds increase in insulin secretion at basal glucose concentration (i.e. 2.8 mM), suggested that *Kalanchoe pinnata* DCM fraction can stimulate insulin production in a glucose independent manner.
Figure 7. Effect of basal glucose on insulin secretagogue action of *Kalanchoe pinnata* DCM fraction

![Graph showing effect of basal glucose on insulin secretagogue action of Kalanchoe pinnata DCM fraction]

Results are mean ± S.D.; n=6. **p<0.0005 significant from 2.8 mM glucose control.

### 7.2 Role of K⁺-ATP channel in insulin stimulatory activity of unknown compound from DCM fraction

The K⁺-ATP channel plays pivotal role in insulin secretion (Ashcroft and Gribble, 1999). Insulin secretagogues like glucose or sulphonylurea lead to insulin secretion by closure of K⁺-ATP channel which lead to series of events like depolarization of cell membrane and increases in intracellular Ca²⁺ concentration through voltage-dependent Ca²⁺ channel. The increased [Ca²⁺]ᵢ which ultimately leads to exocytosis of insulin (Bratanova-Tochkova *et al*., 2002). The insulin stimulatory effect of glucose and sulphonylureas was abolished by diazoxide which prevents the closure of K⁺-ATP channel (Henquin *et al*., 1982; Dunne and Petersen, 1991).

The effect of diazoxide (opener of K⁺-ATP channel) on insulin stimulatory activity of *Kalanchoe pinnata* DCM fraction was shown in Figure 8. Insulin secretion in presence of 11.8 mM was considered as control. The insulin secretion at 11.8 mM glucose was 38.24 µIU. DCM fraction at concentration 10 µg/ml in presence of 11.8 mM glucose stimulates the insulin secretion to 108.61 µIU. When treated with 300 µM
diazoxide (an established opener of K⁺-ATP channel) in presence of 11.8 mM glucose, the secretion was inhibited and insulin secretion dropped from 38.24 to 17.13 µIU. The DCM fraction was not able to augment the insulin secretion in presence of diazoxide.

From the results it is found that presence of diazoxide (150 µM) abolishes the insulin secretagogue action of *Kalanchoe pinnata* DCM fraction. Hence K⁺-ATP channel plays vital role in the insulin secretagogue action of *Kalanchoe pinnata* DCM fraction. The action of *Kalanchoe pinnata* DCM fraction may be due to closure of K⁺-ATP channel.

Figure 8. Effect of diazoxide (opener of K⁺-ATP channel) on insulin secretagogue action of *Kalanchoe pinnata* DCM fraction

Insulin secretion in presence of 2.8 mM glucose was considered as a negative control. The insulin release in other test groups was expressed in terms of fold increase with respect to this 2.8 mM glucose control. Results are mean ± S.D.; n=6. *p<0.05 is significant from 11.8 mM glucose control.
7.3 Role of extracellular Ca\textsuperscript{2+} in insulin stimulatory activity of unknown compound from *Kalanchoe pinnata* DCM fraction

Increase in [Ca\textsuperscript{2+}], whether by influx of extracellular Ca\textsuperscript{2+} or by release of Ca\textsuperscript{2+} from intracellular stores plays important role in insulin secretion (Henquin, 2004; Tengholm and Gylfe 2009). To evaluate Ca\textsuperscript{2+} dependency for insulin secretion, islets were treated with 11.8 mM glucose in presence of 20 µM nifedipine with or without *Kalanchoe pinnata* DCM fraction. Nifedipine is L-type Ca\textsuperscript{2+} channel blocker which prevents the influx of extracellular Ca\textsuperscript{2+} and hence abolishes insulin secretion (Giugliano 1980). Effect of nifedipine on insulin stimulatory activity of *Kalanchoe pinnata* DCM fraction was shown in Figure 9.

Figure 9. Effect of nifedipine (the L-type Ca\textsuperscript{2+} channel blocker) on insulin secretagogue action of *Kalanchoe pinnata* DCM fraction

Insulin secretion in presence of 11.8 mM glucose was considered as a control. Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from 11.8 mM glucose control.

In presence of nifedipine (20 µM) with 11.8 mM glucose, there was inhibition in insulin secretion i.e. insulin secretion drops from 40.75 µIU to 21.68 µIU. In presence of
DCM fraction (10 µg/ml), the insulin secretion inhibition due to nifedipine was released to some extent. In presence of DCM fraction along with nifedipine and 11.8 mM glucose, there is a 64.17 µIU insulin secretion was observed.

It was observed that nifedipine was not able to completely abolish the stimulatory effect of Kalanchoe pinnata DCM fraction. This suggested that the DCM fraction may induce insulin secretion by mobilizing Ca\(^{2+}\) from intracellular stores. Plant extract like Ocimum sanctum leaf extract having similar type of insulin stimulating mechanism was previously reported. Diazoxide inhibited the insulin-releasing effects of the Ocimum sanctum leaf extract and suggests that closure of K\(^{+}\)-ATP channels participates in the overall mechanism of action of O. sanctum. Also presence of verapamil (a voltage-dependent Ca\(^{2+}\) channel blocker) and omission of Ca\(^{2+}\) from buffer did not completely abolish the insulin-secretory effects, suggesting that the extract can induce mobilization of intracellular Ca\(^{2+}\) as well as promoting Ca\(^{2+}\) entry (Hannan et al. 2006).

Effect of all the above test challenges on islets viability was evaluated (Figure 10). The viability of islets was not less than 90% in all the above test conditions.

Figure 10 - Viability assessment of islets by MTT conversion

The viability of islets incubated at 2.8 mM glucose was considered as 100%. Results are mean ± S.D.; n=6. *p<0.05 and **p<0.005 significant from 11.8 mM glucose control.
8 Conclusion

In conclusion, DCM fraction extracted from Kalanchoe pinnata leaves distillate possesses significant antihyperglycemic activity. Prolonged treatment for 45 days also improved the lipid profile, glycated Hb and liver glycogen levels of treated rats. The antihyperglycemic activity and improved plasma insulin levels in long term treated rats is due to the insulin secretagogue action of Kalanchoe pinnata DCM fraction. The plant DCM fraction shows significant and dose dependent insulin secretion in vitro. The insulin secretagogue action of DCM fraction was glucose independent. The $K^+$-ATP channel plays vital role in insulin stimulatory activity of Kalanchoe pinnata DCM fraction and hence the action of the DCM fraction is a $K^+$-ATP channel dependent secretagogue. Blocking the influx of $Ca^{2+}$ could not completely abolishes the insulin secretion which suggested that the DCM fraction may induce insulin secretion by mobilizing $Ca^{2+}$ from intracellular stores. The in vivo and in vitro findings suggest that DCM fraction extracted from Kalanchoe pinnata leaves steam distillate is beneficial in diabetes therapy.

9 References


