CHAPTER 6

a) Effect of Feeding Different Levels of 80% Methanolic Extract of CnI (MEC) in Diabetic Rats

It is important to prevent diabetes and its complications with careful monitoring of glycemic control. Though the oral hypoglycemic agents are valuable in treatment of diabetes mellitus, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects (Bailey et al., 1989). Traditional medicines derived mainly from plants played an important role in the management of diabetes (Zhou et al., 2011). Herbal medicine is thought to provide comparative advantage by reason of the diverse bioactive secondary metabolites present. However, adequate research on medicinal plants beyond screening for biological activity should be conducted with the aim to systematically standardize and develop them into natural therapeutic products or dosage forms which should effectively complement or supplement existing conventional measures (Bauer et al., 1996; Ong, 2004).

As mentioned earlier, one of the etiological factors implicated in the development of diabetes and its complications is the damage induced by free radicals and reactive oxygen species. Hence, an antidiabetic compound with antioxidant properties would be more beneficial. From the previous studies, it is evident that methanolic extract of CnI possesses better antihyperglycemic, antioxidant and antiglycative properties than ethanol and ethyl acetate extracts. But, it is pertinent to find out the effective dose at which CnI methanol extract exerts maximum antidiabetic effect. So the current investigation in furtherance of that previous study evaluated the dose dependent antidiabetic effect of the 80% methanol extract of CnI (MEC) in streptozotocin induced diabetic rats.
Materials and methods

Chemicals

Streptozotocin was purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Methanol was purchased from Merck KGaA, Darmstadt, Germany. Kit for glucose estimation was purchased from Agappe Diagnostics, Thane, India. Insulin assay kit was purchased from SP-BIO, France. All other chemicals used were of the highest analytical grade.

Methods

Collection of CnI and procedure for preparation of methanol extract of CnI were described in chapter 2.

In vivo experiments

Male albino rats of Sprague Dawley strain weighing 150-200 g were used for the study. The animals were housed individually in polypropylene cages in a room maintained at 25±5°C with a 12 h light and 12 h dark cycle. Rats in all the groups were given standard laboratory animal feed and water *ad libitum*. Animals were divided into 5 groups of 6 rats each and treated as follows:

Group I - Normal Control
Group II - Diabetic Control
Group III - Diabetic + MEC (100 mg)
Group IV - Diabetic + MEC (200 mg)
Group V - Diabetic + MEC (400 mg)

Group I rats were served as normal control, while rats in group II were served as diabetic control. Rats in group III were given 80% methanol extract of CnI (MEC) at a dose of 100 mg/kg body weight/day orally by intra gastric intubation. Group IV rats and group V rats were orally administered with 200 mg/kg body weight and 400 mg/kg body weight of MEC respectively per day. Different test doses of MEC were suspended in sterile water prior to intra gastric feeding. Diabetes was induced in rats of groups II,
III, IV and V by a single intraperitoneal injection of streptozotocin at a dose of 45 mg/kg body weight, dissolved in 0.1M citrate buffer pH 4.5. The blood glucose level was checked 72 h after STZ injection. The animals were considered diabetic when the fasting blood glucose level was raised beyond 200 mg/dL.

The treatment lasted for 45 days. After the treatment period, the rats were euthanized and liver was taken out, transferred to cold containers and serum was separated from the blood samples and used for the biochemical analyses. For immunohistochemical analysis, the pancreas was removed from each rat and fixed in Bouin's fixative. The procedures for analysis of biochemical parameters and immunohistochemistry were described in chapter 2.

Results

6.1a Concentration of serum glucose and liver glycogen

Table 6.1a Concentration of serum glucose and liver glycogen

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum glucose (mg/dL)</th>
<th>Liver glycogen (mg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>75.06±8.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.66±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>221.63±20.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>III</td>
<td>121.83±11.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.78±0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>110.48±10.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.02±0.35&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>130.32±11.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.62±0.25&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>F value</td>
<td>101.21</td>
<td>25.88</td>
</tr>
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</table>

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.
Effect of Feeding Different Levels of 80% Methanolic Extract of Cnl (MEC) in Diabetic Rats

There was a significant increase in the concentration of glucose in the serum of diabetic control rats compared to the normal rats. Diabetic rats fed different levels of MEC showed a significant reduction in the serum glucose concentration compared to diabetic control. Liver glycogen in diabetic rats was found to be significantly decreased compared to the normal control. Treatment with MEC significantly increased the levels of liver glycogen in diabetic rats compared to diabetic control animals (Table 6.1a). These effects were predominant in rats treated with MEC at a dose of 200 mg.

