CHAPTER 9

Studies on the Effects of AF and CGA on Mitochondrial Oxidative Phosphorylation and Lysosomal Enzymes in STZ Induced Diabetes

The pathophysiology of diabetes mellitus is varied and complex. Hyperglycemia causes many of the pathological consequences of both types of diabetes and much of this damage is suggested to be a consequence of elevated production of reactive oxygen species by the mitochondrial respiratory chain during hyperglycemia (Green et al., 2004). It is being argued that many of the defects in diabetes either at site of origin (β-cells) or elsewhere (target cells) are mediated by ROS from different sources (West, 2000). Broadly ROS can be defined as overactive fragmented atoms or molecules which are capable of tormenting and fragmenting other molecules (Halliwell, 1997). Superoxide anion radical, often referred as primary ROS, in human body arises from metabolic reactions, irradiation and leakage from electron transport chain (Valko et al., 2007). These radicals are produced in cellular membrane mitochondria, lysosomes, peroxisomes, endoplasmic reticulum and cytoplasm (Singh et al., 2009).

It has been proposed that during the early stages of β-cell destruction, hyperglycemia-induced mitochondrial overwork and alterations in the rate of oxygen utilization by respiratory chain complexes are possible mechanisms for development of diabetes-related complications (Raza et al., 2011). Mitochondria undergo rapid fragmentation with a concomitant increase in ROS formation after exposure to high glucose concentrations (Yu et al., 2006). Increased ROS formation in mitochondria may lead to disturbance of mitochondrial bioenergetics due to mutations in mitochondrial DNA and altered electron flow through respiratory complexes (Kucharska et al., 2000; Wallace, 2005). Activities of mitochondrial electron transport chain complex enzymes were also reported to be decreased in diabetes condition (Akude et al., 2011).
Lysosomal enzymes have generated much interest due to their role as indicators of oxidative stress. Lysosomal enzymes catalyze hydrolytic cleavage of glycosidic bonds of glycosaminoglycans, glycoproteins and glycolipids (Belfiore et al., 1972). Changes have been observed in the lysosomal enzyme activities in the tissues of both experimental animals and human subjects during diabetes (Chougala et al., 2012). Hence, study of sub-cellular oxidative stress and lysosomal enzymes is of great relevance during diabetes and also other stress related diseases.

In the previous study, we isolated and characterized the aqueous fraction of MEC (AF) and found that chlorogenic acid is one of its active components. Since the pharmacological property of AF was attributed to chlorogenic acid, it is pertinent to study its in vivo protective effects. Thus the present study investigates the effects of AF and CGA on mitochondrial oxidative phosphorylation and lysosomal marker enzymes in STZ induced diabetes in rodent model.

**Materials and methods**

**Chemicals**

Decylubiquinol, cytochrome c, NADH, NADP, rotenone, glucose - 6 - phosphate dehydrogenase, 2’, 7’-dichlorofluorescin diacetate were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Kit for glucose estimation was purchased from Agappe Diagnostics, Thane, India. All other chemicals used were of the highest analytical grade.

**Methods**

Preparation of MEC from CnI and procedures for fractionation of MEC, preparation and purification of AF were described in chapter 2.

**Animal experiments**

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

Group I - Normal Control

Group II - Diabetic Control

Group III - Normal + AF (140 mg)

Group IV - Diabetes + AF (140 mg)

Group V - Normal + CGA (2.5 mg)

Group VI - Diabetes + CGA (2.5 mg)

Group I rats were served as normal control, while rats in group II were served as diabetic control. Rats in group III and IV were given aqueous fraction of MEC (AF) at a dose of 140 mg/kg body weight/day orally by intra gastric intubation. Group V rats and group VI rats were orally administered with 2.5 mg/kg body weight of CGA per day, since 200 mg of MEC corresponds to 140 mg of AF by yield and 140 mg of AF corresponds to 2.5 mg of CGA by yield. These test doses of AF and CGA were suspended in sterile water prior to intra gastric feeding. Diabetes was induced in rats of groups II, IV and VI 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 and pancreas were taken out, transferred to cold containers and serum was separated from the blood samples and used for the estimation of glucose. The procedures for analysis of serum glucose, isolation of mitochondria from liver and pancreas and isolation of hepatic lysosomes were described in chapter 2.
Results

