CHAPTER 5

Effect of Different Solvent Extracts of CnI on Antioxidant Status in Streptozotocin Induced Diabetic Rats

Diabetes is one of the most prevalent metabolic disorders and is characterized by increased blood sugar levels and improper primary metabolism. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Raised glucose levels have been linked to reactive oxygen species (ROS) generation and studies indicate that hyperglycemia may be the cause of oxidative stress in organisms, including in pancreatic β-cells (Mohanty et al., 2000; Ihara et al., 1999). ROS generation and oxidative stress lead to β-cell damage and have been associated with the complications in diabetes (Li and Shah, 2003; Maechler et al., 1999). Studies also suggest that increased oxidative stress causes reduced insulin secretion by the islet β-cells (Kajikawa et al., 2002; Sakai et al., 2003).

In diabetes mellitus, hyperglycemia can lead to the depression of the natural antioxidant system (Pavana et al., 2009). Several reports have demonstrated that elevated free radical generation is common in chronic hyperglycemia as well as in both experimental models and human diabetes mellitus (Bhor et al., 2004). In diabetes, free radicals are formed harmfully through the following mechanisms: glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Sudha Rani, 2013). The antioxidant network of the body, due to its wide range of redox potential and localization, allows an efficient protection against reactive oxygen nitrogen species (RONS). The multi-functional properties of the antioxidant network highlight the crucial importance of the dynamic interactions among the components of the network in protecting body fluids from oxidative stress (Serafini et al., 2006). Non enzymatic antioxidant capacity takes into account the antioxidant activity of compounds present in phytonutrients as well as their potential synergistic and redox interactions (Bartosz, 2010).
Besides the non-enzymatic antioxidant components, cells possess comprehensive array of antioxidant defense mechanisms including enzymatic antioxidants (Kharrazi et al., 2008). Deficient functioning of these leads to accumulation of toxic oxidative free radicals and consequent degenerative changes. The therapeutic activity of plant products is mainly related to the antioxidant activity of phytochemical components, which in turn imparts antidiabetic effect (Saito et al., 2008; Buyukbalci and El SN, 2008).

In the previous phytochemical screening study, we find out the different phytochemical and macronutrient constituents present in young inflorescence of coconut palm and also compared the antioxidant and antiglycation potentials of solvent fractions of CnI using in vitro assay models. In view of the observed in vitro antioxidant and antiglycation effects of young inflorescence, further investigation on the in vivo antidiabetic and antioxidant properties of different solvent fractions of CnI was planned. Hence, the present study aimed to evaluate the antidiabetic and antioxidant effects of methanol, ethanol and ethyl acetate extracts of CnI on streptozotocin (STZ) induced diabetic rats.

**Materials and methods**

**Chemicals**

Streptozotocin was purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA. Methanol, ethanol and ethyl acetate were purchased from Merck KGaA, Darmstadt, Germany. Dinitro phenyl hydrazine (DNPH) and ferric chloride were purchased from Sisco Research Laboratories, Mumbai, India. Kit for glucose estimation was purchased from Agappe Diagnostics, Thane, India. Insulin assay kit was purchased from SP-BIO, France. Fructosamine estimation kit was purchased from Spinreact, S.A.U., Spain. All other chemicals used were of the highest analytical grade.

**Methods**

Collection of CnI and procedures for preparation of methanol, ethanol and ethyl acetate extracts of CnI were described in chapter 2.
Animal Experiments

Male albino rats of 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. Rats in all the groups were allowed free access to standard laboratory animal feed (VRK Nutritional Solutions, Pune, India.) and water ad libitum. Animals were divided into 5 groups of 6 rats each and fed as follows:

Group I - Normal Control rats.
Group II - Diabetic Control Rats.
Group III - Diabetic + 80% methanolic extract (200 mg/kg)
Group IV - Diabetic + 95% ethanolic extract (200 mg/kg)
Group V - Diabetic + ethyl acetate extract (200 mg/kg)

Rats in group I were served as normal control, while group II rats were served as diabetic control. Animals in group III were given 80% methanol extract of CnI at a dose of 200mg/kg body weight/day orally by gastric intubation. Group IV rats and group V rats were orally fed with 95% ethanol extract and 100% ethyl acetate extract of Cnl respectively. These different solvent extracts of Cnl 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 concentration 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. This condition was observed at the end of 72 h after STZ injection.