6.2a Concentration of serum insulin

Serum insulin concentrations were significantly decreased in diabetic control rats compared to normal rats. Oral treatment of different levels of MEC to diabetic rats caused a significant increase in the levels of serum insulin compared to diabetic control rats. Serum insulin levels of rats treated with MEC at a dose of 200 mg were higher than those rats treated with 100 and 400 mg MEC (Figure 6.1a).

**Figure 6.1a Concentration of serum insulin (ng/mL)**

![Concentration of serum insulin](image)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.
6.3a Level of serum glycated Hb

A significant elevation in the levels of glycated Hb was observed in the serum of diabetic control rats compared to the normal animals. Oral administration with MEC significantly reversed increasing levels of serum HbA$_1c$ in diabetic rats indicating the potential of MEC in glycemic control. Those rats administered with MEC (200 mg) showed better reduction in glycated Hb levels compared to the other two doses (Figure 6.2a).

**Figure 6.2a Level of serum glycated Hb in percentage of total Hb**

![Graph showing levels of serum glycated Hb](https://via.placeholder.com/150)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.

6.4a Immunostaining of insulin secreting cells in pancreatic islets (magnification x 400)

Photomicrographs of the immunohistochemical staining of insulin producing cells of islets of Langerhans revealed normal number of immunoreactive cells which was evident from strong positive reaction for anti-insulin antibodies as brown granules occupying the cytoplasm of the β-cells in the pancreas of normal control rats. But, the number of insulin positive β-cells showing immunoreactivity for anti-insulin antibodies
was markedly decreased in diabetic control rats, as they were confined to only certain parts of pancreatic islets. However, administration of MEC at a dose of 200 mg to diabetic rats caused an increase in the number of positive immunoreactions of β-cells for anti-insulin antibodies, than the other two doses as evident from the increase in staining particles appeared in many parts of the pancreatic islets (Figure 6.3a). These observations indicate that MEC at a dose of 200 mg effectively alleviated the pancreatic cytotoxicity induced by STZ administration.
Figure 6.3a Immunostaining of insulin secreting cells in pancreatic islets (magnification x 400)

(a) Normal control rats - showing normal number of immunoreactive β-cells
(b) Diabetic control rats - showing lesser number of insulin positive β-cells
(c) Diabetic + MEC (100 mg) - relatively more insulin positive cells than diabetic control
(d) Diabetic + MEC (200 mg) - number of insulin positive cells comparable to normal
(e) Diabetic + MEC (400 mg) - showing more immunoreaction than MEC (100 mg)

Arrows show immunoreactive insulin secreting cells stained with deep brown color; Scale bar 200 μm.
Effect of Feeding Different Levels of 80% Methanolic Extract of CnI (MEC) in Diabetic Rats

Discussion

The current study was undertaken to evaluate the modulation of glucose homeostasis by different doses of 80% methanolic extract of coconut inflorescence (MEC) on streptozotocin (STZ) induced diabetes in rats. Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose; insulin is secreted (Edem, 2009). STZ-induced diabetes may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion caused by the cytotoxic effects of STZ in the pancreatic β-cells (Rosa Martha Perez Gutierrez, 2012).

In this study, streptozotocin-diabetic rats exhibited significant increase in blood glucose level. Oral treatment with different doses of (100, 200 and 400 mg) MEC reduced blood glucose level indicating its antihyperglycemic activity. Though all the three tested dose levels proved to be effective, MEC at a dose of 200 mg had a satisfactory effect to restore blood glucose significantly. This finding is in line with the previous reports where plant extracts decreased blood glucose levels (Oluba et al., 2010). In the current study, serum insulin levels were significantly decreased in the untreated diabetic rats. Pathological changes in serum insulin levels in diabetes reflect abnormalities in β-cell function or structure. Treatment with MEC augmented the serum insulin levels suggesting an improved state of availability of serum insulin to control blood sugar. This might be due to higher secretion of insulin in MEC fed rats. The present study showed that serum insulin augmenting effect was highest at the dose of 200 mg suggesting that serum insulin effect of MEC is depending on this dose. Similar effect was observed in a previous report, where methanol extract of Lippia nodiflora increased insulin secretion in rats (Balamurugan and Ignacimuthu, 2011).