9.1 Effect of AF and CGA on serum glucose levels

There was a significant increase in the concentration of glucose in the serum of diabetic control rats compared to the normal animals. Diabetic rats treated with AF and CGA showed a significant reduction in the serum glucose concentration compared to diabetic control rats (Figure 9.1). There was no significant difference observed between AF and CGA on serum glucose lowering action.

Figure 9.1 Concentration of serum glucose (mg/dL)

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

9.2 Activity of mitochondrial NADH - dehydrogenase in liver and pancreas

The activity of mitochondrial respiratory complex, NADH - dehydrogenase in hepatic and pancreatic mitochondria was significantly decreased in rats administered with streptozotocin alone as compared to normal control animals. However, AF and CGA treatment significantly increased this enzyme activity in diabetic rats as compared
to diabetic control animals (Figure 9.2). The effect of AF and CGA in modulating NADH-dehydrogenase activity was comparable with each other.

**Figure 9.2 Activity of mitochondrial NADH – dehydrogenase* in liver and pancreas**

![Activity graph showing mitochondrial NADH-dehydrogenase activity in liver and pancreas](image)

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

*μmol/min/mg protein

**9.3 Activity of mitochondrial succinate - cytochrome c – reductase in liver and pancreas**

The activity of succinate - cytochrome c – reductase in the mitochondria of liver and pancreas was decreased significantly in streptozotocin induced diabetic control rats. Compared with the diabetic control rats, those diabetic rats treated with AF and CGA showed significantly increased activity of this mitochondrial complex in hepatic and pancreatic tissues (Figure 9.3). AF and CGA showed almost similar effect on modulating the activity of succinate - cytochrome c – reductase.
Figure 9.3 Activity of mitochondrial succinate - cytochrome c - reductase* in liver and pancreas

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

9.4 Activity of mitochondrial ubiquinol - cytochrome c - reductase in liver and pancreas

The activity of electron transport complex III, ubiquinol - cytochrome c - reductase in hepatic and pancreatic mitochondria was significantly decreased in rats administered with streptozotocin alone as compared to normal control animals. However, AF and CGA treatment significantly increased the activity of this complex in diabetic rats as compared to diabetic control animals (Figure 9.4).
**Figure 9.4 Activity of ubiquinol - cytochrome c - reductase* in liver and pancreas**

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

*μmol/min/mg protein

**9.5 Activity of mitochondrial cytochrome c - oxidase in liver and pancreas**

The activity of cytochrome c - oxidase in the mitochondria of liver and pancreas were decreased significantly in streptozotocin induced diabetic control rats. Compared with the diabetic control animals, those diabetic rats treated with AF and CGA showed significantly increased activity of this mitochondrial enzyme complex in hepatic and pancreatic tissues (Figure 9.5). The effect of AF and CGA were comparable with each other.
Figure 9.5 Activity of mitochondrial cytochrome c-oxidase* in liver and pancreas

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

9.6 Activity of mitochondrial ATP synthase in liver and pancreas

The activity of respiratory chain enzyme, ATP synthase in hepatic and pancreatic mitochondria was significantly decreased in rats induced with streptozotocin alone as compared to normal control animals. However, treatment with AF and CGA significantly increased the activity of this enzyme in diabetic rats as compared to diabetic control animals (Figure 9.6). There was no significant difference observed between AF and CGA on mitochondrial ATP synthase activity.
Figure 9.6 Activity of mitochondrial ATP synthase* in liver and pancreas

<table>
<thead>
<tr>
<th>Group</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>a</td>
</tr>
<tr>
<td>II</td>
<td>b</td>
</tr>
<tr>
<td>III</td>
<td>c</td>
</tr>
<tr>
<td>IV</td>
<td>a</td>
</tr>
<tr>
<td>V</td>
<td>c</td>
</tr>
<tr>
<td>VI</td>
<td>c</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.