The feeding experiment lasted for 45 days. After the experimental period, the rats were euthanized and tissues were taken out, transferred to cold containers and serum and plasma were separated from the blood samples collected and used for the biochemical analyses. For histopathological examinations, the pancreas was removed from each rat and fixed in 10% buffered neutral formalin. The procedures for assay of biochemical parameters were described in chapter 2.
Results

5.1 Concentration of serum glucose and serum fructosamine

Streptozotocin (STZ) administration caused increased serum glucose and serum fructosamine levels in diabetic control rats compared to that of normal control animals. Treatment with Cnl extracts to diabetic rats resulted in a reduction in the blood glucose and fructosamine levels when compared with diabetic control rats (Figure 5.1). The levels of glucose and fructosamine were much reduced in animals treated with Cnl methanol extract compared to those animals treated with Cnl ethanol and ethyl acetate extracts indicating a better efficiency of methanolic extract in glycemic control.

Figure 5.1 Concentration of serum glucose (mg/dL) and serum fructosamine (µmol/L)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.
5.2 Concentration of serum insulin and hepatic glycogen

Serum insulin levels were found to be decreased in diabetic rats administered with STZ alone. Oral treatment of different solvent extracts of Cnl to diabetic rats significantly increased insulin levels compared to diabetic control rats. Serum insulin levels of rats treated with Cnl methanol extract were higher than those rats treated with ethanol and ethyl acetate fractions (Figure 5.2). Glycogen levels in the liver of diabetic rats were also found to be significantly reduced compared to the normal control rats. Treatment with Cnl extracts enhanced the glycogen storage capacity of diabetic rats compared to diabetic control animals and this effect was predominant in rats treated with Cnl methanol extract (Figure 5.2).

Figure 5.2 Concentration of serum insulin (ng/mL) and hepatic glycogen (mg/g wet tissue)

Values are expressed as mean ± SD (n = 6). Comparison is between groups. Different alphabets indicate significant difference at p<0.05.
5.3 Effect of treatment with Cnl extracts on oral glucose tolerance test in diabetic rats

Changes in blood glucose levels of oral glucose tolerance test (OGTT) were shown in Figure 5.3a. In STZ-treated diabetic control rats, the peak increase in blood glucose level was observed after 60 min. Even after 120 min, the blood glucose concentration in this group remained high. In normal control and in Cnl extracts treated groups, however, a decrease in blood glucose concentration was observed after 60, 90 and 120 min. The integrated area under curve for glucose (AUCglucose) values for 0–120 minute post glucose load was calculated using trapezoid rule, and the results were given in Figure 5.3b. Estimation of AUCglucose values indicated that Cnl methanol extract treatment to diabetic rats showed 54.62% decrease in blood glucose levels compared to diabetic control group, which were better than ethanol (49.61%) and ethyl acetate (45.27%) extracts.

Figure 5.3a OGTT Curve

![OGTT Curve](image)

Values are expressed as mean ± SD (n = 6). Comparison is between groups.
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Figure 5.3b Area under curve for glucose (AUCglucose) values for 0–120 min

Values are expressed as mean ± SD (n = 6). Comparison is between groups.

Different alphabets indicate significant difference at p<0.05.

AUCglucose is expressed as mg/dL.min

5.4 Concentration of Glycated Hb (HbA$_{1c}$)

Administration of STZ significantly elevated the levels of HbA$_{1C}$ in the serum of diabetic control rats compared to the normal animals. In the present study, oral treatment with Cnl methanol, ethanol and ethyl acetate extracts (200 mg/kg) significantly reversed increasing levels of serum HbA$_{1C}$ in diabetic rats indicating the antiglycative effect of Cnl extracts (Figure 5.4). Those rats administered with Cnl methanol extract showed better reduction in HbA$_{1C}$ levels compared to the other two fractions.
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Figure 5.4 Concentration of HbA1c in percentage of total Hb

![Bar chart showing the concentration of HbA1c in different groups.]