Diabetes mellitus is known to impair the normal capacity of liver to synthesize glycogen (Ramesh and Pugalendi, 2005). In diabetic condition, the concentration of liver glycogen was found to be decreased (Stalmans et al., 1997). Insulin is the main regulator for glycogenesis in liver. The decrease of liver glycogen observed in this study may be due to lack of insulin in diabetic state or oxidative stress induced by STZ in diabetes may inactivate the glycogen synthase system. Since destruction of β-cells of islets of Langerhans results in marked decrease in insulin levels, it is rational that
glycogen level in liver tissue decrease as they depend on insulin for influx of glucose (Grover et al., 2002). Treatment with MEC (100, 200 and 400 mg) significantly increased liver glycogen indicating that the defective glycogen storage in the diabetic state was partially normalized by MEC. This effect was more with the rats administered 200 mg of MEC.

In diabetes mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with hemoglobin in a non-enzymatic process to form glycosylated hemoglobin. Since the glycation rate is directly proportional to blood glucose concentration, level of glycosylated hemoglobin indicates glycemic control in the diabetic state (Monnier, 1982). In the present study, administration of MEC significantly reduced the elevated glycosylated hemoglobin levels in streptozotocin-diabetic rats, further substantiating its potential in long term glycemic control of diabetes mellitus. The effect was predominant in those diabetic rats fed MEC at a dose of 200 mg.

The antidiabetic activity of MEC was also evidenced from the immunohistochemical changes of the insulin-producing cells. STZ is known to induce severe histopathological changes in the pancreas (Mitra et al., 1996), which gives rise to hypoinsulinemia and hyperglycemia features similar to those of clinical diabetes (Howarth et al., 2000). This was indicated by the observed histopathological alterations and decreased immunoexpression of insulin-secreting cells in diabetic control rat pancreas. The increase in circulating glucose level itself is reported to contribute to β-cell dysfunction (Hansen, 1998). Amelioration of histomorphometrical changes in β-cells by MEC is considered to be direct evidence that MEC at a concentration of 200 mg protects the β-cells from the STZ action, which corresponds to the results for serum insulin levels in this study. This may be considered as one of the possible mechanism of antidiabetic activity of MEC.

Hence, it can be concluded that 200 mg/kg body weight is the most effective dose of MEC, which has potent antihyperglycemic effect in STZ diabetic rats. So, this dose of MEC was selected for further studies.
b. Effects of MEC on Tricarboxylic Acid Cycle Enzymes, Advanced Oxidation of Protein, Peroxidation of Lipid and Erythrocyte Membrane Stability in Diabetic Rats

Diabetes is associated with several mechanisms, and one of which is oxidative stress. Increased oxidative stress is a widely accepted event in the development and progression of diabetes and its complications (Ceriello, 2000). The processes; glycooxidation and lipid peroxidation has been proposed as important pathways and these lead, via several mechanisms, to multiple biochemical sequels, which display a disturbance of oxidative-antioxidative balance, and create glycooxidative and peroxidative molecular damage (Kostolanská et al., 2009). The majority of the glycooxidation products can accumulate in biological systems and bring about tissue degeneration, particularly in areas of blood vessels and thus take part in the origin of the diabetic vascular late complications such as nephropathy (Piwowar et al., 2008). Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be increased in diabetes (Sato et al., 1979; Collier et al., 1992). The increased concentration of lipid peroxidation induces oxidative damage by increasing peroxyl radicals and hydroxyl radicals (Levy et al., 1999). Thus, lipid peroxidation is one of the characteristics features of chronic uncontrolled diabetes.

Red blood cells (RBCs) play an important role in the onset of complications associated with diabetes (Carroll et al., 2006). In chronic diabetic condition, RBCs are susceptible to attacks by reactive oxygen species because of their high polyunsaturated fatty acid (PUFA) content and their abundance of iron (Fe\(^{2+}\)) rich hemoglobin (Rizvi et al., 2005). Ionic Fe\(^{2+}\) acts as a catalyst in redox reactions and lipid peroxidation and forms malondialdehyde (MDA) as the end product (Draper and Hadley, 1990). Diabetes mellitus also weakens the components of antioxidant defense systems such as superoxide dismutase, catalase and glutathione peroxidase in RBC membrane (Martí’n-Galla’n et al., 2003).