*nmol of Pi/min/mg protein

9.7 Levels of adenosine triphosphate (ATP) in liver and pancreas mitochondria

Concentration of ATP in the mitochondria of liver and pancreas was decreased significantly in streptozotocin induced diabetic control rats. Oral gavage of AF and CGA to diabetic rats significantly increased the concentration of ATP in hepatic and pancreatic mitochondria compared to diabetic control animals (Figure 9.7).
9.7 Levels of ATP in liver and pancreas mitochondria (nM/mg protein)

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

9.8 Reactive oxygen species (ROS) production in liver and pancreas mitochondria

The levels of ROS in the mitochondria of liver and pancreas were increased significantly in streptozotocin induced diabetic control rats. Oral administration of AF and CGA significantly decreased the amount of ROS produced in the hepatic and pancreatic mitochondria of diabetic rats compared to diabetic control animals (Figure 9.8).
Figure 9.8 Levels of ROS production in liver and pancreas mitochondria (fluorescence units/min/mg protein)

![Bar chart showing ROS production levels in liver and pancreas mitochondria for different groups.](chart.png)

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

9.9 Activities of β-glucuronidase, β-glucosidase and β-hexosaminidase in hepatic lysosomes

The activities of lysosomal β-glucuronidase, β-glucosidase and β-hexosaminidase in liver tissue were decreased significantly in diabetic control rats compared to the normal control rats. Oral treatment with AF and CGA significantly increased the activities of these lysosomal enzymes in diabetic rats compared to the diabetic control animals (Figure 6.3b).
Table 9.1 Activities of $\beta$-glucuronidase, $\beta$-glucosidase and $\beta$-hexosaminidase in hepatic lysosomes

<table>
<thead>
<tr>
<th>Groups</th>
<th>$\beta$-glucuronidase*</th>
<th>$\beta$-glucosidase*</th>
<th>$\beta$-hexosaminidase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25.29±2.9$^a$</td>
<td>7.77±0.7$^a$</td>
<td>7.11±0.6$^a$</td>
</tr>
<tr>
<td>II</td>
<td>11.16±1.0$^b$</td>
<td>2.09±0.2$^b$</td>
<td>2.81±0.2$^b$</td>
</tr>
<tr>
<td>III</td>
<td>25.28±2.9$^a$</td>
<td>7.74±0.7$^a$</td>
<td>7.18±0.6$^a$</td>
</tr>
<tr>
<td>IV</td>
<td>16.59±1.9$^c$</td>
<td>3.86±0.3$^c$</td>
<td>4.14±0.3$^c$</td>
</tr>
<tr>
<td>V</td>
<td>26.70±2.4$^a$</td>
<td>8.11±0.7$^a$</td>
<td>7.49±0.7$^a$</td>
</tr>
<tr>
<td>VI</td>
<td>17.77±1.6$^c$</td>
<td>4.04±0.5$^c$</td>
<td>4.33±0.3$^c$</td>
</tr>
<tr>
<td>F value</td>
<td>44.91</td>
<td>117.19</td>
<td>84.80</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.

*μmoles of p-nitrophenol liberated/min/mg protein.
9.10 Activities of acid phosphatase and Cathepsin D in hepatic lysosomes

The activities of lysosomal acid phosphatase and cathepsin D in hepatic tissue were decreased significantly in diabetic control rats compared to the normal control animals. Treatment with AF and CGA significantly increased the activities of these lysosomal enzymes in diabetic rats compared to the diabetic control animals (Figure 6.3b). The effects were similar for AF and CGA in protecting these lysosomal enzyme activities.