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

5.5 Activity of superoxide dismutase in liver, heart and kidney

Figure 5.5 Activity of superoxide dismutase in liver, heart and kidney (*Units/mg protein)

The activities of superoxide dismutase (SOD) in hepatic, cardiac and renal tissues were decreased significantly in diabetic control rats compared to the normal. Treatment with CnI extracts significantly increased the activity of SOD in these tissues compared to the diabetic control rats. Among the three extracts of CnI, methanol extract showed a better increase in SOD activity (Figure 5.5).
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.

5.6 Activity of catalase in liver, heart and kidney

Intraperitoneal injection of streptozotocin to diabetic control rats caused a drastic reduction in the activities of catalase in tissues such as liver, heart and kidney. Intra gastric administration of CnI extracts to diabetic rats for a period of 45 days caused significant increase in the activities of catalase in liver, heart and kidney compared to the diabetic control rats (Figure 5.6). CnI methanol extract had a better effect on catalase activity than ethanol extract while, CnI ethyl acetate extract showed only a moderate effect.
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Figure 5.6 Activity of catalase in liver, heart and kidney (x10^{-3} *Units/mg protein)

Values are expressed as mean ± SD (n = 6). Comparison is between groups.

Different alphabets indicate significant difference at p<0.05.

*One unit is defined as the velocity constant per second.

5.7 Activity of glutathione peroxidase (GPx) in liver, heart and kidney

Glutathione peroxidase activity in liver, heart and kidney were decreased significantly in diabetic control rats administered with STZ. Compared with the diabetic control animals, those diabetic rats fed with Cnl extracts showed significantly increased glutathione peroxidase activity in hepatic, cardiac and renal tissues (Figure 5.7). There was no significant difference observed for the activity of GPx in diabetic rats fed Cnl ethanol extract compared to those fed Cnl ethyl acetate extract. Cnl methanol extract showed much more increase in GPx activity compared to the other two extracts.
Figure 5.7 Activity of glutathione peroxidase in liver, heart and kidney (x10^{-2} *Units/mg protein)

Values are expressed as mean ± SD (n = 6). Comparison is between groups.
Different alphabets indicate significant difference at p<0.05.
*One unit is defined as the µmoles of glutathione oxidized per minute.

5.8 Activity of glutathione reductase in liver, heart and kidney

The activities of glutathione reductase (GRd) in hepatic, cardiac and renal tissues were decreased significantly in diabetic control rats compared to normal control animals. Daily oral treatment with Cnl extracts to diabetic rats for a period of 45 days significantly increased the activity of GRd in these tissues compared to the diabetic control rats (Figure 5.8). Among the three extracts of Cnl, methanol extract showed a better increase in GRd activity.
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Figure 5.8 Activity of glutathione reductase in liver, heart and kidney (x10^{-2} *Units/mg protein)

Values are expressed as mean ± SD (n = 6). Comparison is between groups.

Different alphabets indicate significant difference at p<0.05.

*One unit is defined as the µmoles of glutathione reduced per minute.

5.9 Levels of reduced glutathione in liver, heart and kidney

A significant decrease in the levels of reduced glutathione (GSH) in liver, heart and kidney were observed in diabetic control animals administered with streptozotocin alone. Cnl methanolic extract treatment significantly increased the concentration of GSH in liver, heart and kidney of diabetic rats compared to ethanolic and ethyl acetate extracts (Figure 5.9). While, ethanol and ethyl acetate extracts treated rats showed almost similar levels of GSH content in these tissues.
Figure 5.9 Levels of reduced glutathione in liver, heart and kidney (mM/100g)