In diabetes mellitus, various agents like inflammatory cytokines and streptozotocin could operate by forming free radicals that could attack the mitochondria and thereby decrease oxygen consumption and respiratory ratio (Sener et al., 1990). The
increased production of free radicals in mitochondria may damage β-cells, which is known to be very sensitive to free radicals (Sakai et al., 2003). It has been suggested that the diabetogenicity of streptozotocin is dependent on the inhibition of the activities of citric acid cycle enzymes such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase (Boquist et al., 1985).

In STZ induced diabetes, prior to β-cell destruction, STZ induces a non-specific islet inflammation called insulitis, where inflammatory macrophages and pancreatic endothelium are activated and release several inflammatory mediators such as prostaglandins (PGs), leukotriens (LTs) and hydroxylated derivatives; hydroxyeicosatetraenoic acids (HETEs), which are lipoxygenase (LOX) and cyclooxygenase (COX) derived eicosanoids (Roselló-Catafau et al., 1994). The roles played by COX and LOX enzymes in β-cells have not been fully established, but there is good evidence that both COX and LOX play roles in cytokine-mediated damage of β-cells (Tabatabaei et al., 2000; Chen et al., 2005).

Observations from the previous study indicate that MEC at a dose of 200 mg is more effective in controlling hyperglycemia. Thus, the present study investigates the effects of oral administration of 200 mg of MEC on TCA cycle enzymes, advanced oxidation of protein, lipid peroxidation, erythrocyte membrane antioxidant status and marker enzymes in arachidonic acid pathway in experimental diabetes.

Materials and methods

Chemicals

Streptozotocin, sodium linoleate, diethyl dithiocarbamate and arachidonic acid were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Methanol was purchased from Merck KGaA, Darmstadt, Germany. Reduced glutathione, thiobarbituric acid and glycylglycine were purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals used were of the highest analytical grade.
Methods

Collection of CnI and procedures for preparation of MEC were described in chapter 2.

Experimental design

Male albino rats (Sprague Dawley strain) weighing 150-200 g were used for the study. The animals were individually housed under hygienic conditions in polypropylene cages at 12 hour light and dark cycle. The animals were maintained at a temperature of 25±5°C. Rats in all the groups were given free access to standard laboratory animal feed and water *ad libitum*. Animals were divided into 3 groups of 6 rats each and treated as follows:

Group I - Normal Control

Group II - Diabetic Control

Group III - Diabetic + MEC (200 mg)

Group I rats were served as normal control, whereas rats in group II were served as diabetic control. Rats in group III were given MEC suspended in sterile water at a dose of 200 mg/kg body weight/day orally by intra gastric intubation. Diabetes was induced in rats of groups II and III by a single intraperitoneal injection of streptozotocin at a dose of 45 mg/kg body weight, dissolved in 0.1M citrate buffer pH 4.5. The blood glucose level was checked 72 h after STZ injection. The animals were considered diabetic when the fasting blood glucose level was raised beyond 200 mg/dL.

The experiment lasted for 45 days. After the experimental period, the rats were euthanized and tissues were taken out, transferred to cold containers and blood samples were collected for the preparation of erythrocyte membrane and used for the biochemical analyses. The procedures for isolation of liver mitochondria, preparation of erythrocyte membrane and analyses of biochemical parameters were described in chapter 2.
Results

6.1b Activities of succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) and \(\alpha\)-ketoglutarate dehydrogenase (\(\alpha\)-KGDH) in liver

The activities of TCA cycle enzymes such as succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase and \(\alpha\)-ketoglutarate dehydrogenase in hepatic mitochondria were significantly decreased in rats administered with streptozotocin alone as compared to normal control animals. However, MEC treatment significantly increased these enzyme activities in diabetic rats as compared to diabetic control animals (Table 6.1b).

Table 6.1b Activities of SDH, ICDH, MDH and \(\alpha\)-KGDH in liver

<table>
<thead>
<tr>
<th>Groups</th>
<th>SDH(^*)</th>
<th>ICDH(^\ddagger)</th>
<th>MDH(^#)</th>
<th>(\alpha)-KGDH(^\wedge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35.08±4.09(^a)</td>
<td>707.72±67.89(^a)</td>
<td>328.75±30.00(^a)</td>
<td>45.67±4.16(^a)</td>
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<tr>
<td>II</td>
<td>16.07±1.54(^b)</td>
<td>428.00±41.06(^b)</td>
<td>199.97±18.24(^b)</td>
<td>24.09±2.20(^b)</td>
</tr>
<tr>
<td>III</td>
<td>25.09±2.88(^c)</td>
<td>607.89±69.83(^c)</td>
<td>277.36±25.30(^a)</td>
<td>33.49±3.05(^c)</td>
</tr>
<tr>
<td>F value</td>
<td>59.26</td>
<td>32.37</td>
<td>40.37</td>
<td>66.77</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at \(p<0.05\).