Table 9.2 Activities of acid phosphatase and Cathepsin D in hepatic lysosomes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acid phosphatase*</th>
<th>Cathepsin D#</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>128.42±11.7^a</td>
<td>31.92±3.0^a</td>
</tr>
<tr>
<td>II</td>
<td>83.12±7.9^b</td>
<td>19.54±1.8^b</td>
</tr>
<tr>
<td>III</td>
<td>128.62±11.7^a</td>
<td>33.59±3.0^a</td>
</tr>
<tr>
<td>IV</td>
<td>96.03±9.2^c</td>
<td>26.62±2.4^c</td>
</tr>
<tr>
<td>V</td>
<td>129.94±11.8^a</td>
<td>33.62±3.0^a</td>
</tr>
<tr>
<td>VI</td>
<td>97.10±9.3^c</td>
<td>26.65±2.4^c</td>
</tr>
<tr>
<td>F value</td>
<td>21.47</td>
<td>24.94</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.
* µmol of phenol liberated/min/mg protein  
# µmol of tyrosine liberated/h/100 mg protein.
Discussion

The current study deals with the effects of aqueous fraction of MEC (AF) and the active component present in AF, chlorogenic acid (CGA), on mitochondrial electron transport chain complexes, oxidative stress and lysosomal marker enzymes in STZ induced experimental diabetes in rats. Streptozotocin induced hyperglycemia in rodents is considered to be a good model for diabetes mellitus with insulin deficiency condition and a relevant example of endogenous chronic oxidative stress (Sudhakara et al., 2012). Hyperglycemia itself induce oxidative stress, can leads to more complicated damages to β-cells and other tissues (Brownlee, 2005). The development of hyperglycemia in rats following STZ injection is primarily due to direct pancreatic β-cell dysfunction (Spinas, 1999). STZ is a toxic glucose analogue that preferentially accumulates in pancreatic β-cells through glucose transporter 2 (Lenzen, 2008). In addition, STZ has deleterious effects on the liver and other tissues (Laguens et al., 1980).

Administration of STZ to rats caused an increase in the fasting blood glucose beyond the normal levels indicating the presence of diabetes. The serum glucose concentration of diabetic control rats in this study was found to be significantly increased compared to normal rats. The oral administration of aqueous fraction of MEC and CGA to diabetic rats improved the glycemic control and significantly reduced the serum glucose levels compared to the diabetic control. Our findings suggest that aqueous fraction of MEC has a protective effect against STZ induced diabetes and that CGA is the major factor responsible for this effect. These results are in agreement with previous studies where, green tea aqueous extract significantly alleviated hyperglycemia in rats (Ramadan et al., 2009) and epigallocatechin gallate (EGCG), one of the catechins in green tea, improved the blood glucose in severely diabetic db/db mice (Wolfram et al., 2006).

STZ induced diabetes is characterized by severe derangements in intracellular metabolism and structural alterations of cell membranes (Brasitus and Dudeja, 1985). Mitochondrion is now gaining importance in diabetes because of its central role as a regulator of energy balance. Disruption of mitochondrial functions has been implicated in more than 40 known diseases, including diabetes, cancer, atherosclerosis, ischemic heart disease and neurodegenerative disease such as Alzheimer’s disease and
Parkinson’s disease (Schon, 2000). It is well established that mitochondrial function is required for normal glucose-stimulated insulin release from pancreatic β-cells (Wallace, 1999). Recent magnetic resonance spectroscopy (MRS) studies of humans suggest that more subtle defects in mitochondrial function might play a role in the pathogenesis of insulin resistance in diabetes (Lowell and Shulman, 2005).

Mitochondrion generates most of the energy in animal cells through oxidative phosphorylation, a process in which electrons are passed along a series of carrier molecules called the electron transport chain (Kwak et al., 2010). The electron transport chain consists of four respiratory enzyme complexes arranged in a specific orientation in the inner mitochondrial membrane (Vonck and Schafer, 2009). The mitochondrial matrix contains the components of the TCA cycle and of the β-oxidation pathway, which provide reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH$_2$) to the electron transport chain (ETC) (Benard and Rossignol, 2008; Stuart, 2008). The electrons from NADH and FADH$_2$ enter the electron transport chain through complex I and complex II, respectively (Saraste, 1999). Mitochondria isolated from diabetic rats showed decreased activities of ETC complexes I and II (Rolo and Palmeira, 2006). Similar finding was also observed in this study, where the mitochondria isolated from diabetic rats showed significantly decreased activities of NADH-dehydrogenase and succinate - cytochrome c - reductase compared to control rats’ mitochondria. Upon treatment with AF and CGA to diabetic rats, the enzyme activities of complex I and II were increased significantly. This indicates that AF and its active compound CGA beneficially modulated the utilization of glucose in diabetic condition.