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

5.10 Levels of plasma α-tocopherol and ascorbate

Streptozotocin induced diabetes caused depletion of serum α-tocopherol and ascorbate levels in diabetic control rats compared to normal control rats. CnI methanolic extract administration significantly increased α-tocopherol and ascorbate level in serum of diabetic rats compared to ethyl acetate extract (Figure 5.10). There was no significant difference observed in the levels of α-tocopherol and ascorbate in the serum of diabetic rats fed with CnI methanol and ethanol extracts.
Figure 5.10 Levels of plasma α-tocopherol and ascorbate (mg/dL)

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

5.11 Concentration of plasma ceruloplasmin

The concentration of ceruloplasmin in plasma was found to be increased in diabetic rats administered with streptozotocin alone. Compared with the diabetic control animals, diabetic rats administered with CnI extracts showed a significant decrease in the levels of plasma ceruloplasmin (Figure 5.11). The effect was more with rats fed CnI methanol extract.
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Figure 5.11 Concentration of plasma ceruloplasmin (mg/dL)

Values are expressed as mean ± SD (n = 6). Comparison is between groups.

Different alphabets indicate significant difference at p<0.05.

5.12 Histopathology of pancreas stained with hematoxylin and eosin (x 400)

The histopathology of pancreas of diabetic rats treated with CnI extracts were showed in figure 5.12. In the histology of pancreatic sections of diabetic rats (B), the islets were less and their shapes and structure were altered drastically compared to normal control rats (A). In CnI extracts fed diabetic rats (C, D and E), there were more islets and the architecture of those islets were comparable to normal rat islets, although there were individual differences.
Figure 5.12 Histopathology of pancreas stained with hematoxylin and eosin (x 400)

(A) Normal control
(B) Diabetic control
(C) Diabetic + CnI methanolic extract
(D) Diabetic + CnI ethanolic extract
(E) Diabetic + CnI ethyl acetate extract

Arrow shows islets β-cells.
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Discussion

The present study described the antidiabetic and antioxidant effects of methanol, ethanol and ethyl acetate fractions of CnI on streptozotocin (STZ) induced experimental diabetes mellitus. STZ is a classical broad-spectrum antibiotic obtained from Streptomyces achromogenes. Since the finding that STZ possess diabetogenic properties mediated by pancreatic β-cell destruction, this drug has been widely used to induce diabetes in experimental animals (Junod et al., 1969; Singh et al., 2001). STZ is a preferred agent to induce experimental diabetes since it has some advantages over alloxan such as relatively longer half-life, sustained hyperglycemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality (Ozturk et al., 1996; Rajashree et al., 2011). Evidence suggested that STZ induces oxidative stress (Wright, 1999). STZ can selectively destruct pancreatic β-cells by producing free radicals of oxygen, nitrogen monoxide, and reducing intracellular NAD and NADP, which are essential for the electron delivery and energy metabolism in β-cells (Gutierrez et al., 2011).

Intraperitoneal administration of STZ to rats caused a decrease in serum insulin and a concomitant increase in serum glucose levels. Oral feeding of CnI extracts after STZ treatment resulted in a significant reduction of serum glucose levels and improved serum levels of insulin as compared with rats administered streptozotocin alone. This indicates the efficacy of CnI extracts in regulating glucose homeostasis by controlling circulating levels of insulin. The results obtained in the current study are in accordance with those of other investigations using different plant extracts (Norfarizan-Hanoon et al., 2009; Abdel-Sattar et al., 2011). Treatment with CnI extracts showed a significant effect on orally administered glucose load in diabetic rats without inducing the hypoglycemic state. The glucose load was well tolerated in CnI extracts treated groups. The glucose tolerance was more with CnI methanol extract, indicating its antihyperglycemic action and better glycemic control over the other two extracts. In this context, a previous study also had a similar effect on the oral glucose tolerance test in experimental diabetic rats (Pari et al., 2001).
The liver plays an important role in buffering postprandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen. Aberration of liver glycogen synthesis or glycogenolysis in diabetes may be due to a lack of or resistance to insulin, which is essential to activate the glycogen synthase system (Whitton and Hems, 1975; Chackrewarthy et al., 2010). In the diabetic control rats, the glycogen content in the liver was reduced compared to normal control animals due to the lack of insulin. Similar observations were also reported in studies using STZ-diabetic rats (Vats et al., 2003). Oral administration with CnI extracts prevented the depletion of glycogen in the liver of STZ-diabetic rats. This could be due to the increased circulatory insulin concentrations observed in the CnI extracts treated rats. These effects were predominant in rats fed with CnI methanolic extract.