SDH\(^*\) - \(\mu\)moles of succinate oxidized /min/mg protein

ICDH\(^\ddagger\) - nmol of \(\alpha\)-ketoglutarate liberated/min/mg protein

MDH\(^\#\) - nmol of NADH oxidized/min/mg protein

\(\alpha\)-KGDH\(^\wedge\) - \(\mu\)mol of ferrocyanide liberated/min/mg protein.
6.2b Concentration of serum advanced oxidation of protein products (AOPP)

There was a significant elevation in the levels of AOPP in the serum of STZ induced diabetic control rats compared to the normal animals. Oral administration with MEC (200 mg) significantly reduced the levels of serum AOPP in diabetic rats compared to diabetic control rats (Figure 6.1b).

Figure 6.1b Concentration of serum AOPP (μmol/L)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.

6.3b Levels of thiobarbituric acid reactive substances (TBARS) in liver, kidney, pancreas and erythrocyte membrane

The levels of malondialdehyde estimated as TBARS in liver, kidney, pancreas and erythrocyte membrane were increased significantly in streptozotocin induced diabetic control rats. Compared with the diabetic control animals, those diabetic rats treated with MEC showed significantly decreased concentration of TBARS in hepatic, renal and pancreatic tissues and erythrocyte membrane (Figure 6.2b).
Figure 6.2b Levels of TBARS in liver, kidney, pancreas (mM/100g wet tissue) and erythrocyte membrane (mM/100g protein)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.

6.4b Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in erythrocyte membrane

The activities of SOD, CAT and GPx in erythrocyte membrane were decreased significantly in diabetic control rats compared to the normal control rats. Oral treatment with MEC (200 mg) significantly increased the activities of these enzymes in diabetic rats compared to the diabetic control animals (Figure 6.3b).
Figure 6.3b Activities of SOD (U/mg protein), CAT(x10³ Units/mg protein) and GPx (x10² Units/mg protein) in erythrocyte membrane

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.

*Enzyme concentration required to inhibit the NBT to 50% in one minute

#One unit is defined as the velocity constant per second

^One unit is defined as the µmoles of glutathione oxidized per minute.

6.5b Levels of reduced glutathione (GSH) in erythrocyte membrane

Concentration of reduced glutathione (GSH) in erythrocyte membrane was decreased significantly in streptozotocin induced diabetic control rats compared to normal control rats. MEC oral administration significantly increased the concentration of GSH in RBC membrane of diabetic rats compared to diabetic control animals (Figure 6.4b).
Figure 6.4b Levels of GSH in erythrocyte membrane (mM/100mL)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.

6.6b Activities of 5-lipoxygenase (5-LOX) and total cyclooxygenase (COX) in pancreas

The activities of inflammatory marker enzymes such as 5-LOX and COX were found to be increased in the pancreas of diabetic control rats in comparison with the normal animals. Diabetic rats administered with MEC showed a significant decrease in the activities of these inflammatory marker enzymes compared to the diabetic control rats (Figure 6.5b).
Effect of Feeding Different Levels of 80% Methanolic Extract of CnI (MEC) in Diabetic Rats

Figure 6.5b Activities of 5-LOX and COX in pancreas

Discussion

The present study investigated the effects of oral administration of 200 mg/kg body weight of MEC on TCA cycle enzymes, advanced oxidation of proteins and lipids, erythrocyte membrane stability and inflammatory marker enzymes in streptozotocin induced experimental diabetic rats. STZ is a commonly employed compound for induction of diabetes in animal models especially in rats and mice (Rerup and Tarding, 1969). STZ causes diabetes by rapid depletion of β-cells which leads to reduction in insulin release (Tomlinson et al., 1992). Diabetogenic effect of STZ was observed due to the excess production of reactive oxygen species, which led to cytotoxicity in pancreatic β-cells (Arulselvan and Subramanian, 2007).