In respiratory electron transport chain, from the complex I and complex II, the electrons are transported sequentially to complex III through the coenzyme Q and then to complex IV through cytochrome c (Saraste, 1999). The transport of electrons is accompanied by release of large amounts of free energy, most of which is harnessed for the translocation of protons from the matrix to the intermembrane space; the remainder is dissipated as heat. The proton gradient formed across the inner membrane creates the electrochemical gradient, which acts as the driving force of ATP generation in complex V (Mitchell, 1961). Thus, oxidative phosphorylation in mitochondria results from electron transport, the generation of a proton gradient, and subsequent proton flux.
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coupled to the ATP synthase. A reduction in the activities of complex III (cytochrome c reductase), IV (cytochrome c oxidase) and V (ATP synthase) of the respiratory chain, leading to the impairment of the cell energy state is observed in diabetes mellitus (Mastrocola et al., 2005). In line with this finding, the current study also observed a significant reduction in the activities of cytochrome c reductase, cytochrome c oxidase and ATP synthase of the respiratory chain in diabetic control rats compared with the normal ones. Treatment with either AF or CGA caused a significant increase in the activities of these mitochondrial complexes in the diabetic rats indicating an improvement in the cellular energy status in diabetic condition. These observations from the present study are in agreement with a previous report where, *Semecarpus anacardium* treatment to diabetic rats improved the cellular energy state (Aseervatham et al., 2011).

Mitochondria are important for adenosine triphosphate (ATP) production, which is vital for all living organisms. During diabetes, the mitochondrial damage induced by streptozotocin in pancreatic β-cells cause a diminished production of ATP (Kang et al., 2009). In the present study, mitochondria of diabetic control rats showed significantly decreased levels of ATP. Administration of AF and CGA to diabetic rats caused a significant increase in the amount of ATP. Thus it can be inferred that CGA treatment increased both glucose oxidation and ATP generation upon glucose stimulation in diabetes. This suggests that at the cellular level, the mechanism of action of CGA may associate with enhanced glucose metabolism and energy production. This is in relation with the literature (Makom Ndifossap et al., 2010).

Apart from ATP production, mitochondria are the major source of endogenous ROS (Raha and Robinson, 2000). When electron transport is impaired in the electron transport chain, it can be transferred to O$_2$ and generates superoxide. Complex I of the electron transport chain is the predominant site of donating electrons to O$_2$ and producing superoxide (O$_2^-$). The superoxide can be dismutated to H$_2$O$_2$ and subsequently converted to the hydroxyl radical, OH$^-$ (Murphy, 2009). These three products constitute the major ROS formed during respiration. Emerging evidence shows that the increased oxidative stress and consequent oxidative damage observed in hyperglycemic conditions begins in the mitochondria, which are the major site of ROS production (Raha et al., 2000; Duchen, 2004). Reactive oxygen species are byproducts
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of the mitochondrial respiratory chain that are physiologically counteracted by the intracellular antioxidant systems (Smith et al., 1999; Green et al., 2004). Recently, it has been suggested that increased mitochondrial ROS production during hyperglycemia may be central to much of the pathology of diabetes (Nishikawa et al., 2000). Furthermore, because β-cell mitochondria play a central role in glucose-stimulated insulin secretion, damage to β-cell mitochondria will attenuate this response (Sakai et al., 2003). Therefore, mitochondrial ROS production and oxidative damage may contribute to the onset, progression, and pathological consequences of both types of diabetes. Mitochondrial ROS generation in insulin deficient diabetic condition is found to be increased (Yoon et al., 2005; Yu et al., 2013). In the present investigation, the diabetic control rats’ mitochondria showed significantly increased production of ROS compared to the normal control animals. Oral administration of AF and CGA to diabetic rats resulted in a significant decrease in the formation of subcellular ROS associated with the respiratory chain reaction. This finding suggests that CGA beneficially modulated the ETC enzymes and thereby ameliorated the mitochondrial ROS generation. This is in close agreement with a previous report in which Terminalia chebula extract attenuated the mitochondrial ROS production in STZ diabetic rats (Senthilkumar and Subramanian, 2007).