In diabetes, the glycation and subsequent Maillard reaction are enhanced by increased glucose levels, and there is evidence that glycation itself may induce the formation of oxygen-derived free radicals (Gupta et al., 1997). Serum HbA1c is a glycated protein which has crucial roles in the progression of many pathological conditions including diabetes (Gabbay, 1976). The levels of fructosamine, an early glycation marker and glycated haemoglobin, an advanced glycation marker, are monitored as a reliable index of glycemic control in diabetes (Cerami et al., 1978). They result from spontaneous nonenzymatic condensation of excess glucose present in blood and a number of proteins including albumin and hemoglobin due to uncontrolled or poorly controlled diabetes (Misciagna et al., 2004). The increased levels of serum fructosamine and HbA1c were found to be directly proportional to the fasting blood glucose level (Jackson et al., 1979). In line with previous studies (Montilla et al., 2004), the present investigation also revealed significant elevation in serum fructosamine and HbA1c levels in diabetic control rats in comparison with normal ones. Ingestion of the CnI extracts effectively reduced the increasing levels of serum fructosamine and glycated Hb, which may attributed to their potential glycemic control together with they may have an important role in preventing protein glycation. Among the three extracts, CnI methanol extract had a more potential effect on inhibiting protein glycation.
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The increase in oxidative stress in diabetes could be related to raise in blood glucose levels, leading to auto-oxidation of glucose to generate free radicals. Hyperglycemia-induced oxidative stress may also cause damage to tissues such as liver, heart and kidney (Murugan and Pari, 2006). Several studies showed that STZ produces a decrease in the activity of the antioxidant enzymes during the development of STZ-induced diabetes in liver, heart and kidney (Rathod et al., 2009). Superoxide dismutase (SOD) is an important antioxidant defense enzyme, which catalyses the dismutation of superoxide radicals (Bolzan and Bianchi, 2002). It plays a pivotal role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide, which is easily reduced to water by catalase (CAT). CAT is a hemeprotein that catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. The free radical scavenging activity of SOD is effective only when it is followed by the increased CAT activity (Rodriguez et al., 2004). The present study revealed that SOD and CAT activities were decreased in the tissues of diabetic control rats. Reduction in the activities of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide (Aragno et al., 1997). These antioxidant enzymes SOD and CAT levels significantly increased after the oral administration of Cnl methanol and ethanol extracts in STZ-induced diabetic rats indicating the free radical scavenging activity and their protective effect against tissue damage.

Reduced glutathione (GSH) is the major endogenous antioxidant that counters balance free-radical-mediated damage in diabetes mellitus. It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions (Rotruck et al., 1973). The physiological role of GSH is as an essential intracellular reducing agent for the maintenance of thiol groups on intracellular protein and antioxidant molecules. It was well established that GSH, the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation and hydroperoxide reduction (Dickinson et al., 2003). In addition, the GSH antioxidant system plays a fundamental role in cellular defense against reactive oxygen species and other free radicals (Masella et al., 2005). This system consists of GSH and an array of functionally related enzymes,
of which glutathione reductase (GRd) is responsible for the regeneration of GSH, whereas glutathione peroxidase (GPx) worked together with GSH in the decomposition of hydrogen peroxide or other organic hydroperoxides (Mak et al., 1996). Thus, the levels of GSH and activities of GPx and GRd were used to monitor the peroxidative balance. In diabetic condition, the tissue levels of GSH were reported to be decreased (Gregus et al., 1996). A marked reduction in the activities of GPx and GRd were also reported in diabetes (Ravi et al., 2004). In the present study, the GSH levels and the activities of GPx and GRd were significantly decreased in tissues like liver, heart and kidney of STZ-induced diabetic control rats. The decrease in GSH levels and a concurrent decrease in GPX and GRd activities may represent increased utilization of GSH antioxidant system due to oxidative stress induced by STZ. After treatment with different solvent fractions of Cnl in diabetic rats, the activities of these antioxidant enzymes GPx and GRd and the levels of GSH were significantly increased compared to diabetic control rats. These observations indicate that Cnl extracts possess protective effect on endogenous antioxidants against STZ induced oxidative damage.