Peroxidation of lipids is a free radical induced process, which is found to be an important pathophysiological event in variety of diseases including diabetes and cardiovascular disorders (Karpen et al., 1982). There are reports that an elevated lipid peroxidation and lowered antioxidants in streptozotocin induced diabetes mellitus (Prakasam et al., 2003). The major pathological consequence of free radical induced
membrane lipid peroxidation includes increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity (Kolanjiappan et al., 2002). The present results showed a significant increase in lipid peroxidation in erythrocyte membrane as well as in tissues of rats treated with STZ. The observed increase in the concentration of malondialdehyde in the tissues and in the RBC membranes of diabetic rats, were consistent with the results of previous studies (Hussein et al., 2011). The administration of MEC to diabetic rats reduced MDA, a marker of lipid peroxidation in the tissues and erythrocytes suggesting that the extract possesses potent antioxidative properties which protects the tissues and RBC membranes from oxidative damage during diabetic condition.

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic scavenger system. SOD, CAT and GPx constitute a mutually supportive first line of defense against free radicals (Nonaka and Manabe, 1991). Functions of these antioxidant enzymes are interconnected and a decrease of their activities resulting in the accumulation of lipid peroxides and increase in oxidative stress in diabetic rats (Sen and Hanninen, 1994). SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical, converting it into H$_2$O$_2$ and molecular oxygen, both of which damage the RBC membrane and other biological structures (Arivazhagan et al., 2000). Catalase is responsible for the detoxification of significant amounts of H$_2$O$_2$ (Cheng et al., 1981). The activities of SOD and CAT are reported to be low in diabetes mellitus (Vucic et al., 1997). Erythrocytes were probably affected more due to the higher vulnerability thus causing inhibition of erythrocyte SOD and CAT activities in diabetic rats (Yadav et al., 1997). The levels of enzymatic antioxidants such as SOD and CAT were significantly restored after treatment of MEC to diabetic rats. This indicates the modulatory activity of MEC since antioxidant enzymes such as superoxide dismutase and catalase, which have a direct role in scavenging free radicals, were also partly restored by the extract.

Glutathione peroxidase, an enzyme with selenium, works together with glutathione in the decomposition of H$_2$O$_2$ or other organic hydro peroxides to non-toxic products at the expense of the GSH (Bruce et al., 1982). Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975). Further, insufficient availability of GSH may also reduce the activity
of GPx (Illing et al., 1951). Glutathione is a tripeptide, intracellular antioxidant and protects the cellular system from adverse effects of lipid peroxidation. The decreased GSH level may be associated with reactive oxygen species generation by mechanisms creating oxidative stress in chronic hyperglycemia (Scholz et al., 1997). Furthermore, the depletion in GSH level may be related to the apparent increase in lipid peroxidation in the tissues of rats exposed to STZ (Gumieniczek, 2005). In this study, the activity of GPx and concentration of GSH in erythrocyte membrane was much reduced in the diabetic rats. These findings are similar to those of other researchers studying GSH in relation to risk factors in diabetic subjects and animals (Damasceno et al., 2002). In diabetic rats treated with MEC, a significant increase in the concentration of GSH and activity of GPX in erythrocyte membrane was observed. This might reflect the antioxidant potency of MEC in cell membranes as in erythrocytes.