Lysosomes are a distinct group of cytoplasmic organelles, known to occur in numerous animal tissues and characterized by their content of a variety of hydrolytic enzymes (Novikoff, 1963). The involvement of lysosomal apparatus in diabetes was reported for the first time in 1965 by Woollen and Turner. Since then, many studies have further strengthened this phenomenon which has suggested direct connection between the lysosomal apparatus and insulin controlled metabolic pathways and a potential role for lysosomal enzymes as indicators of the metabolic complications during diabetes (Burlina et al., 1987). In normal physiological conditions the lysosomal system shows high stability. Disruption of lysosomal membranes can result in the release of lysosomal enzymes causing cellular digestion and various pathological conditions including arthritis, allergic response, several muscle diseases and drug induced tissue destruction (Burdan et al., 2000). Under controlled conditions, lysosomal enzymes are secreted from the cell for the digestion of extracellular material (Hegele et al., 1993). Lysosomal enzymes have generated much interest due to their role as
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indicators of stress due to which they are considered as markers of diabetic complications and also other stress related diseases (Kiffin et al., 2004). One of the factors responsible for the damage of lysosome could be oxidative stress which occurs during diabetes due to various reasons reported (Johansen et al., 2005). The extent of the cellular damage varies with the amount of the oxidation, which determines the degree of disruption of the lysosomal membrane. Thus, nutrient deprived cells show augmented sensitivity to hydrogen peroxide induced oxidative stress (Ollinger and Roberg, 1997). Alteration in the activity of hepatic lysosomal enzymes was observed in diabetic rats (Rajasekaran et al., 2007).

Besides improving the ETC enzyme activities, modulation in the lysosomal enzyme activities like N-acetyl-β-D-hexosaminidase, N-acetyl-β-D-glucuronidase, N-acetyl-β-D-glucosidase, cathepsin D and acid phosphatase were observed by feeding of AF and CGA. It has been proposed that the diabetic state itself and the associated long-term metabolic derangement, in addition facilitating the release of lysosomal enzymes in the extracellular fluid, does interfere with the mechanisms that control their half-life (Wiese et al., 1997). In the present study, activities of lysosomal enzymes such as N-acetyl-β-D-glucuronidase, N-acetyl-β-D-glucosidase, N-acetyl-β-D-hexosaminidase, cathepsin D and acid phosphatase were decreased in the liver of diabetic control rats in comparison to normal control rats. This is in accordance with a previous report in which feeding banana flower increased lysosomal enzyme activities in STZ diabetic rats (Bhaskar et al., 2011). Oral administration with either AF or CGA resulted in a significant increase in the activities of these enzymes in diabetic rats indicating better lysosomal stability in diabetes condition after treatment with CGA. This suggests that CGA beneficially decreased the intracellular oxidative stress. This is in line with a previous report where administration of curcumin and quercetin to diabetic rats improved lysosomal enzyme activities (Chougala et al., 2012). On contrary, there are other reports where the lysosomal enzyme activities were increased in diabetic condition (Abd El-Azim et al., 2013).

In conclusion, treatment with the aqueous fraction of MEC and the active compound, chlorogenic acid to diabetic rats caused significant antidiabetic potential and effectively improved the respiratory chain complex enzyme activities by reducing the mitochondrial ROS production thereby beneficially modulated glucose utilization. In
addition, AF and CGA increased the lysosomal stability by normalizing the altered lysosomal enzyme activities. Furthermore, it can be inferred that the antidiabetic effect of CGA and AF is comparable with each other.