In diabetic condition, non-enzymatic antioxidants in plasma are altered (Bajaj and Khan, 2012). The most important non-enzymatic hydrophobic antioxidant in the cell membrane is α-tocopherol; it interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxyl radicals, thus protecting the cell structures against damage (Takenaka et al., 1991). In the current study, rats injected with STZ showed a decreased level of vitamin E. This result is in line with the previous report, where decreased level of plasma α-tocopherol was observed in STZ induced diabetic rats (Garg et al., 1996). The decreased level of α-tocopherol found in the diabetics as compared with control rats could be due to the increased oxidative stress which accompanies the decrease in the level of antioxidants, and may be related to the cause of diabetes mellitus. While, feeding Cnl methanol and ethanol extracts caused an increase in the concentration of vitamin E in diabetic rats compared to the ethyl acetate fraction.

Vitamin C is an excellent plasma hydrophilic antioxidant because it disappears faster than other antioxidants, when plasma is exposed to reactive oxygen species (Frei et al., 1989). It functions as a free radical scavenger of active and stable oxyradicals. Ascorbate is capable of regenerating α-tocopherol from tocopheroxyl radical that is formed upon the inhibition of lipid peroxidation by vitamin E (Niki et al., 1982). In this
study, a significant decrease in the levels of ascorbate was observed in the plasma of diabetic control rats. The observed decrease in plasma vitamin C might be due to increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C (Wefers and Sies, 1988). Intra gastric supplementation of Cnl methanol and ethanol extracts to diabetic rats resulted in the improvement of plasma levels of ascorbate in comparison with the diabetic control animals. Previous studies have shown that under all types of oxidative stress, ascorbic acid successfully prevents detectable oxidative damage and helps to prevent degenerative diseases in which oxidative stress plays a causative or exacerbation role (Zhang and Omaye, 2001).

The plasma protein ceruloplasmin is a powerful free radical scavenger. It is a copper containing oxidase, which serves as a copper transporter in tissues. Ceruloplasmin has been established as a chain breaking antioxidant with a potential to scavenge peroxyl radicals (Halliwell and Gutteridge, 1984). It was shown that ceruloplasmin levels increase under conditions leading to the generation of oxygen products, such as the superoxide radical and hydrogen peroxide (Dormandy, 1980). Thus, the increase in ceruloplasmin levels is an indication of increased antioxidant defense to compensate for the loss of other antioxidants. In the present study, the levels of ceruloplasmin in the plasma of diabetic control rats were significantly increased. The observed rise in plasma ceruloplasmin in diabetic rats might be due to increased peroxyl radicals. Cnl extracts treatment to diabetic rats beneficially reversed the plasma concentrations of ceruloplasmin to near normal level.

Histopathological analysis of pancreas also supported all these biochemical results in the present study. In the diabetic control rats, a decrease in the number of β-cells of the islets of Langerhans was observed in comparison to the normal control group. The damage or necrosis of β-cells was caused by the administration of streptozotocin. Few functional β-cells were also observed. The damaged β-cells seen after the initial induction of diabetes were no longer observed after treatment with Cnl extracts. The recovery of necrotic β-cells was especially more pronounced after treatment with methanol extract of Cnl than in the groups treated with ethanol and ethyl acetate extracts.
To summarize, the present study proved that methanolic extract of Cnl was more effective in controlling hyperglycemia and oxidative stress induced by STZ than the other two fractions and it also possesses a better antiglycative property.