The role of oxidative protein damage in the pathogenesis of the diabetic state is being investigated extensively (Telci et al., 2000). The protein oxidation provoked by free radicals has been demonstrated to play a significant role in the pathogenesis of diabetes (Altomare et al., 1997). Radical-mediated damage to proteins may be initiated by electron leakage, metal-ion dependent reactions and autoxidation of lipids and sugars. Accumulation of modified proteins disrupts cellular function either by loss of catalytic and structural integrity or by interruption of regulatory pathways (Dean et al., 1997). The occurrence of protein oxidative damage in diabetic patients was confirmed by a novel marker, advanced oxidation protein products (AOPP) which is a good reflection of excess free radical generation in vivo (Witko-Sarsat et al., 1996). Hyperglycemia induced oxidative stress may play an important intermediary role in the pathogenesis of diabetes complications, thereby, AOPP are much higher in diabetic patients (Pan et al., 2010). In this study, it was observed that serum AOPP levels in experimental animals increased after STZ injection, suggesting that, as reported previously, AOPPs spontaneously generated in diabetes (Martin-Gallan et al., 2003). Intra gastric treatment with MEC, however, significantly reduced AOPP contents in the serum of diabetic rats compared to diabetic control. This clearly indicate that MEC, by decreasing oxidative stress, may be effective in preventing oxidative protein damages which are thought to be involved in cellular damages under diabetic condition.
Glucose metabolism, the citric acid cycle and oxidative phosphorylation are central biochemical pathways in cellular energy metabolism. TCA cycle plays an important role in diabetes mellitus since the activity of Kreb’s cycle enzymes act as indicators of glucose utilization (Sener et al., 1990). STZ acts by forming free radicals thereby damaging the mitochondrial membrane and resulting in decreased activities of citric acid cycle enzymes such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase and succinate dehydrogenase (Paneerselvam and Govindaswamy, 2002). Isocitrate dehydrogenase is an enzyme that catalyzes the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO₂ while converting NAD⁺ to NADH in TCA cycle (Cherbavaz et al., 2000). α-ketoglutarate dehydrogenase is a highly regulated enzyme, which could determine the metabolic flux through the Kreb’s cycle. It catalyses the conversion of α-ketoglutarate to succinyl-CoA and produces NADH directly, providing electrons for the respiratory chain (Hansford, 1980). The activities of these enzymes were found to be decreased in STZ induced diabetic animals (Chen and Ianuzzo, 1981). Treatment using MEC to diabetic rats significantly restored the activities of ICDH and α-KGDH suggesting that MEC improved glucose utilization in diabetic condition.

Succinate dehydrogenase is an important enzyme that participates in both the citric acid cycle and the electron transport chain. SDH activity was reported to be inhibited in the tissues of STZ induced diabetic animals (Boquist et al., 1985). Decreased SDH activity in diabetic condition affects succinate-fumarate conversion, which indicates depressed oxidative metabolism in mitochondria (Oyedotun and Lemire, 2004). Malate dehydrogenase is another important enzyme in the citric acid cycle. This provides oxaloacetate for the formation of citrate with acetyl-CoA for generating malate which can feed the cytosolic gluconeogenic pathway (Murray et al., 1998). The activity of malate dehydrogenase was decreased in diabetic rats (Hikino et al., 1989; Ramudu et al., 2011). The decrease in MDH activity as a consequence of diabetes significantly decreased the utilization of malate. The reduction in the functioning of these mitochondrial enzymes may lead to defect in mitochondrial energy production which would impair β-cell function (Maiti et al., 2005). The decreased activities of these citric acid cycle enzymes in diabetic rats were significantly enhanced upon administration of MEC, which suggests that Cnl may be beneficial in
mitochondrial energy production. These findings are in agreements with a previous report where treatment with *Catharanthus roseus* extract improved the activities of TCA cycle enzymes in diabetic rats (Singh *et al.*, 2001). Thus, our findings suggest that there is increased mitochondrial oxidative potential and energy production when diabetic rats are treated with MEC.

Studies indicate that hyperglycemia-induced diabetic complications are likely from oxidative dysfunction and inflammatory effect (Dandona *et al.*, 2004; Madarász *et al.*, 2009). Lipid peroxide-mediated damage has been observed in the development of type 1 and type 2 diabetes mellitus. Insulin secretion is also closely associated with lipoxygenase-derived peroxides (Walsh and Pek, 1984). Low levels of lipoxygenase mediated peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in diabetes (Metz, 1984). Evidence has been accumulated; suggested that arachidonic acid stimulates insulin release from rat islets β-cells (Band *et al.*, 1992; Jones *et al.*, 1993). It has been reported that an influx of pancreatic cyclooxygenase and lipoxygenase mediated metabolites is involved in the insulitis associated with STZ-induced diabetes (Roselló-Catafau *et al.*, 1994). In this study, LOX and COX activities in the pancreas were found to be increased in STZ diabetic rats. MEC treatment to diabetic rats caused a reduction of LOX and COX activities indicating its efficacy in the inhibition of these enzymes, which would possibly minimize β-cell dysfunction by eicosanoid mediated inflammation.

In summary, MEC administration to STZ diabetic rats significantly ameliorated the advanced oxidation of protein in serum, lipid peroxidation in tissues and erythrocytes and maintained the antioxidant status in RBC membrane. In addition, MEC beneficially modulated the activities of TCA cycle enzymes and also alleviated LOX and COX mediated inflammatory damage of islets β-cells.